بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Serologic Prevalence of Brucellosis in Horses (*Equus Caballus)* Stables in Khartoum State - Sudan

الإنتشار المصلي لداء البروسيلا في إسطبلات الخيول (إكوس كابالوس) بولاية الخرطوم - السودان

By

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بسمِ ٱللهِ ٱلرَّحۡمَنِ الرّحِيَمِ

قَالَ تَعَالَىٰ:

﴿ وَوَهَبۡنَا لِدَاوُۥدَ سُلَيۡمَٰنَۚ نِعۡمَ ٱلۡعَبۡدُۖ إِنَّهُۥٓ أَوَّابٌ ٣٠ إِذۡ عُرِضَ عَلَيۡهِ بِٱلۡعَشِيِّ ٱلصَّٰفِنَٰتُ ٱلۡجِيَادُ ٣١ فَقَالَ إِنِّيٓ أَحۡبَبۡتُ حُبَّ ٱلۡخَيۡرِ عَن ذِكۡرِ رَبِّي حَتَّىٰ تَوَارَتۡ بِٱلۡحِجَابِ ٣٢ رُدُّوهَا عَلَيَّۖ فَطَفِقَ مَسۡحَۢا بِٱلسُّوقِ وَٱلۡأَعۡنَاقِ ٣٣ ﴾

ص: ٣٠ – ٣٣

صَدَقَ اللهُ العَظيم

Dedication:

To my Darling Father

To my Darlingness Mom

To my Darlings Brothers and sisters

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Praise be to Allah, by which good deeds are done.

I’m wish to express my sincere gratitude to my supervisor professor. Dr. Galal El Dean El Azhari Mohammed El Hassan Professor and the Dean of the college of Veterinary Medicine Sudan University of Science and Technology, or his guidance, keen interest sound advice and kind supervision throughout this work.

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ABSTRACT

The Rose Bengal Plate Test (RBPT) and serum agglutination test (SAT) were conducted to determine the serologic prevalence of brucellosis in horses in the Khartoum State Sudan. A total of 150 horses that were on stable management system was sampled. During the period from December 2016 to February 2017. Using cross sectional study localities State this included Khartoum, Bahri and Omdurman. The objectives of the study were to determine the prevalence of *Brucella* antibodies in horses as well as the distribution of the infection according to the certain risk factors. The samples collected comprised of 109 males and 41 females from three localities in Khartoum State Sudan. Sera was screened by Rose Bengal Plate Test (RBPT) and the positive or week positive such as further processed by serum agglutination test (SAT) for confirmation. The overall Sero-prevalence of brucellosis in horses was 16.7% by RBPT and 4% by SAT. Source wise Sero-prevalence of brucellosis was 44.7%, 30.7%, and 24.7%, in horses of Khartoum, Bahri and Omdurman respectively. Gender wise Sero-prevalence in horses was 72.7% males and 27.3% females. In relation to age, Sero-prevalence was 93.3% adults and 6.7%, young horses. Prevalence of brucellosis in different breeds of horses was 88% and 12%, in cross and local breed of horses, respectively. Depending upon the body condition, the Sero-prevalence was 88.7%, and 11.3% in normal and emaciated body conditioned horses, respectively. And Sero-prevalence was conducted in horses according to absent and present the wool sorter this was 78.0% and 22.0%, respectively. The statistical analysis using are Chi-square Test and Logistic Regression. Chi-square showed high significant association between disease and risk factors such as location and gender, and significant association in breed, wool sorter and body condition. But no significant association between disease and risk factors in age and contact. The Logistic Regression Test was conducted the all results significant from Chi square was no significant in the Logistic Regression.

الخلاصة:

تم إجراء إختبار فحص البنقال وإختبار تراص المصل لتحديد الإنتشار المصلي لمرض البروسيلا في الخيول في ولاية الخرطوم في دولة السودان. تم أخذ عينات من 150 خيل كانت علي نظام إدارة مستقرة. في الفترة من ديسمبر2016 إلي فبراير2017. بإستخدام المقاطعات دراسة المقطعية الدولة هذا شمل الخرطوم, بحري وام درمان. كانت أهداف الدراسة هي تحديد مدى إنتشار الأجسام المضادة للبروسيلا في الخيول فضلآ عن توزيع الإنقسام وفقآ لعوامل الخطر القصيرة. العينات التي تم جمعها من 109 ذكر و 41 أنثى من ثلاث مناطق في ولاية الخرطوم السودان. تم فحص المصل من قبل إختبار لوحة بنقال روز والإيجابية أو ضعيفة الإيجابية مثل مزيد من المعالجة من قبل إختبار تراص المصل للتأكيد. كان الإنتشار المصلي الكلي لداء البروسيلا 44.7%, 30.7% و24.7% في الخيول في الخرطوم, بحري و ام درمان بالتوالي. وكانت نسبة الإنتشار المصلي الحكمي بين الجنسين في الخيول 72.7 في المائة من الذكور و27.3 في المائة من الإناث. فيما يتعلق بالإنتشار المصل للسن كان 93.3% من البالغين و6.7% من الخيول الشابة. كان إنتشار داء البروسيلا في سلالات مختلفة من الخيول 88% و 12% في السلالة المحلية, والخيول علي التوالي. إعتمادآ علي حالة الجسم, كان إنتشار المصلي 88.7% و11.3% في الخيول الجسم العادية وهزال مكيفة علي التوالي. وأجرى سيروبريفلانس في الخيول وفقآ لغياب وعرض فارز الصوف كان هذا 78.0% و22.0% علي التوالي. والتحليل الإحصائي هي إختبارمربع كاي واللوجستك. واظهرت مربع كاي إرتباط كبير جدآ بين المرض وعوامل الخطر مثل الموقع ونوع الجنس. وإرتباط كبير في سلالة فرز الصوف وحالة الجسم. ولكن لا علاقة كبيرة بين المرض وعوامل الخطر في العمر والإتصال. أجري إختبار الندم اللوجستي كل النتائج الهامة من مربع كاي لم تكن كبيرة في إختبار اللوجستي.

Table of content

List of tables:

List of figure:

Introduction

Brucellosis is an important zoonotic disease worldwide causing serious human health problems and substantial economic losses for the livestock industry (Corbel, 1997).

Brucellosis is still one of the world's major problems as a disease of both human beings and in animals, it is caused by various species of *Brucella* (boussetta, 1991).

The infection by *Brucella*, naturally acquired in horses associated with infected cattle *B. abortus* and swine *B. suis* and horizontal transfer has been demonstrated (Forbes, 1990).

The disease remains an uncontrolled problem in regions with poor animal and public health standards leading to high endemic in Africa, Middle East, the Mediterranean, parts of Asia and Latin America (Capasso, 2002; Refai, 2002).

Equine infection most frequently involves *B. abortus*, but *B. suis* was isolated from horses with septic bursitis and from the internal organs of a mare without external signs of disease (Cvetnic *et al.*, 2005).

Serological surveys have indicated that many horses may be exposed to *B. abortus* without developing clinical signs of disease (Göz *et al*., 2007).

The sero-prevalence of brucellosis in various animals such as cattle, camels, sheep, goats, human and poultry were described in Iran, (Bokaie, 2008; Rabbani *et al.*, 2008; Sabbaghian., 1973; Makarem *et al.*, 1982).

Horses are accorded special attention, due to the immense role they play in polo games, cultural festivals and security (Ehizibolo *et al.*, 2011).

Human infection is contracted from the infected animals and closely linked to poor animal husbandry methods, feeding habits, and hygiene standards (Pascual *et al*., 1998).

In the Sudan, the brucellosis occurs in all animal species including wildlife and humans. *B .abortus,* biovars 1, 3, 6 and 7 and *B. melitensis,* biovars 2 and 3 were found associated with the disease (Musa *et al.,* 2008).

There are indications of introduction of some of these biovars through exportation of infected or latently infected animals. Occurrence of *B. abortus* and *B. melitensis* in different animal's species throughout the country has important implications in control of the disease (Musa *et al.,* 2008).

Scientific justification:

Brucellosis a zoonotic disease has been eradicated from most of the developed countries through test and culling policy. When the disease exists in horses, which is a reservoir .Vaccination in animal against this disease has been used successfully in most countries. Therefore be considered because the diseases important from the public health standpoint (Acosta *et al*., 2006). There has a significant public health impact for people who are in direct contact with low awareness animals and poor hygienic conditions which favor for infection (Megersa *et al*., 2008).

Objective:

The objectives of this study was:

1. To detect Seroprevalence of brucellosis in horses in Khartoum state, Sudan.
2. To identify the risk factors associated with the disease.

Chapter One

Literature Review

1.1 Brucellosis:

Brucella is Gram-negative coccobacilli, an infectious and contagious organism. The natural hosts of this organism are mainly cattle, horses and humans. Brucellosis was considered to be occupational disease that mainly affects slaughter-house workers, butchers, and veterinarians, (Acha and Szyfer, 1987).

Species of genus *Brucella* and their major hosts are *B. abortus* (cattle), *B. melitensis* (goats), *B. ovis* (sheep), *B. suis* (swine). *B. abortus* causes infection in horses and is commonly found in chronic bursal enlargements as a secondary invader rather than a primary pathogen, (Radostits *et al.*, 2000). Transmission typically occurs through contact with infected animals or materials with skin abrasions. Symptoms in human brucellosis can be highly variable ranging from flu-like, non-specific symptoms (acute form) to undulant fever, orchitis, arthritis and epididymitis, (Gul and Khan, 2007).

All domesticated species can be affected with brucellosis except cats which are resistant to *Brucella* infection. Considering the damage by the infection in animals in terms of decreased milk production, infertility, weight loss, abortion, weak off-spring and lameness, it is one of the most serious disease of livestock. It's also a major impediment for the trade. Death may occur as result of acute metritis, followed by retained fetal membranes, (Radostits *et al.*, 2000).

The ability of *Brucella* to replication and persist in host cells is directly associated with its capacity to cause persistent disease and circumvent innate and adaptive immunity, (Fichi, 2003).

1.2 Synonyms:

In domestic animals brucellosis known as bovine contagious infection, Enzootic abortion, Epizootic abortion, contagious abortion, infectious abortion, Bang's disease, slinking of calves and ram epididymitis. In the case of human brucellosis including Malta fever, undulant fever, Mediterranean gastric fever, Mediterranean fever, gastric fever, Gibraltar Rock fever, Neapolitan fever, Cyprus fever, intermittent gastric fever or intermittent typhoid fever, pseudo typhus, febris typho- malariae and fievre sudorale, (Ray and Steele, 1979).

1.3 History of the disease:

History shows that the disease was first identified in Malta in the year 1887 by Sir David Bruce who isolated *Brucella melitensis*, the then *Micrococcus melitensis* from a soldier died due to Maltese fever (Godfroid *et al.*, 2005). Brucellosis occurs naturally in domestic animals. It's still an important public health problem throughout the world, but principally, and in particular, in the Mediterranean region, including the Arabian Peninsula, Turkey, Mexico, the Indian subcontinent, and parts of Central and South America (Young 1995, Colmenero *et al.*, 1996).

1.4 Definition:

Brucella is one of major zoonotic pathogen worldwide, it is responsible for enormous losses as well as considerable human morbidity in endemic areas. The organism infects animals such as cattle, sheep, goat, swine and dogs. It may also infects other ruminants and marines mammals. Human can be infected indirectly through contact with infected animals or animal products, (Lopes *et al.*, 2010).

Brucellosis is a disease of great economic importance, affects the productive and reproductive potential of the animal in terms of loss of young ones,

infertility and reduction or complete cessation of milk after abortion (Radostits *et al.,* 2000).

1.5 Etiology and epidemiology:

The bacteria of the genus *Brucella* are nonmotile, aerobic, intracellular Gram negative cocci, coccobacilli or short rods. *Brucella spp*. are transmissible to a wide range of species, and among the domesticated animals include the cattle, sheep, goats and pigs are most commonly affected (Godfroid *et al.* 2004).

Wild animal species are also occasionally infected (Godfroid 2002).

The most important clinical manifestations are reproductive failure (include mid to late term abortions, infertility in cows, orchitis and inflammation of the accessory sex glands in bulls). Chronic infections can result in arthritis. In cattle the primary sources of infection are fetal membranes, fluids, vaginal discharges, milk and semen. Placental samples from brucellosis induced abortions in cattle have yielded 1010 organisms/g (Alexander *et al.* 1981).

Major six of the *Brucella species* have been classically characterized include *B. abortus, B .melitensis, B. suis, B. canis, B. ovis* and *B. neotamae*. Recently, two new species, *B. ceti* and *B. pinnipedialis*, have been isolated from marine species (Foster *et al*. 2007).

Most *Brucella spp*. have strong host preference, which was evident in their ability to establish the chronic infection in individuals and maintain transmission and infection in populations of specific animal species (Glynn and Lynn 2008).

The brucellosis in cattle is usually caused by biovars of *B. abortus,* although in southern Europe and in the Middle East, *B. melitensis* may cause abortion in cattle kept close contact to infected sheep and goats (Godfroid *et al.* 2004).

Brucellosis is an important zoonotic disease in the man, human brucellosis

(undulant fever), is seen primarily in veterinarians, stock inspectors, abattoir worker, etc. (Anon 1986).

Human brucellosis is dependent on the presence of *Brucella spp*. Among animals with which people have direct or indirect contact (Glynn and Lynn 2008).

The *B. abortus* infections in domestic animals have been reported worldwide, but they have been effectively eradicated from several European countries, Japan and Israel, (Nicoletti 2007).

There are no apparent age, gender or breed predisposition to infection in horses, although most cases have been reported in horses aged more than three years (Nicoletti 2007).

Brucella spp. are fairly hardy; organisms have been recovered from fetal and manure samples that remained in a cool environment for more than two months. However, exposure to sunlight kills the organisms within a few hours, and the organisms are susceptible to many common disinfectants (Glynn and Lynn 2008).

1.6 The horses in Sudan:

1.6.1 The entry of horses to Sudan:

The date of the entry of horses to Sudan was not known, however, has been seen some pharaonic inscriptions and drawings that symbolize the equestrian, which came in the form of a knight on horseback, indicating that the relationship between the horse and the exploiters of the cavalry were present and not surprising as this the drawings were in northern Sudan, the largest of Egypt where he lived the Sudanese pharaohs who were known to fight wars. These drawings support that the horse's relationship with the Sudanese, especially the pharaohs in the north of the country was ancient and known and exploited in the invasions and wars, although the drawings in the Egyptian caves were referring to a horse dragging a cart even in wars. It is

certain that the horse entered Sudan with the entry of the Arab tribes that entered Egypt through Isthmus in Suez and from there to Sudan or those tribes of the Jihani that arrived in Sudan from the west and across the Sahara and all, the numbers that have settled for Sudan found the environment suitable for breeding and the human being keen to care and knows what it can do and what it can provide for it in terms of aid and assistance in all the harsh conditions of life it faces. (Shaddad. 2014).

1.6.2 Stable:

One of the basics of horse breeding is to secure the place where comfort and protection are available. Divide the stables into several sections for horses to live in groups or at self that are consistent and harmonious. The horses, the Persians, the Persians with their wits, the broken seas, mourning in the age of grooming and horses towing strollers. The temperature suitable for horse comfort is between 10-15 degrees and horses prefer low heat on high. The horses may be tied to the feeder or left free in their movement inside the cabin. The slope of the ground behind the horse should be between 2-2.5%. This tendency ends in a small channel that prevents the pool of urine at the feet of horses. (Shaddad 2014).

Figure 1: stable

1.6.3 Feeding:

It is preferable to use the stone feeders at an altitude of 80 – 100 cm, from the ground 40 -50 cm and a depth of 25 -30 cm should not be used to feed the horse. (Mealia) is used for fodder in western Sudan. The stripes are the best to drink the horse and give the water to the horse 3 -5 times daily, and in the summer 6-10 times due to increase the temperature. Green cannabis and dries are placed in special receptacles in front of the horse's front legs when presented. (Shaddad 2014).

1.6.4 Ground floor:

The soil should preferably be cemented with a brush, provided that the thickness of the bedding is not less than 90 cm. the thick bedding is removed from the comfort of the horses and helps keep the hoofs intact and prevents tumors from occurring in the menus. The sawdust and wheat straw are used in Sudan. (Shaddad 2014).

1.7 Brucellosis in horses:

The *B. abortus* and *B. suis* have been isolated from the horses. The disease usually manifests itself in the form of fistulous bursitis, "poll evil" and "fistulous withers". But the abortions are rare they do occur (Robertson *et al.*, 1973).

The *B. abortus* has been isolated from horse feces, but this is uncommon. The horses acquire the infection from cattle or swine, but transmission from horses to cattle has also been proven. Man can contract the infection from horses that have open lesions. In general, horses are more resistant to the infection. The cases of horse to horse transmission are unknown. In areas where there is a high rate of infection, it is common to find horses with high agglutination titers. Brucellosis is generally asymptomatic in horses (Denny, 1972).

Equine infections usually involve the cattle pathogen *B. abortus*, although infection with *B. suis* has been reported (Nicoletti 2007).

Late to mid-term abortion in mares has been reported, but this appears to be rare (McNutt and Murray 1924; McCaughey and Kerr 1967; Shortridge 1967; Robertson *et al.* 1973; Hinton *et al.* 1977).

Abortion, infertility (Denny, 1972), and arthritis (Carrigan *et al.*, 1987).

1.7.1 Description of the Bursa:

The bursa or supraspinous bursa is located between the funicular portion of the nuchal ligament and the dorsal spinous processes of the second to fifth thoracic vertebrae (Hawkins and Fessler 2000).

1.7.2 Horse fistulous Withers and Poll Evil:

Also Known As

Saddle sore, Wither sinus inflammation:

The horse Fistulous withers and poll Evil, is a chronic inflammatory disease of the supraspinatus bursa and associated tissues, (Gaughan *et al.,* 1988; Rashmir-Raven *et al.,* 1990; Cohen *et al.,* 1992).

Fistulous withers and poll evil are the most common clinical manifestations in the horse, also associated with the variety of other clinical manifestations, including vertebral osteomyelitis, (Collins *et al.,* 1971; Cohn *et al*., 1992),

Although the infection by *B. abortus* has been associated with the condition and other infectious organisms, and trauma can also cause the disease, (Duff 1937; O'Sullivan 1981).

In geographical areas with a low prevalence of brucellosis in cattle, *B. abortus* is rarely isolated from the fistulous withers cases (Gaughan *et al.,* 1988; Cohen *et al*., 1992).

Fistulous withers or poll evil are rare, inflammatory conditions of horses that differ essentially only in their location in the respective supra-spinouts or supra-atlantal bursa. The supraspinous bursa is located between the funicular portion of the nuchal ligament and the dorsal spinous processes of the second to fifth thoracic vertebrae (Hawkins and Fessler 2000).

Other than *B. abortus,* organisms commonly isolated from clinical cases include, *Streptococcus equi, Staphylococcus aureus, Staphylococcus epidermidis, Proteus mirablis, Escherichia coli, bacteroides fragilis, Streptococcus zooepidemicus, Pasteurella spp*. and *Coryne bacterium spp.* (Guard 1932; Gaughan *et al.* 1988; Cohen *et al.* 1992; Hawkins and Fessler 2000).

1.7.3 Clinical signs:

Most classical clinical signs of brucellosis observed in horses due to fistulous withers (septic supraspinatous bursitis) and poll evil (septic supra-atlantal bursitis). Draining sinuses are seen as these problems are chronic in case of equines (Crawford *et al.,* 1990).

Initial signs include swelling, pain, heat in the bursal region leading to stiffness of the neck, later stages the bursa ruptures and pus material gets discharged through one or more opening. These fistulas may heal slowly and there are always chances that they reappear (Cohen *et al.*, 1992).

Draining sinuses seen in these problems are chronic in case of equines (Crawford *et al.,* 1990).

The clinical signs due to brucellosis in equines are mostly noticed in the musculoskeletal system mainly as the organism localise in the bursa (causing septic bursitis), tendon sheaths (causing septic tenosynovitis) and joints (causing septic arthritis), (Denny, 1972, Denny, 1973; Carrigan *et al.*, 1987; Ocholi *et al.,* 2004).

The granulomatous lesions type was observed in lung, liver, testes and metatarsophalangeal synovial membranes (Megid *et al*., 2010).

Serum antibody level was evident after 7 to 12 days of infection and intermittent bacteraemia are observed for 2 months (MacMillan *et al.,* 1982).

Horses affected from the reproductive organs of mares without apparent clinical signs (Cvetnic *et al.,* 2005).

And affected with septic bursitis (Portugal *et al.,* 1971).

1.7.4 Treatment the fistula withers:

The fistulous withers is difficult to treatment because of the often deep seated nature. Antibiotics are effective in early stages. Vaccination with *Brucella* vaccine may help to resolve the disease when it is determined that *Brucella abortus* is the cause of the disease. The administration of the

Brucella strain 19 vaccine tracts with antiseptic solutions and dimethyl sulphoxide may be helpful, (Cohen *et al*. 1992).

If the condition becomes chronic, surgical removal of devitalized and infected tissue may be necessary for a permanent cure. In any case, the veterinarian should be consulted both as to the exact cause of the condition and the best treatment available. Although *Brucella spp.* are generally sensitive to tetracyclines, chloramphenicol, streptomycin and some sulphonamides, there may be insufficient to penetration into infected tissues to achieve resolution of the infection, (Nicoletti 2007).

The successful treatment of 3 horses using clofazimine has been reported (Knottenbelt *et al.*, 1989).

1.8 The disease in humans:

Man is susceptible to infection caused by *B. melitensis, B. suis, B. abortus,* and *B. canis.* The most pathogenic and invasive species for man is *B. melitensis,* followed in descending order by *B. suis*, *B. abortus*, and *B. canis.* The incubation period is one to three weeks, but May sometimes be several months, the disease is septicemic, with sudden onset, and is accompanied by continued, intermittent, or irregular fever. The symptomatology of acute brucellosis, like that of many other febrile diseases, includes chills and profuse sweating, weakness is an almost constant symptom, and any exercise produces pronounced fatigue, temperature vary from normal in the morning to 40°C in the afternoon, sweating is characterized by a peculiar odor occurs at night. The common symptoms are insomnia, sexual impotence, constipation, anorexia, headache, arthralgia, and general malaise. Disease has a marked effect on the nervous system, evidenced by irritation, nervousness, and depression, many patients have enlarged peripheral lymph nodes or splenomegaly and often hepatomegaly, but rarely jaundice. Hepatomegaly or

hepato-splenomegaly is particularly frequent in patients infected by *B. melitensis* (Pfischner *et al.,* 1957).

Brucella organisms localize intra-cellularly in tissues of the reticulo-endothelial system, such as lymph nodes, spleen, liver, and bone marrow. Tissue reaction is granulomatous. The duration of the disease can vary from a few weeks, months to several years. Modern therapy has considerably reduced the disease's duration as well as the incidence of relapses. At times, it produces serious complications, such as encephalitis, meningitis, peripheral neuritis, spondylitis, suppurative arthritis, orchitis, seminal vesiculitis, vegetative endocarditis, and prostatitis. The chronic form of the disease occurs in some patients and may last many years, with or without the presence of localized foci of infection. The symptoms are associated with hypersensitivity. Diagnosis of chronic brucellosis is difficult. Separate mention should be made of human infection caused by the *B. abortus* strain 19 vaccine, which is the vaccine used most often to protect cattle. Cases have been described of accidents among those administering the vaccine (veterinarians and assistants), who have pricked a finger or hand with the syringe needle or have gotten aerosol in their eyes. If someone has no prior exposure to *Brucella* and has no antibodies to the agent, the disease sets in abruptly after a period of 8 to 30 days. The course of the disease is usually shorter and more benign than that caused by the field strains of *B. abortus*, but there are severe cases that require hospitalization. In individuals who have been exposed to *Brucellae*, as is usually the case with veterinarians and vaccinators, a different, allergic-type syndrome appears that is characterized by painful swelling at the inoculation site. After some hours, the patient may experience systemic symptoms similar to those described in individuals infected by strain 19 without prior exposure. The symptoms usually abate in a few days with or without treatment. Local and general symptoms may recur if the person has another accident (Young, 1989).

The human disease in Sudan was first reported in 1904 in Berber in the North of the country (Haseeb, 1950), (Simpson, 1908) followed by another incident in Blue Nile. Erwa (1966) was the first to isolate *B. abortus* from an infected person. Later, reported prevalence of (14.7%) in veterinary workers and their families were serologically positive for the disease Omer *et al*. (1977). Other reported 10% prevalence rate in abattoir workers and milkers in Omdurman abattoir and dairy farms in Khartoum North, Al Sharif (1994). Examined 372 of the people in Darfur and found that 49 (13.2%) of them were positive for the disease, Musa (1995). The surveyed eight states in the country from 1998 _ 2002 and reported 99 cases of the disease, Osman (2004).

Brucellosis in humans is usually associated with the consumption of unpasteurized milk and soft cheeses made from the milk of infected animals, primarily goats, infected with *Brucella melitensis* and with occupational exposure of laboratory workers, veterinarians and slaughter house workers. Some vaccines used in livestock, most notably *B. abortus* strain 19 also cause disease in humans if accidentally injected. Most of the human brucellosis cases are caused by *B. melitensis* but *B. abortus* also accounts for some (Tun, 2007).

Occurrence of the disease in humans is largely dependent on the animal's species, when brucellosis exists in sheep and goats; it causes the greatest incidence of infection in the humans (Shrestha, 2004).

1.8.1 Transmission of Brucellosis to humans:

The Brucellosis in human was once thought to be predominantly transmitted through animals contact. However, it's now being increasingly realized that animal's 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 the high

concentration of the bacteria and consumption of these, an important cause of brucellosis, (Kumar, 2010).

The disease has been recognized as one of the common laboratory transmitted infections and has been reported to occur in the clinical, research, and the production laboratories, (Kumar, 2010).

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

Although there is no reported case of the bio-terrorism using *Brucella spp*. Nevertheless, *Brucella* are not difficult to grow and disperse (the American military weaponized *Brucella suis* in 1954). The transmission to humans may result in prolonged illness and long-term sequela, (Kumar, 2010).

1.8.2 Pathogenesis:

The *Brucella spp*. are facultative intracellular pathogens and establish infection by invading macrophages, the evading macrophage-induced host defense mechanisms (Gorvel and Moreno 2002).

These are characteristics contribute to the clinical signs and therapeutic considerations, including the difficulty in both diagnosis and treatment (Glynn and Lynn 2008).

Following an ingestion of the organism, the bacteria travel through the oral mucosa to the regional lymph nodes. Infection leads to bacteraemia, which is usually transient; the organisms ultimately settle in the reproductive tissues or the musculoskeletal system. Horses usually become infected by the ingestion of *B. abortus* contaminated feed, and most reported cases indicate a

history of the contact with cattle (Duff 1937; McCaughey and Kerr 1967; Denny 1973; O'Sullivan 1981; Ocholi *et al*., 2004).

There are no evidence to suggest that horses are a reservoir of brucellosis in endemic areas or they are an important source of infection for other animals (Acosta-Gonzalez *et al*. 2006).

1.8.3 Diagnosis:

Diagnosis in man is the clinical diagnosis of brucellosis based on symptoms and history, should always be confirmed in the laboratory. Isolation and typing of the causative agent is definitive and may also indicate the source of the infection. Blood or sternum marrow or ileal crest taken while the patient is febrile is cultured in appropriate media. Culture material may also be taken from lymph nodes, cerebrospinal fluid and abscesses. It's recommended that the cultures be repeated several times, especially in enzootic areas of *B. abortus*. Due to widespread use of antibiotics before diagnosis in febrile patients, bacteriologic examinations, particularly of blood, often yield negative results, and serologic tests become increasingly necessary. The serum agglutination test, preferably in tubes, is the simplest and most widely used procedure. A high titer (more than 100 international units, IU) and increasing titers in repeated serum samples provide a good basis for diagnosis. The cross reactions in serum agglutination have been observed in the cases of cholera, tularemia (or as a result of vaccination against these diseases) and in infections caused by *Yersinia enterocolitica* 0:9, as well as *Escherichia coli* 0:157 and 0:116, and *Salmonella* serotypes of Kauffmann-White group N, (Corbel *et al.,* 1984).

1.8.4 Diagnostic Tests:

The most human infections are diagnosed by serology. The tests used include serum agglutination, a modified Coombs' (antiglobulin) technique, ELISAs and immunoblotting (Western blotting). Serologic diagnosis is complicated

by previous exposures and other factors. The definitive diagnosis usually requires a fourfold rise in titer. The immune-staining can sometimes demonstrate the presence of the *Brucella spp*. in a clinical specimen. The PCR techniques can also be used for diagnosis. The chronic brucellosis can be extremely difficult to diagnose, if the serologic results are equivocal and the organism cannot be cultured (Center of Food Security and Public Health 2009).

1.8.5 Treatment of the human:

The human disease treatment, WHO oral regimen of 200 mg doxycycline plus, 600-900 mg rifampicin daily for the minimum of 6 weeks, whereas alternate oral/ parenteral scheme replaces rifampicin with 15 mg/kg streptomycin daily for first 2-3 weeks of treatment only, (Franco *et al*, 2007). If the Jarisch-Herxheimer reaction upon starting antibiotic treatment, the intravenous administration of cortisol is recommended. If the antibiotic therapy is not successful, a chronic focus of infection should be sought, particularly in the infections caused by *B. melitensis* and *B. suis* (WHO, 1986).

The steroids may be administered to counteract toxicity in patients who are very ill (Benenson, 1992).

1.9 Other Animal Brucellosis:

Brucellosis in cattle usually caused by *B. abortus.* In some countries, particularly in south Europe and western Asia, where cattle are puts in close association with sheep or goats, infection can also be caused by *B. melitensis.* Occasionally, *B. suis* may cause a chronic infection in the mammary gland of cattle, but it has not been reported to cause abortion or spread to other animals, (Lopes *et al,* 2010).

The infection rate was higher in intensive camel production system where large numbers of animals are kept in a farm. In countries with extensive form of husbandry the rate is low, (Abbas and Agab, 2002).

The main etiologic agent of brucellosis in goats is *B. melitensis* with its three biovars. All types of goats are susceptible to infection by *B. melitensis*. Infection by *B. suis* and *B. abortus* has occasionally been found. The symptomatology is similar to that observed in other species of animals and the main symptom is abortion, which occurs most frequently in the third or fourth month of pregnancy. In natural infections occurring in the field, other symptoms, such as mastitis, arthritis, orchitis and spondylitis are rarely found. These symptoms can be seen when the animals are inoculated experimentally with large doses of the agent. Sexually mature female goats that are not pregnant are susceptible and suffer from a chronic infection that may have no clinical symptoms, but that represents a risk for the other animals in the flock. Infection of the mammary gland is common (Alton, 1985).

While sheep brucellosis are similar in its symptomatology to the disease in goats, sheep appear to be more resistant to infection and, in mixed flocks, fewer sheep than goats are found to be infected. Susceptibility varies from breed to breed. Maltese sheep are very resistant, while Middle Eastern Awassi (fat tail) sheep are very susceptible (Alton, 1985). *B. canis* is a widespread infection of the dogs in many countries, it's infrequently associated with human disease. Reported cases have usually been mild. *Brucella* infection occurs in many species of wild animals but there are rarely implicated as sources of human disease, (Corbel, 2006).

Wild animal species are also occasionally infected (Godfroid, 2002).

1.10 prevention and control in livestock animals:

Serological based on testing and culling is carried out at Government farms for the eradication of brucellosis. However, the desired objectives cannot be achieved until the disease status in other domestic animals is also known, (Ahmed and Munir, 1995a).

Eradication only be achieved by test and slaughter policy combined with effective prevention measures and control of animals movement, (Corbel, 2006).

However, exposure to sunlight kills the organisms within a few hours, and the organisms are susceptible to many common disinfectants (Glynn and Lynn 2008).

In endemic areas all dead fetuses and placentas should be buried as a routine practice (Radostits *et al.,* 2006).

All new animals should be serological tested before introduction to the flock. Ram and bucks should be tested yearly before start of the breeding season, (Pugh and Baird, 2002).

Chapter Two

Material and methods

2.1 Study area;

Khartoum state is the capital city of Sudan. Also the political center of Sudan, which is considered a Republic, and home to its Executive head of state. Located in the central Sudan, comprising the semi- desert zone between the latitude 15.08^{o} N to 16.39^{o} N and longitude of 31.36^{o} E to 34.25^{o} E. the resident live stock in Khartoum is about 1579 (0.20%) head of horse, (Ministry of animal recourses and fisheries informational center 2015).

2.2 Study population:

Equines population in Khartoum is about 1579 (0.20%) head of horses, (Ministry of animal recourses and fisheries informational center 2015).

2.3 Study design:

A cross sectional study was conducted to estimate serological prevalence of brucellosis in horses (*Equus caballus*) stables.

2.4 Sampling method;

The samples were collected by multistage sampling method, stage one begin selecting from (Khartoum), stage two selecting from (Bahri), stage three selecting from (Omdurman), by simple random sampling. The individual sample of animals was selected by simple random sampling methods, (Martin *et al*., 1987).

2.5 Experimental animals:

For this study, the one hundred and fifty 150 horses of both sexes were selected randomly from the stables include specialized and government

official in Khartoum state Sudan. For each animal information about age (young and adult), the body condition (poor and good), and history of abortion if present, was noted. And breed (local and cross), and gender (male and female), the position (stable and as group), an infection (presence of signs and absence), the source of water (tape water, canal), the breed source (sport and transport) and the inbreeding (natural and artificial), the wool sorter (present and absent). Blood samples were collected from these horses by punctured the jugular vein and sucking blood without anticoagulant, in vacuum tube, serum was harvested and stored in container content ice board and transport to lab- college of vet. Medicine, Sudan University. Then separation of serum by centrifuge 3000 rpm/min at 5-10 minute then putted in eppendorf tube, conservation at -4^{o}C in refrigerator and transported to central laboratory soba for sero-diagnosis.

2.6 Sample size determination:

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

Required sample size (n)

$$\mathbf{n = 1.96^2 P_{exp} (1-P_{exp}) / d^2}$$

n = required sample size

P_{exp} = expected prevalence

d = desired absolute precision

Expected prevalence was conducted as 4.9% in Darfur western Sudan (Musa, 2004). Desired absolute precision was 5% and confidence interval 95%. The total sample size was one hundred and fifty 150 individual animals.

Formula:

$n = 1.96^2 Pexp\ (1\text{-}Pexp) / d^2$

exp = 4.9% = 0.049

d^2 = 5% = 0.05

n = (1.96^2 x 0.049 (1-0.049))/ 0.0025 = 72x2 = 144 also 150

2.7 Sample collection:

The total number of 150 blood Samples was collected from horses stables in Khartoum state Sudan from three locality include Khartoum, Bahri, and Omdurman. The sixty seven 67 horse from Khartoum locality stables, forty six 46 horse from Bahri locality stables, and thirty seven 37 horse from Omdurman locality stables. Approximately 4 ml of blood was collected from the punctured jugular vein of the one hundred and fifty 150 horse of different ages and sex from stables, selected from three localities. Using plain vacationer tubes without anticoagulant, each animals was in properly labelled by name or number of the stable to know hose of them is infected or carrier bacteria *brucella abortus*. The blood siphoned and pouring in sterile vacutainer tubes and then to the thermo flask with ice board. The blood samples was allowed to clot, and then centrifuged at 3000 rpm for 5-10 minute or live to separate alone in room temperature during 8 hours, in the college of veterinary medicine, Sudan university of science and technology in parasitic laboratory. Sera were decanted into properly labelled eppendorf tubes, and conservation in bacteriology lab at –20°C until tested. Same samples are difficult to separate the serum from blood corpuscle due to high cool by ice, was separate it by addition of time to be 10-20 minute in 3000 rpm/min. and in other samples the serum of it be like gel, may be due to fatty degeneration or fatty degradation, was deliquescent by exposure to sun light. And then transported in thermo flask to Central Veterinary Research

Laboratory (CVRL) to the *Brucella* department, (soba) and frozen at -20ºC until testing. Sera were tested within a week of collection.

2.8 laboratory procedures:

The serum samples was first screened using buffered Rose Bengal stained antigen obtained from the Central Veterinary Laboratory Khartoum using the technique by Alton *et al.*, (1975) and each positive sample was examined with RBPT and serum agglutination test (SAT) (Corner, A.L; 1987).

2.9 Serological test:

Sera collected was tested for *B. abortus* antibodies using the Rose Bengal Plate Test (RBPT) described by Alton *et al*., (1975). As screening test and Serum Agglutination Test (SAT).

2.9.1 The Rose Bengal Plate Test:

The Rose Bengal plate test is a fast, simple and sensitive assay used as screening test. Always should be confirmed by other sensitivity bacteriological and serological test.

2.9.1.1 Principle of the test:

It is a card test for the detection of agglutinating antibodies by using inactivated *Brucella* cells, stained with Rose Bengal and suspended in an acid buffer. The acid pH of the suspension prevents the non-specific agglutination of the bacteria, increasing the specificity of the test.

2.9.1.2 Materials required and supplied:

- Ceramic plate.
- Mixing rods.
- Precision micropipette
- Rocking shaker (100 rpm/min) optional.
- Tips

- Gloves
- Rose Bengal antigen
- Serum
- Cotton

2.9.1.3 Specimen collection and handling:

Blood should be collected aseptically through puncture of jugular vein in horse, using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum samples are to be refrigerated (-4ºC) upon collection or frozen (-20ºC) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use haemolysed or contaminated sera. Samples containing particles should be clarified by centrifugation.

2.9.1.4 Serum Sample:

The blood sample was collected without addition of anticoagulant and left to clot. Then centrifuged at 3000 rpm for 5-10 or 20 -10 minutes Take the clear serum and separate it in a clean sterile plastic container for examination (eppendorf tube) and conservation in refrigerator at -4ºC.

2.9.1.5 Procedure:

1. Bring all reagent to room temperature.

2. Carefully shake the antigen suspension

3. Dispense 30mμ of each sample and controls (Positive and negative controls) and the individual on to circles of the ceramic plate.

4. Add onto each circle, close to the sample or control being analyzed, one drop of the Rose Bengal stained *Brucella* suspension (drop equal 30mμ)

5. Mix both drops with the aid of a mixing rod until it covers all the circle surface.

6. Carefully shake the card by hand or on a rocking shaker (100 rpm.) for 4 min. Read the presence or absence of agglutination after this time.

2.9.1.6 Interpretation:

The presence of agglutination in the edge of circle or clump implies the presence of anti-*Brucella* antibodies in the sample. The absence of agglutination or clump indicates a negative reaction and implies the absence of agglutinating anti-*Brucella* antibodies in the sample.

2.9.2 Serum Agglutination Test (SAT):

The antigen for the sero-agglutination in tubes is a concentrated suspension of *brucella abortus* (Weybridge 99 Strain), inactivated by heat and phenol this antigen allows the serological diagnostic of the following Brucellosis; *Brucella abortus* and *B suis*. This antigen is supplied at 10x working concentration after dilution the antigen is standardized to give 50% of agglutination with a final dilution of 1/650 of the international *Brucella abortus* standard serum (OIEISS)

2.9.2.1 Principle:

Wrights sero-agglutination is a technique of slow agglutination in tubes. Successive dilutions of serum to be titrated are brought into contact with constant concentrations of Brucellosis antigen.

2.9.2.2 Materials required and supplied:

- Test tubes
- Racks
- Micropipette
- Tips 30mu and 500mu
- Gloves

- Incubator
- SAT antigen
- Serum
- Falcon sheet

2.9.2.3 Antigen dilution:

1 antigen add 12 phenol saline equal 13mml. SAT 1000mμ and phenol saline 12ml.

2.9.2.4 Tube method:

The test may be done in the glass sterile tubes suitable for working with 1000mμ volumes. At least seven tubes are normally used for each serum under test. Pipette into the tube No.1 of all sets 800mμ of phenol saline. To each of the remaining tubes (2 to 8) add 500mμ of phenol saline. To the tube No.1 tube in each row add 200mμ of the serum sample to be tested and mix well. Transfer 500mμ of the diluted serum from tube No.1 to tube No.2 and mix well. Transfer 500mμ of the diluted sample from tube No.2 to tube No.3 and mix well. Continue this serial dilution till tube No.7 in each set. Discard 500mμ of the diluted serum from tube No.7 of each set. Tube No.8 in all the sets, serves as a saline control. To each tube is then added 500mμ of antigen at the recommended dilution and the contents of tube are thoroughly mixed, thus giving final serum dilutions of 1:10, 1:20, 1:40, 1:80, 1:160, etc. Cover the tubes and incubate at 37° C overnight (approximately 24 hours).

2.9.2.5 Results:

The degree of agglutination is assessed on the amount of clearance tacked place in the tubes as compared with standard tube. The tube is examined without being shaken against in the black background with source of light from above and behind the tubes, Corner, A.L., (1987). Complete

agglutination and sedimentation with water clearance is recorded as ++++, nearly complete agglutination and nearly water clearance recorded as +++, poor agglutination and poor water clearance recorded as ++, less agglutination and turbid resulting as +, and not clearance only SAT colure resulting as -ve. The accuracy and reliability of the reading are much with standard tube. Standard should be prepared at the time the tests are done and incubated with them, Corner, A.L., (1987). Presence of clearance clumps indicates a positive result. Suspected particle is present. Absence of clearance clumps indicates a negative result. Suspected particle is not present.

2.9.3 Interpretation:

Result of the agglutination tests due to the international units (IU), was interpreted according to the recommendations contained in the fifth report of the FAO/WHO Expert Committee of Brucellosis (FAO/WHO, 1971), the six 6 positive tested by SAT all of them adult. Five 5 of them are stallions and one is mare.

2.9.3.1 Conversion of Titres to international units-european method:

Positive sample	Final dilution of serum	End point reading	IU/ML
1-male	1:20	++++	53
	1:40	++++	106
	1:80	++	106
2-male	1:20	++++	53
	1:40	++	80
3-male	1:10	++++	27
	1:20	++++	53
4-male	1:10	++++	27
	1:20	+++	47
5-male	1:40	++++	106
6-female	1:10	++++	27
	1:20	+++	47

These results indicated the horse is carrier of the brucellosis disease and not show any clinical signs E.g wools sorter, laminitis, arthritis, abortion, etc. because due to very long incubation period in horses two years or the numbers of bacteria is low to induction the disease.

2.10 Questionnaires survey:

The potential risk factors of the questionnaire information such as owner data, animals data and management data was filled on a developed format during blood sampling (gender {stallion/mare}, Age {young/Adult}, Breed {local/cross}, body condition {normal/emaciated}, history of abortion {yes/No}, history of retention placenta {yes/No}, infertility {yes/No}).

2.11 Questionnaire survey of horse stables:

Questionnaire survey was administered to individual stables doctors or horse stableman together information on stable managements, breeding type, water source, veterinary service, feeding type, source of new horse, awareness of brucellosis, type of floor, waste disposal.

2.12 Data management:

The questionnaire data was record into a Microsoft Excel sheet. Its Descriptive data, collected from questionnaire survey was analyzed by using descriptive statistical methods. Association between risk factor and disease in stables.

2.13 Statistical analysis:

The descriptive data was collected from questionnaire survey in the stables, used univariate analysis Chi-square and multivariate analysis which was done by logistic regression. Chi- square test was used for univariate analysis with p- value 0.25, and each factors the p- value equal or less than 0.25 was enter to multivariate Logistic Regression and each factor in Logistic with p-value less than or equal 0.05 was considered statistically significant. All statistical analysis was conducted using Statistical Package for Social Sciences (SPSS) Windows version 21.

Chapter Three

The Results

3. Result of Serological survey:

The RBPT result 25 seropositive out from 150 sample. Are the overall brucellosis stables prevalence, the positive samples showed that to RBPT was identified in stables resulting in 16.7% prevalence. All sera positive to RBPT (25 samples) was subjected to SAT to read the titer of positive. Six of them was seropositive out from 25 positive RBPT, resulting in 4.0% stable prevalence (Table 1).

Frequency Tables:

3.1 Prevalence of equine brucellosis among 150 horse examined by RBPT in Khartoum State;

Table 1:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	+ve	25	16.7	16.7	16.7
	-ve	125	83.3	83.3	100.0
	Total	150	100.0	100.0	

All sera positive to rose Bengal plat test RBPT (25 samples) were subjected to further confirmatory test using serum agglutination test SAT. Six 6 sera (4.0%) of sera was confirmed positive by SAT (Table 2).

3.2 Prevalence of equine brucellosis among 150 horse examined by SAT in Khartoum State;

Table 2

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid +ve	6	4.0	4.0	4.0
-ve	144	96.0	96.0	100.0
Total	150	100.0	100.0	

3.3 Prevalence of equine brucellosis among 150 horse according to location in Khartoum State;

Sixty seven (67), serum samples were examined from Khartoum locality, forty six (46) from Bahri locality and thirty seven (37) from Omdurman locality. The prevalence among these localities the rate of infection was high in the Khartoum 44.7%, followed by 30.7% in Bahri and 24.7% in Omdurman (Table 3). Chi square test showed that there was highly significant association between rate of infection and location of the animals (p- value = 0.000), (Table 12).

Table 3:

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid Khartoum	67	44.7	44.7	44.7
Bahri	46	30.7	30.7	75.3
Omdurman	37	24.7	24.7	100.0
Total	150	100.0	100.0	

3.4 Prevalence of equine brucellosis among 150 horse according to Age in Khartoum State;

The total of 150 horses of various age were examined in this study the results showed that one hundred and forty 140 of horses were adults and the ten 10 of them is young. The prevalence among the age showed the adult horse has a prevalence of 93.3%, and young 6.7% (Table 4). Chi square test showed that there was no significant association between infection and age (p- value = 0.143), (Table 12).

Table 4:

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid adult	140	93.3	93.3	93.3
Young	10	6.7	6.7	100.0
Total	150	100.0	100.0	

3.5 Prevalence of equine brucellosis among 150 horse examined by gender in Khartoum State;

The results of 150 horses examination for brucellosis disease by sex, the total number of the stallion examined was one hundred and nine (109) male, while the total number of mares examined was forty one (41) females. The prevalence among stallion was 72.7%, while the mare prevalence was 27.3% (Table 5). Chi square teat showed highly significant association between the infection by brucellosis and gender, (p- value = 0.001), (Table 12).

Table 5:

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid Male	109	72.7	72.7	72.7
Female	41	27.3	27.3	100.0
Total	150	100.0	100.0	

3.6 Prevalence of equine brucellosis among 150 horse examined by breed in Khartoum State;

This study showed the distribution of the horse's brucellosis infection by the breeds. The number of local breed account was eighteen (18) animals and the total of cross breeds was (132) one hundred and thirty two, the prevalence in the local breeds was 12.0%, while the prevalence among cross breeds was 88.0% (Table 6). And Chi square test showed that was significant association between infection and the breed, (p- value= 0.043), (Table 12).

Table 6:

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid cross breed	132	88.0	88.0	88.0
local	18	12.0	12.0	100.0
Total	150	100.0	100.0	

3.7 Prevalence of equine brucellosis among 150 horse according to body condition in Khartoum State;

A normal or good body condition was (133) animals, other which was emaciated or poor body condition was (17) seventeen animals. The prevalence of normal and poor body condition was 88.7%, 11.3% respectively (Table 7). Chi square test showed that there was significant association results between brucellosis infection and body condition, the (p-value= 0.050), (Table 12.)

Table 7:

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid normal	133	88.7	88.7	88.7
emaciated	17	11.3	11.3	100.0
Total	150	100.0	100.0	

3.8 Prevalence of equine brucellosis among 150 horse according to feeding status in Khartoum State;

Animals feeding together was 10 animals, and separated feeding collected was 140 animals. In the prevalence of feeding separate was 93.3%, while the prevalence of feeding together was 6.7% (Table 8). Chi square test showed that are no significant association between group feeding or separate, (p-value= 0.143), (Table 12).

Table 8:

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid Separate	140	93.3	93.3	93.3
Together	10	6.7	6.7	100.0
Total	150	100.0	100.0	

3.9 Prevalence of equine brucellosis among 150 horse according to the stable contact in Khartoum State;

This results of the study showed the distribution of equine brucellosis infection by the separate animals or not separate. One hundred and thirty two 132 was each in one stable, and eighteen (18) present together in one large stable. Their prevalence of it, separate and together was 88.0%, 12.0% respectively (Table 9). Chi square test showed that there was significant association between infection by disease and contact, (p- value = 0.043), (Table 12).

Table 9:

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid No	132	88.0	88.0	88.0
yes	18	12.0	12.0	100.0
Total	150	100.0	100.0	

3.10 Prevalence of equine brucellosis among 150 horse examined by the present of wool sorter in Khartoum State;

This results of the study was identified the present of injury in horses (wool sorter), due to brucellosis. The total number of the horses with absent the wool sorter was 117 one hundred and seventeen. And number with present of the wool sorter was 33. There prevalence are 78.0% and 22.0%, by absent and present of wool sorter respectively (Table 10). Chi square test showed there was significant association between the wool sorter and infection by brucellosis. The (p- value =0.004). (Table 12).

Table 10:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Absent	117	78.0	78.0	78.0
	present	33	22.0	22.0	100.0
	Total	150	100.0	100.0	

3.11 Chi square test:

The Chi square unvaried test was conducted between the outcome and the risk factors Couse's the infection by the *coccobacilli* bacteria of genus *brucella spp.*, was examination among 150 samples equine (horses), from the stable. Chi square showed the highly significant association between the infection by bacteria and risk factors include location and gender, while there significant association between bacterial infection and the risk factors are the breed, body condition, wool sorter and the contact. And no significant association between the bacterial infection and there risk factors, include the feedings and ages. Show in the (table 11).

3.12 Logistic regression:

The multivariate analysis was tested between outcome and all significant result from Chi square the result are no significant.

Table 11: Summary cross tabulation for the prevalence of Brucellosis in equine with potential risk factors.

Risk factor	No. tested	No. positive	Percentage (%)
Location			
Khartoum	67	25	37.3%
Bahri	46	0	
Omdurman	37	0	
Total	150		
Gender			
Male	109	25	22.9%
Female	41	0	
Total	150		
Age			
Adult	140	25	17.9%
Young	10	0	
Total	150		
Breed			
Cross	132	25	18.9%
Local	18	0	
Total	150		
Body condition			
Normal	133	25	18.8%
Emaciated	17	0	
Total	150		
Feeding			
Separate	140	25	17.9%
Together	10	0	
Total	150		
Contact			
No	132	25	18.9%
Yes	18	0	
Total	150		
Wool sorter			
Absent	117	25	21.4%
Present	33	0	
Total	150		

Table 12: Summary of univariate analysis for potential risk factors of the equine brucellosis in 150 horse examined in the Khartoum state Sudan using the Chi square test (x^2);

Risk factors	No. tested	No. positive (%)	df	X^2	p- value
1.Gender					
male	109	25 (22.9)	1	11.284	0.001
female	41				
2.Age					
adult	140	25 (17.9)	1	2.143	0.143
young	10				
3.Breed					
Cross	132	25 (18.9)	1	4.091	0.043
Local	18				
4.Body condition					
Normal	133	25 (18.8)	1	3.835	0.050
Emaciate	17				
5.Feeding					
Separate	140	25 (17.9)	1	2.143	0.143
Together	10				
6.Contact					
No	132	25 (18.9)	1	4.091	0.043
Yes	18				
7. wool sorter					
Absent	117	25 (21.4)	1	8.462	0.004
Present	33				

Table 13: Summary of multivariate analysis for the risk factors of equine brucellosis in 150 horse examined in Khartoum state Sudan using Logistic Regression test;

Risk factor	No. tested	No. positive (%)	p-value	Exp(B)	95% C.I for Exp(B)
1.Location					
Khartoum	67	25 (37.3)	0.000	1.000	0.000
Bahri	46	0			
Omdurman	37	0			
2.Gender					
Male	109	25 (22.9)	0.001	1.000	0.000
Female	41	0			
3.Age					
Adult	140	25 (17.9)	0.143	1.000	0.000
Young	10	0			
4.Breed					
Cross	132	25 (18.9)	0.043	1.000	0.000
Local	18	0			
5.Body condition					
Normal	133	25 (18.8)	0.050	1.000	0.000
Emaciated	17	0			
6.Wool sorter					
Absent	117	25 (21.4)	0.004	1.000	0.000
Present	33	0			

Note: 95% C.I for Exp(B) all lower.

Figure 2: RBPT Result

Figure 3: SAT Result

Chapter four

4.1 Discussion:

The current study provides the information on the serologic prevalence of Brucellosis among 150 horses on the Khartoum state Sudan. In this study three different locality of Khartoum Sudan was included. Was studied serologically as well as through Rose Bengal plate test RBPT and serum agglutination test SAT. The RBPT is highly sensitive test, therefore agglutinates with antibodies of other pathogens that was similar to *brucella* antibodies, whereas, SAT is highly specific and can discretely segregate between simulate antibodies of other pathogens similar to *brucella* and there agglutinates only with *brucella* specific antibodies, as reflected in the results of previous study, (OIE 2008).

The prevalence was detected by screening Rose Bengal plate test RBPT are 25 (16.7%) and prevalence was detected by serum agglutination test are 6 (4%). In this study the prevalence of RBPT 16.7%, it's higher than result obtained in Darfur 4.9% by (Musa, 1995.). And was higher than those from other studies carried out in Nigeria (14.7% for a study that used the RBPT (Ehizibolo, and Ocholi, 2011). And nearly similar to study 16% used the RBPT in (Mohammed and Dauda, 2016).

Serum was sampled 33 of the showed clinical signs e.g fistula wither, arthritis. Believed that *B. abortus* tends to localized in the bursa, muscles, tendons and joints rather than tissue of reproductive tract, Ray (1977). And addition the organism also produces abortion in horses and donkys, such that abortion could be a source of infection to the cattle, Nicoletti (1980).

In this our study the all positive brucellosis indicated in adult horses. A Sexual matured animals are more prone to *Brucella* infection than immature animals of either sex (Radostits *et al.*, 2007).

There may be due to fact that erythritol and sex hormones, which stimulate the growth and multiplication of *Brucella* organisms, tend to increase in concentration with age and sexual maturity (Radostits *et al.*, 2007).

In this study all positive result included normal body condition, because may be due to horses is carrier, equines are potential source of infection for other animals and man, and may be a possibly shedder of *Brucella* (Ocholi *et al*,. 2004: Refai 2002).

This study the all positive result to infection *brucella* among horses was detected in stallion, in comparison to females, because due to maximum number sampled are males, However, the reported higher prevalence of brucellosis in females than in males, Ahmed and Munir (1995b) and Solmaz *et al.* (2004). And these results are in accordance to who reported that seroprevalence of brucellosis was not associated with sex. Muma *et al.* (2006).

And highly significant with location, due to the brucellosis one of the endemic disease in Sudan.

4.2 Conclusion:

The results of sero-prevalence of equine brucellosis in stables in the Khartoum State Sudan indicated that the brucellosis is not prevalence widely among horse. The presence of brucellosis in horses in this study may have been exposed to *B. abortus* through the ingestion of infected foods from origins. Or may be exposed to the bacterial infection at an early age or from imported origin or other things that was not identified in this study. The Identification of horse's brucellosis makes a good strategic planning for the control of brucellosis in our country Khartoum State Sudan.

4.3 Recommendations:

1) Preventive measure should be done every year by local veterinary authorities for controlling the disease in horse's stables in Khartoum State.

2) Management programs is very important for health, biosecurity and eradication of the disease in Khartoum State.

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Appendix:

Questionnaire of horse stable:

SUDAN UNIVERSITY FOR SCIENCE AND TECHNOLOGY

COLLEGE OF VETERINARY MEDICINE

MASTER DEGREE

1- Date …… Serial No……

2- Locality ……

3- Governorate: ……

Personal data of stable:

Name: ……

Address: …….

*Occupation:

a- Government official ( ) B- specify ( ) c- other ( ).

*Education:

a- Primary ( ) b-high secondary ( ) C- university ( ).

d- Postgraduate ( ).

Animal data:

1* Type of horse breed:

a- local ( ) B- imported ( ) c- mixed ( ).

2* Gender:

a- Mare ( ) b- stallion ( ).

3* Breed source:

a- Sport ( ) b- transport ( ).

4* Age:

a- small ( ) b- adult ( ).

5* History of abortion:

a- yes ( ) b- No ( ).

6* history of Retained placenta:

a- yes ( ) b- No ( ).

7* infertility:

a- yes ( ) b- No ( ).

8* Do you know about brucellosis?

a- Yes ( ) b- No ( ).

Management data:

9* Feed and water:

a- Common container ( ) b- separate container ( ).

10* Source of fodder:

a- Green food ( ) b- Dura ( ) c- mix ( ).

11* Source of water:

a- Common canal ( ) .b- tap water ( ).

12* Medical care:

a- Yes ( ) b- No ( ) c- type……

13* Affected:

a- Lameness ( ) b- wound ( ).

14* Body condition:

a- Normal ( ) b- emaciated ( ).

15* In breeding:

a- Natural ( ) b- artificial ( ).