بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Appraisal of Brucella Infection Disease in Dairy Cattle in Khartoum North

تقييم الإصابة بمرضي البروسيلا في أبقار اللبن بمحافظة الخرطوم بحري

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الآية

قال تعالى

بسم الله الرحمن الرحيم

﴿وَإِنَّ لَكُمْ فِي الأَنْعَامِ لَعِبْرَةً نُّسْقِيكُم مِّمَّا فِي بُطُونِهَا وَلَكُمْ فِيهَا مَنَافِعُ كَثِيرَةٌ وَمِنْهَا تَأْكُلُونَ﴾

صدق الله العظيم

سورة المؤمنين ﴿21﴾

Dedication

To my parents, my angels and the candles of my life

To my lovely wife, who stood beside me

through thick and thin

To the beautiful colleagues I had, who I learnt from them the real

meaning of solidarity and cooperation

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Abstract

The study was conducted to assess and provide an overview to find out about the burden and impact of brucellosis on milking dairy cattle, and to study the suitable diagnostic techniques to detect brucellosis according to the economical point of view in Khartoum North, Sudan. The present study showed the serological result for brucella by different methods of diagnostic techniques, which applied on 100 cows, selected randomly from 20 farms, from each five blood samples. The study were showed in comparison between two categories, professional and non-professional farms management. It recorded a highly occurrence of infection in non-professional farms 30%, and low occurrence 6% in professional farms. The tests results of the study was obtained by four different serological diagnostic methods, Rose Bengal Plate Test, Modified Rose Bengal Plate Test, Buffer Acidified Plate Antigen and Serum Agglutination Test, and was concluded that the overall sero-prevalence of brucellosis on milking dairy cattle was 13% by RBPT, 16% by mRBPT, 16% by BAPA and 17% by SAT. The statistical analysis recorded that there was no significant difference between three diagnostic techniques depicted in the study, mRBPT-BAPA and SAT, which gave similar results 16%-17%, but the results 13% which obtained by RBPT had a high significant difference. This implies that the former three techniques mRBPT-BAPA and SAT are more sensitive and reliable to be adopted than the latter one RBPT, which gave some false negative results. The present recorded a highly incidence by brucellosis recorded 16%-17%, which considered as a high infection by brucella, and can pose a considerable potential risk to the public health, animal health, welfare and production. Application of bio-security is very weak, the absence or lack of control and hygiene measures and inadequate application of vaccination programme had played a major roles in the spread of the disease. The study concluded that Khartoum North area should be considered as endemic with bovine brucellosis, and the situation should be tackled seriously considering its negative impact and the zoonotic nature of the disease.

ملخص البحث

أجريت هذه الدراسة لتقييم وتقديم لمحة عامة لمعرفة العبء والتأثير لمرض البروسيلا علي الأبقار الحلوب في مدينة الخرطوم بحري، السودان. ودراسة التقنيات التشخيصية المناسبة للكشف عن البروسيلا وفقا للظروف الإقتصادية الراهنة.

وأظهرت هذه الدراسة نتيجة فحص الأمصال للكشف عن البروسيلا بطرق مختلفة من تقنيات التشخيص، والتي طبقت على 100 بقرة، تم اختيارها عشوائيا من 20 مزرعة، من كل مزرعة 5 عينات دم. وقد أظهرت الدراسة مقارنة بين فئتين، المزارع التي تدار بطريقة احترافية والمزارع التي تدار بطرق غير احترافية، وسجلت والمزارع التي تدار بطرق غير احترافية متوسط إصابة عالي بمرض البروسيلا 30٪، بينما انخفض معدل الإصابة إلي 6٪ في المزارع التي تدار بطرق احترافية. تم الحصول على نتائج الاختبارات في هذه الدراسة بواسطة أربعة أساليب تشخيصة مختلفة, وهي الروز بنغال، والروز بنغال المعدل، واختبار التلازن الشريحي المخمد، واختبار التراص الأنبوبي البطئ. وخلصت إلى أن انتشار مرض البروسيلا في الأبقار الحلوب كان 13٪ بواسطة اختبار الروز بنغال، و 16٪ بواسطة اختبار الروز بنغال المعدل، و 16٪ بواسطة اختبار اختبار التلازن الشريحي المخمد، و 17٪ بواسطة اختبار واختبار التراص الأنبوبي البطئ.

أوضح التحليل الإحصائي عدم وجود فروق معنوية بين ثلاث من التقنيات التشخيصة المستخدمة, وهي الروز بنغال المعدل, واختبار التلازن الشريحي المخمد, واختبار التراص الأنبوبي البطئ, وقد أعطت نتائج مماثلة 16٪ -17٪، ولكن النتائج المتحصلة بواسطة الروز بنغال 13٪ كان الفرق المعنوي عالي بينها وبين التقنيات الثلاث السابقة, وهذا يوضح أن الروز بنغال المعدل, واختبار التلازن الشريحي المخمد, واختبار التراص الأنبوبي البطئ هي تقنيات تشخيصية أكثر حساسية ويمكن الاعتماد عليها أكثر من الروز بنغال الذي أعطي بعض النتائج السلبية الكاذبة.

سجلت هذه الدراسة لمرض البروسيلا عن وجود نسبة اصابة عالية 16%-17%, ويمكن أن تشكل خطرا كبيرا محتملا على صحة الأبقار الحلوب وانتاجها, وأيضا الإنسان. تطبيق الأمن الحيوي ضعيف جدا، وغياب أو عدم وجود تدابير الرقابة والنظافة، وعدم تطبيق برنامج التطعيم بشكل سليم, كلها أسباب تلعب دورا كبيرا في انتشار المرض. وخلصت الدراسة إلى أن منطقة الخرطوم بحري ينبغي النظر لها كمنطقة موبوءة بمرض البروسيلا, وينبغي التعامل مع الوضع بجدية, آخذين في الإعتبار التأثير السلبي للمرض وقابلية انتقاله للبشر.

CHAPTER ONE

INTRODUCTION

Brucellosis is consistently ranked among the most economically important zoonosis globally (WHO, 2009). It is a 'multiple burdens' disease with economic impacts attributable to human, livestock and wildlife disease. The epidemiology and economic impact of brucellosis vary by geography and livestock system. In many high-income countries, brucellosis has been successfully controlled or eliminated in livestock populations. Where it persists, wildlife populations have become the main reservoirs, for example, bison and elk in North America. In emerging middle-income countries, the brucellosis picture is much more variable. Middle-income countries tend to report the greatest number of outbreaks and animal losses (World Bank, 2011). Economic impacts vary depending on the main livestock species, management systems, and on the capacity of the country's veterinary and medical systems. In low-income countries, brucellosis is endemic and neglected, with large disease and livelihood burdens in animals and people and almost no effective control (McDermott and Arimi, 2002). Infectious diseases is a diseases transmitted from animal to another animal or human by any means of various means of infection. Infectious diseases are caused by pathogenic microorganisms, such as bacteria, viruses, parasites or fungi. More than 300 diseases can be transmitted from animals to humans; only a limited number of zoonotic diseases can be transmitted person-to-person. Animal diseases represent an important threat to human health, since the emergence of human diseases is dominated by zoonotic pathogens, about 75% of recently emerging infectious diseases affecting humans are diseases of animal origin, and approximately 60% of all human pathogens are zoonotic (NCEZID, 2014).

Therefore, direct impact of Infectious Animal Diseases in agriculture and public health, constitute a serious limitation to export living animals and their products, as well for international trade. Moreover, seriously compromised food security and causing a high socioeconomic impact on agricultural exporting nations (Andrés, 2012).

According to reports of the General Directorate of Veterinary Quarantine and Meat Hygiene**,** The Ministry of Livestock, Fisheries and the Rangeland, there are some of the infectious diseases that have an impact on exports of animals in Sudan. Brucellosis is one of the most common zoonotic diseases in the present time in both developed and developing countries alike according to the classification of global organizations WHO, FAO and OIE (Mohamed, 2011). The importance of this disease is due to the high economic losses as well as the danger to human health and safety (General Administration of Livestock, Kassala State, 2012).

1-1 Objectives of the study:

The objectives behind this assessment are to provide an overview to find out about the burden and impact of brucellosis, and to study suitable diagnostic techniques to detect brucellosis on dairy cattle in Khartoum North, Sudan.

CHAPTER TWO

LITERATURE REVIEW

2-1 The economic importance of the livestock sector:

Livestock is the single most important component of the agricultural sector in Sudan, consistently accounting for nearly half of the gross domestic product (GDP) of the sector (47%), almost equal to that of all crops combined. The livestock sector makes an important contribution to the food security of the country, employment, export earnings, means of transport of goods and individuals, draught power, manure for soil fertilization and a means of accumulation of capital assets. It is considered an important element in poverty alleviation programmes in the country. The livestock population of Sudan is estimated at some 140 million heads of animals, composed of 41.5 million cattle, 51.5 million sheep, 43 million goats and 4.5 million camels (It may be noted that out of the total livestock population of the Arab world, the Sudanese cattle constitute about 70%, sheep 31%, goats 49 %, and camels 25%).

The prevailing livestock production system in Sudan is the traditional nomadic and transhumant herding. In addition there are sedentary and semi-sedentary livestock production systems, small intra-urban backyard production, and integrated livestock/crop production operations. Livestock population is concentrated in the Western States, comprising 36% of cattle, 40% of sheep, 36% of goats and 33% of camels (These figures are Ministry of Animal Resources and Fisheries estimates based on data of the last animal census carried out in 1975/76. Considerable ecological changes have taken place since then which might have affected the present livestock population and distribution. It is of paramount importance to conduct a new census) (Panos, 2009).

2-2 Brucellosis:

Undulant Fever, Malta Fever, Mediterranean Fever, Enzootic Abortion, Epizootic Abortion, Contagious Abortion, Bang's Disease. Brucellosis is essentially a disease of animals, especially domesticated livestock, caused by bacteria of the brucella group with humans as an accidental host. It is also known as "Undulant fever", "Mediterranean fever" or "Malta fever" and transmitted by direct or indirect contact with infected animals or their products. In other words it is a zoonatic disease (Corbel, 2006).

Because of the major economic impact on animal health and the risk of human disease, most countries have attempted to provide the resources to eradicate the disease from the domestic animal population (Radostits *et al*., 2006).

2-2-1 Importance:

Brucellosis, a bacterial disease caused by members of the genus Brucella, is an important zoonosis and a significant cause of reproductive losses in animals. Brucellosis is usually caused by *B. abortus* in cattle, *B. melitensis* or *B. ovis* in sheep, *B. suis* in pigs and *B. canis* in dogs. Abortions, placentitis, epididymitis and orchitis are the most common consequences, although other syndromes are also reported. The main impact is economical, deaths are rare except in the fetus and neonate. Some brucella species are also maintained in wildlife populations (Cutler, 2005). Most species of brucella can infect animals when they come in close contact, *B. abortus*, *B. melitensis*, *B. suis* and *B. canis brucella* species are human pathogens. In humans, brucellosis can be a serious, debilitating and sometimes chronic disease that may affect a variety of organs. Most cases are caused by occupational exposure to infected animals or the ingestion of unpasteurized dairy products. However, this disease remains a common and serious problem in some parts of the world. (Kahn, 2003).

It is generally transmitted to consumers through raw milk and milk products, causing a considerable impact on human and animal health, along with wide socio-economic impacts. Some specific occupational groups including farm workers, veterinarians, animal caretakers and butchers are considered at higher risk to brucellosis. Brucellosis in milking dairy cattle, mainly affects reproduction and fertility, causes late abortion, reduces survival chances of newborns and reduces milk yield (Islam *et al*., 1983; Rahman *et al*., 2006; Samad, 2008).

2-2-2 Etiology:

Brucellosis results from infection by various species of brucella, a Gram negative, facultative intracellular coccobacillus or short rod in the family Brucellaceae. Six named species occur in animals: *B. abortus, B. melitensis, B. suis, B. ovis, B. canis and B. neotomae.* One or more unnamed species of brucella have been found in marine mammals. Some species of brucella contain biovars. Five biovars have been reported for *B. suis*, three for *B. melitensis*, and up to nine for *B. abortus* (Aguirre *et al*., 2007).

Each brucella species is associated most often with certain hosts. *B. abortus* usually causes brucellosis in cattle, bison and buffalo. *B. melitensis* is the most important species in sheep and goats, but *B. ovis* can also cause infertility in rams. *B. canis* causes disease almost exclusively in dogs. *B. neotomae* is found in rodents, but has not been linked to disease. *B. suis* contains more diverse isolates than other brucella species, and these isolates have broader host specificity. In humans, brucellosis can be caused by *B. abortus*, *B. melitensis*, *B. suis* biovars 1-4 and, rarely, *B. canis* or marine mammal brucella. Live vaccines for *B. abortus* and *B. melitensis*, as well as the *B. canis* M- strain, a less virulent strain used as an antigen for serological testing, are also pathogenic for humans. *B. ovis*, *B. neotomae* and *B. suis* biovar 5 have not been linked to human disease (Alton, 1996).

Genetic and immunological evidence suggests that all members of the genus Brucella are closely related, and some microbiologists have proposed that this genus be reclassified into a single species, *B. melitensis*, which contains many biovars. This proposal is controversial, and both taxonomic systems are currently in use (Godfroid, 2002).

2-2-3 Transmission:

B. abortus, B. melitensis, B. suis and B. canis are usually transmitted between animals by contact with the placenta, fetus, fetal fluids and vaginal discharges from an infected animal. Animals are infectious after either an abortion or full term parturition. Although ruminants are usually asymptomatic after their first abortion, they can become chronic carriers, and continue to shed brucella in milk and uterine discharges during subsequent pregnancies. Dogs may also shed *B. canis* in later pregnancies, with or without symptoms. Entry into the body occurs by ingestion and through the mucous membranes, broken skin and possibly intact sking. Most or all brucella species are also found in semen (Giannacopoulos *et al.*, 2002).

Brucella can be spread on fomites including feed and water. In conditions of high humidity, low temperatures, and no sunlight, these organisms can remain viable for several months in water, aborted fetuses, manure, wool, hay, equipment and clothes. Brucella can withstand drying, particularly when organic material is present, and can survive in dust and soil. Survival is longer when the temperature is low, particularly when it is below freezing. Humans usually become infected by ingesting organisms or by the contamination of mucous membranes and abraded skin. In the laboratory and probably in abattoirs, brucella can be transmitted in aerosols. Common sources of infection for people include contact with animal abortion products; ingestion of unpasteurized dairy products from cows, small ruminants or camels; ingestion of undercooked meat, bone marrow or other uncooked meat products; Human to human transmission is rare, but has been reported after

blood transfusion, bone marrow transplantation or sexual intercourse (Hollett, 2006).

2-2-4 Infections in humans:

The disease occurs worldwide in both animals and humans (Samad, 2008). Five out of the nine known brucella species can infect humans (Seleem *et al*., 2010). Brucellosis in humans can involve any organ or organ system, and have an insidious onset with varying clinical signs. The incubation period in humans is variable and can range from 5 to 21 days up to three months. This often adds to the difficulty of diagnosis due to the latency of clinical signs. The one common sign in all patients is an intermittent/irregular fever of variable duration, thus the term undulant fever. The acute form (<8 weeks from illness onset) is characterized by symptomatic, nonspecific, or flu-like symptoms, including fever, malaise, anorexia, headache, myalgia, and back pain. Drenching sweats can occur, particularly at night. Splenomegaly, hepatomegaly, coughing, and pleuritic chest pain are sometimes seen. Gastrointestinal signs, including anorexia, nausea, vomiting, diarrhea, and constipation, occur frequently in adults but less often in children. In many patients, the symptoms last for two to four weeks and are followed by spontaneous recovery. Others develop an intermittent fever and other persistent symptoms that typically wax and wane at 2 to 14 day intervals. Most people with this undulant form recover completely in three to 12 months. A few patients become chronically ill. Relapses can occur months after the initial symptoms, even in successfully treated cases (Danelle Bickett *et al*., 2012).

2-2-4-1 Communicability:

Rare congenital infections have also been documented. In some cases, the infant appeared to be infected through the placenta and in others by the ingestion of breast milk. Brucellosis was reported in an obstetrician who

swallowed secretions while trying to clear a congenitally infected infant's respiratory tract at birth (Kahn and Line, 2003).

2-2-4-2 Treatment:

Antibiotics are usually the mainstay of treatment; long-term treatment may be required. Some forms of localized disease, such as endocarditis, may require surgery (CDC, 2005).

2-2-4-3 Prevention:

Human brucellosis is usually prevented by controlling the infection in animals. Pasteurization of dairy products is an important safety measure where this disease is endemic. Good hygiene and protective clothing/ equipment are very important in preventing occupational exposure. Precautions should be taken to avoid contamination of the skin, as well as inhalation or accidental ingestion of organisms when assisting at a birth, performing a necropsy, or butchering an animal for consumption. Particular care should be taken when handling an aborted fetus or its membranes and fluids. Risky agricultural practices such as crushing the umbilical cord of newborn livestock with the teeth or skinning aborted fetuses should be avoided (CDC, 2005).

2-2-5 Infections in animals:

Incubation Period: The incubation period varies with the species and stage of gestation at infection. In cattle, reproductive losses typically occur during the second half of the pregnancy; thus, the incubation period is longer when animals are infected early in gestation. In this species, abortions and stillbirths usually occur two weeks to five months after infection. In pigs, abortions can occur at any time during gestation. In dogs, abortions are most common at approximately 7 to 9 weeks of gestation, but early embryonic deaths have also been reported after 2 to 3 weeks (Garner *et al*., 2003).

2-2-5-1 Clinical signs:

2-2-5-1-1 Bovine brucellosis (*B. abortus*):

In cattle, *B. abortus* causes abortions, stillbirths and weak calves; abortions usually occur during the second half of gestation. The placenta may be retained and lactation may be decreased. After the first abortion, subsequent pregnancies are generally normal; however, cows may shed the organism in milk and uterine discharges. Epididymitis, seminal vesiculitis, orchitis and testicular abscesses are sometimes seen in bulls. Infertility occurs occasionally in both sexes, due to metritis or orchitis/epididymitis. Hygromas, particularly on the leg joints, are a common symptom in some tropical countries. Arthritis can develop after long-term infections. Systemic signs do not usually occur in uncomplicated infections, and deaths are rare except in the fetus or newborn. Infections in non-pregnant females are usually asymptomatic. Similar symptoms occur in other ruminants including camels, bison and water buffalo; however, experimentally infected moose develop more serious disease and die rapidly (Schnurrenberger *et al*, 1985).

2-2-5-1-2 Ovine and caprine brucellosis (*B. melitensis*):

B. melitensis mainly causes abortions, stillbirths and the birth of weak offspring. Animals that abort may retain the placenta. Sheep and goats usually abort only once, but reinvasion of the uterus and shedding of organisms can occur during subsequent pregnancies. Milk yield is significantly reduced in animals that abort, as well as in animals whose udder becomes infected after a normal birth. However, clinical signs of mastitis are uncommon. Acute orchitis and epididymitis can occur in males, and may result in infertility. Arthritis is seen occasionally in both sexes. Many non-pregnant sheep and goats remain asymptomatic (European Commission, 2001).

2-2-5-1-3 Ovine epididymitis (*B. ovis*):

Brucella. ovis affects sheep but not goats. This organism can cause epididymitis, orchitis and impaired fertility in rams. Initially, only poor quality semen may be seen; later, lesions may be palpable in the epididymis and scrotum. Epididymitis may be unilateral or, occasionally, bilateral. The tests may atrophy. Some rams shed *B. ovis* for long periods without clinically apparent lesions. Abortions, placentitis and perinatal mortality can be seen in ewes but are uncommon. Systemic signs are rare. *B. ovis* can also cause poor semen quality in red deer stags (FAO, 2003)

2-2-5-2 Diagnostic tests:

There are several serological tests available for brucellosis diagnosis and surveillance. Among these tests, the rose bengal plate test (RBT) is the recommended method for the screening of samples to determine herd and flock prevalence. A variety of tests have been developed for diagnosis of brucellosis, in particular (RBT), the complement fixation test (CFT) and the indirect enzyme-linked immunosorbent assay (ELISA) test are recommended by the International Office of Epizootics (OIE) (Corbel and MacMillan, 1995).

2-2-5-3 Treatment:

There is no practical treatment for infected cattle. Antibiotic treatment has also been used successfully in some valuable rams, but it is usually not economically feasible. Fertility may remain low even if the organism is eliminated (Kortepeter *et al.*, 2001).

2-2-5-4 Prevention:

Brucellosis is usually introduced into a herd by an infected animal, but it can also enter in semen. Herd additions should come from brucellosis-free areas or accredited herds. Animals from other sources should be isolated and tested

before adding them to the herd. Domesticated animals should always be kept from contact with wild animal reservoirs. Commercial *B. abortus* and *B. melitensis* vaccines are available for cattle, sheep and goats. Vaccination can interfere with serological tests; this is minimized when only young animals are vaccinated. Good management can reduce the incidence of infection in an infected herd. Whenever possible, animals should give birth in individual pens. Transmission is reduced by immediate disposal of the placenta, contaminated bedding and other infectious material, followed by thorough cleaning and disinfection (Moreno and Moriyon, 2002).

2-3 The distribution of the disease in Sudan:

Brucellosis caused by *B. abortus* was first reported in Khartoum state. The prevalence of the disease was 160 (80%) of 200 Friesian and 49 (38%) of 130 local zebu cattle (Bennet, 1943). Subsequently the disease was reported by many investigators all over the country. Musa, (1990) reviewed its situation from 1943 – 1990 and found its prevalence in individual animals varying from low (0 – 5%), moderate (6 – 15%), high (16 – 25%) and very high (above 25%), according to the criteria of Thimm and Wundt, (1976). Most of the herds examined in East, West, Central and South (previously) of the Sudan were infected with brucellosis. The prevalence of the disease in cattle and camels was medium and high but low in sheep and goats. *B. abortus* biovars 1, 3, 6 and 7 and *B. melitensis* biovars 2 and 3 were isolated in the Sudan. *B. abortus* biovar 6 and *B. melitensis* biovar 3 are associated with infection in indigenous animals throughout the country, but the other biovars occurred in cross breed dairy cattle in Khartoum town only. Prevalence of *B. melitensis* in sheep and goats and its spread to the secondary hosts, specially cattle and camel poses health and control problems. Work is going on in South Darfur, El Gazera, South Kordofan and Sennar to reveal the present situation of the disease and brucella species biovars associated with infections (Musa *et al*., 2008).

CHAPTER THREE

MATERIAL and METHODS

3-1 Study area:

The farms were targeted randomly in Khartoum North, Sudan to collect blood samples for different serological tests of brucellosis, applied on 100 cows, selected randomly from 20 farms, from each five blood samples, according to two categories, professional and non-professional farms management (Appendix 1). The blood samples had been analyzed in Brucella research division in Veterinary Research Institute (VRI), Soba.

3-2 Material used:

3-2-1 Collection of samples:

Collection of samples needed vacutainer tubes, needle holder, needles and ice bag. The blood samples were withdrawal aseptically from jugular vein into sterile vacutainer tubes conveyed immediately to the laboratory and allowed to stand at upright position at room temperature.

3-2-2 In laboratory:

Working inside lab needed centrifuge, eppendorf tubes, cylinder, flask, test tubes, racks, pipette, tips, incubator, gloves and lab coat. The separated sera by centrifuge were stored in refrigerator at -20°C until needed.

3-2-3 Antigen preparations:

The antigens used for these serological tests were prepared from *B. abortus* biovar 1, strain 99, Weybridge, United Kingdom, and standardized according to EU requirements (Hendry *et al.*, 1985).

3-3 Methods of diagnostic techniques for brucella tests:

Four diagnostic techniques were used, Rose Bengal Plate Test, Modified Rose Bengal Plate Test, Buffer Acidified Plate Antigen and Serum Agglutination Test.

3-3-1 Rose Bengal Plate Test (RBPT):

Brucella colored antigen used in this test was donated by Division of Brucella research in Veterinary Research Institute (VRI) Soba. The antigen and the serum samples were removed from the refrigerator to room temperature and shaken properly before use. Equal quantity of serum sample and (RBPT) antigen (25 μ l) were taken on an enamel plate, mixed thoroughly with metal stick and rotated clockwise and anti clockwise. The result was read immediately after 4 minutes. Definite agglutination was considered as positive reaction. Agglutination appeared as weak positive, positive, strong positive or very strong positive (Alton *et al*., 1988).

3-3-2 Modified Rose Bengal Plate Test (m-RBPT):

It used to detect brucella (antigen + antibody reaction) in serum. About 60 microlitre of the serum were put into the plate. Thirty microlitre of the antigen were added into the plate. It was mix gently, rotated and interpreted for agglutination within 4 minutes. In positive case, the agglutination occurs taking ring shape surrounding the sample inside the plate (Blasco *et al*., 1994).

3-3-3 Buffer Acidified Plate Antigen (BAPA):

It used to detect brucella (antigen + antibody reaction) in serum. Three drops of the serum were put, each one in a separate square of the plate. The first drop of the serum = 20 microlitre, the second drop of the serum = 40 microlitre, the third drop of the serum = 80 microlitre. Thirty microlitre of the antigen were added to each drop. They were mixed gently, rotated the plate

and interpreted for agglutination within 8 minutes. In positive case, the agglutination occurs taking ring shape surrounding the sample inside the plate (OIE Terrestrial Manual, 2012).

3-3-4 Serum Agglutination Test (SAT):

The SAT antigen was prepared and standardized in Division of Brucella research in Veterinary Research Institute (VRI) Soba, The antigen was diluted 1:12 using phenol saline (Buxton and Fraser, 1977).

Eight test tubes were placed in a rack in one raw for each sample. 0.8 ml of 5% NaCl solution was added to the first tube and 0.5 ml into each of the remaining seven tubes using 1 ml graduated pipette. 0.2 ml of serum was added to the first tube of each raw mixed well with the 5% NaCl by sucking and expelling gently to avoid producing bubbles. 0.5 ml of mixture transferred from the first tube to the next tube, mixed well with the 5% NaCl, and then 0.5 ml was transferred to the third tube and so on. Doubling the dilution was continued up to the 8th tube then 0.5 ml from the last tube was discarded. 0.5 ml of the diluted antigen was added to each tube. Control positive tubes containing equal amounts of antigen and known positive serum were included in the test. Control negative tubes containing equal amounts of antigen and known negative serum were included in the test. After shaking, the tubes were incubated at 370 C overnight.

The test was read by examining the tubes against a black background with light coming from behind the tubes. A positive reaction is one in which the serum – antigen mixture is clear and agglutinated antigen appears at the bottom of the tube. Gentle shaking does not disrupt the floculi. This is a complete agglutination and is recorded as ++++. In partial agglutination serum-antigen mixture is partially clear and gentle shaking does not disrupt the floculi, this was recorded as +++ or ++. Some sedimentation as + and no clearing as negative reaction (Alton, 1975).

3-4 Questionnaire:

The survey of the study was accompanied by questionnaire form (Appendix 2), included questions to had general information about the farm, herd type, occurrence of the diseases mainly brucellosis, details on brucella infection prevalence, detection, vaccination and control.

3-5 Statistical analysis:

Data generated from the results of the four serological diagnostic techniques of brucella, were subjected to (CRD) tests, using (SAS) software, and then Duncan's Multiple Range tests was used for means separation between tests.

CHAPTER FOUR

RESULTS

4-1 Serological result according to the two categories:

The present study showed the serological result for brucella by different methods of diagnostic techniques, according to two categories, professional and non-professional management, which applied on 100 cows, selected randomly from 20 farms, from each five blood samples (Table 1) (Figure 1,2).

In Sudan University of Science and Technology farm (Shambat), Albatihani farm, Ali Saad Alrayh farm, Mohammed Sakin farm, Abdalraheem farm, Mamoon farm and Yonis Omer farm, these farms were found free from infection by brucella.

In Khartoum University farm, Sudan University of Science and Technology farm (Kuku), Abomidian farm, Mohammed Ali farm, Almerafabi farm, Alajab farm, Mohammed Alnaeem farm, Wad alfaw farm and Ahmed Alsideeg farm, test results showed one infected cow out of five in each farm, the infection rate was 20%.

In Almahjoob farm, Futihalrahman farm and Hassan Alarabi farm, test results showed two infected cow out of five in each farm, the infection rate was 40%. In Mustafa Ibraheem farm, test results showed three infected cows out of five, the infection rate was 60%.

Farms under professional management showed lower infection percentage (6%), compared to non-professional management (30%). This is a call to exert more efforts to improve private sector status of management so as to decrease infection occurrence.

Table 1. Serological result according to two categories

Mode of Management	**Professional Management**										
Case: Farm Type	1	2	3	4	5	6	7	8	9	10	Average
Infected (+)	0	1	1	0	0	0	0	0	1	0	0.3
Non-infected (-)	5	4	4	5	5	5	5	5	4	5	4.7
Infection rate(%)	0	20	20	0	0	0	0	0	20	0	6.0
Mode of Management	**Non-Professional Management**										
Case	1	2	3	4	5	6	7	8	9	10	Average
Infected (+)	3	1	1	1	2	1	1	2	2	1	1.5
Non-infected (-)	2	4	4	4	3	4	4	3	3	4	3.5
Infection rate(%)	60	20	20	20	40	20	20	40	40	20	30

Figure 1. Serological result according to **professional management**

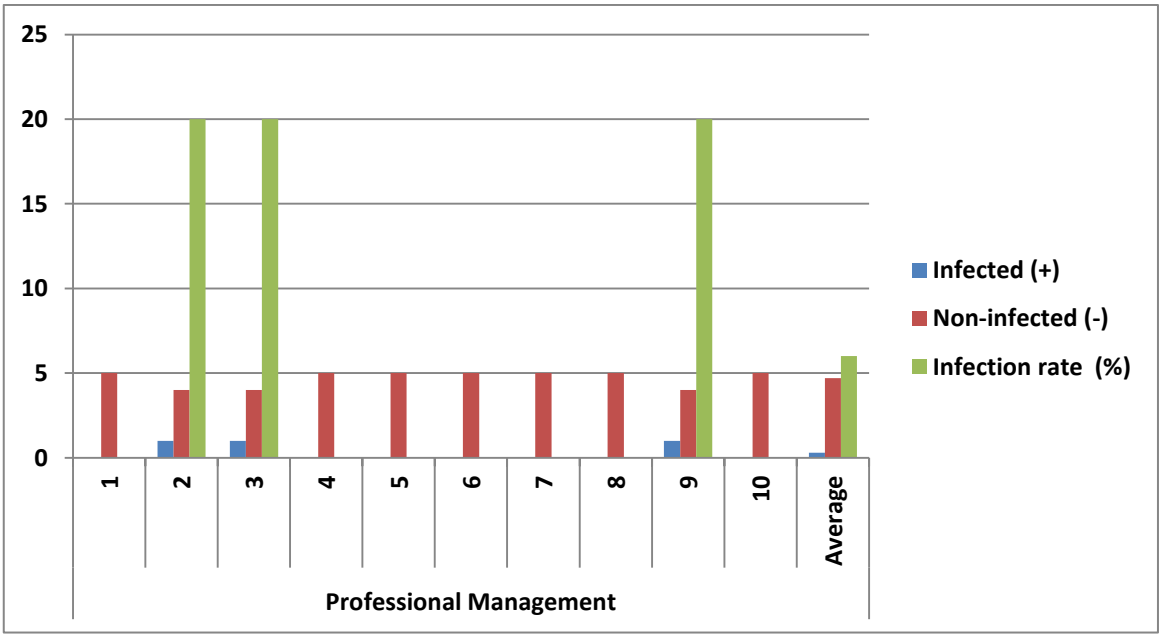
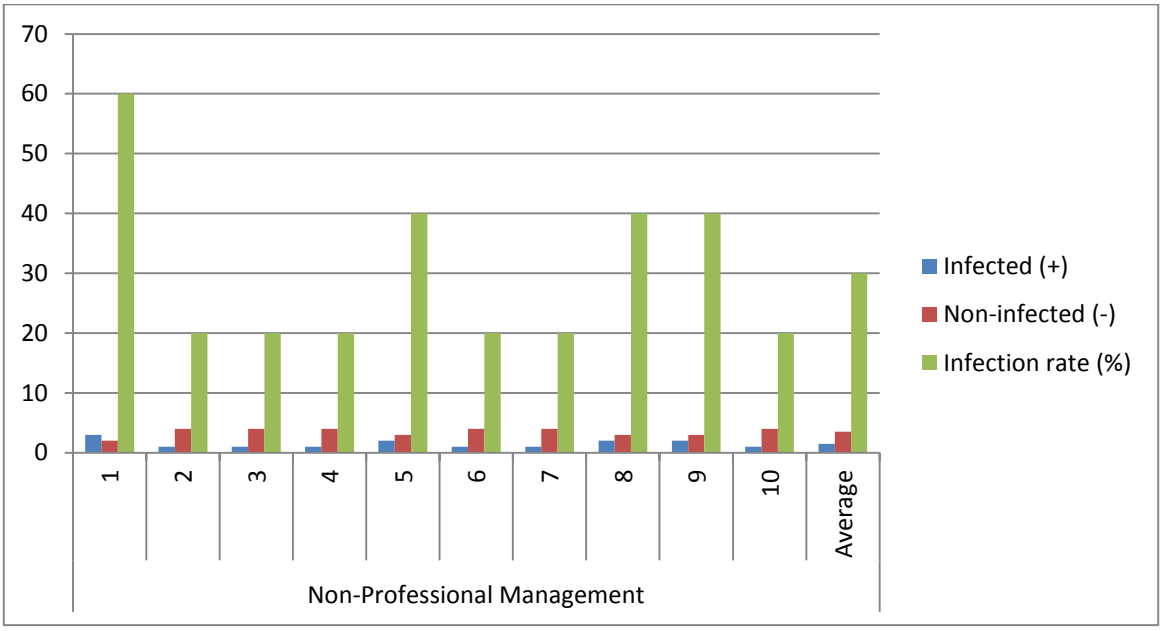


Figure 2. Serological result according to **non-professional management**



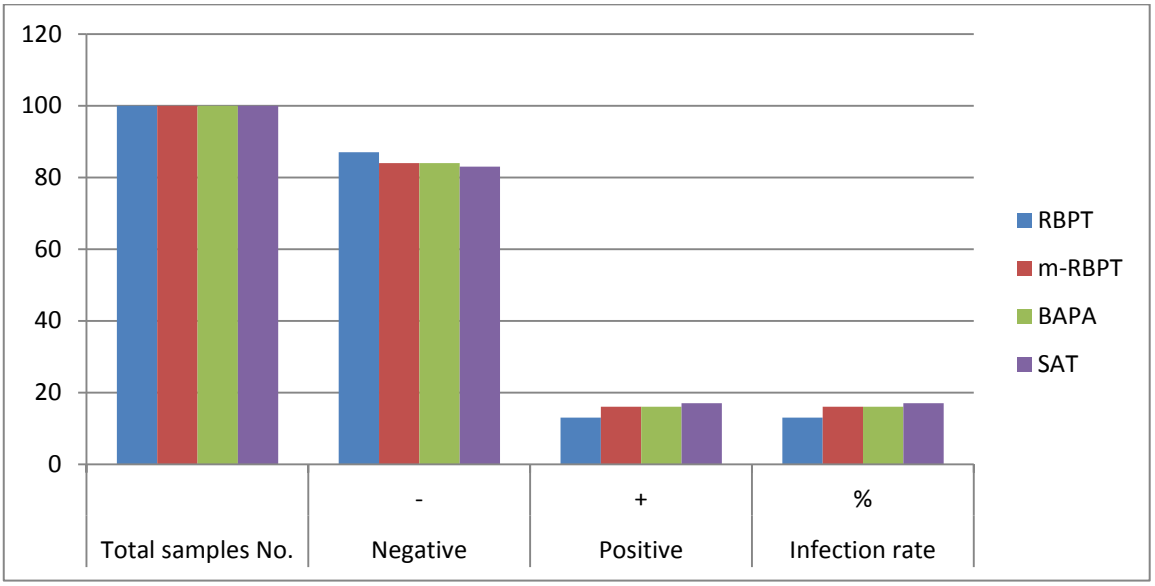
4-2 Serological result according to diagnostic techniques:

Blood samples result and infection rate of brucella according to the four diagnostic techniques (Rose Bengal Plate Test, Modified Rose Bengal Plate Test, Buffer Acidified Plate Antigen and Serum Agglutination Test) showed in (Table 2) (Figure 3). The infection rates of brucella, which applied by RBPT, mRBPT, BAPA and SAT were 13%, 16%, 16% and 17% respectively.

Table 2. Serological result according to diagnostic techniques

Tests	Total samples No.	Negative -	Positive +	Infection rate %	LSD	Cost per sample SDG
RBPT	100	87	13	13	1.883	10
m-RBPT	100	84	16	16		10
BAPA	100	84	16	16		10
SAT	100	83	17	17		10

Figure 3. Serological result according to diagnostic techniques



4-2-1 Statistical analysis of the results:

Highly significant difference was noticed between (RBPT) and (mRBPT-BAPA and SAT), in respect of degree of infection at $P < 0.05$ level.

4-3 Data collected from questionnaire:

The study was accompanied by questionnaire. The collected data (Table 3) were used to evaluate the farms management Performance.

It was found that Cross-breeds recorded 95%, local breeds 5% and no foreign breed. From the observation, all farms were using hand milking. The percentage of private milkers was 80%, and the other 20% worked in more than one farm. Owners who didn't know if they have brucella infection in their farms or not were about 75%, and owners who did know were 25%. Most of the farms do not make brucella test periodically 85%. Milkers and owners usually detect brucella by observation 85%. All milkers stated that they were checking for cow's udders health during dry period 100%. Most of the farms clean the cow's pen once a week 90%. The farms that received an annual vaccinations against the infectious diseases, Anthrax,

Black leg, Contagious bovine pleuropneumonia and hemorrhagic septicaemia were about 45%, and who didn't receive, or even knew that annual vaccination must be taken were about 55%. The most common disease in the area was Mastitis (40%).

Table 3. Questionnaire data

No.	Questionnaire	Options	No. of farms	%
01	Breed	Local	1	5
		Cross	19	95
		Foreign blood	-	-
02	Milking method	Manual	20	100
		Mechanical	-	-
03	Place of milking	Inside pen	18	90
		Outside pen	1	5
		Milking parlour	1	5
04	Private milkers	Yes	16	80
		No	4	20
05	Brucella cases in the farm	Yes	5	25
		No	15	75
06	Periodic inspection for brucella	Yes	3	15
		No	17	85
07	Brucella detection	By observation	17	85
		In laboratory	3	15
08	Check for udders health during dry period	Yes	20	100
		No	-	-
09	Bio-security procedures	Daily	2	10
		Weekly	18	90
		Monthly	-	-
10	Annual vaccination	Yes	9	45
		No	11	55
11	Common diseases	Theileria	5	25
		Mastitis	8	40
		Brucella	1	5
		FMD	3	15
		CBPP	3	15

CHAPTER FIVE

DISCUSSION

The present study on brucella in milking dairy cattle in Khartoum North, Sudan were showed in comparison between two categories, professional and non-professional farms (Table 1), it recorded a highly average of infection in non-professional farms management (30%). The result is in agreement with Gen *et al*., (2005) who reported a higher result of brucellosis sero-prevalence in dairy cows in Turkey, the antibodies against *B. abortus* were detected in the serum samples as 68.1%, 65.6%, 58.9% and 55.2%. While in professional farms management the infection registered an average of (6%). This result is similar with Chivandi, (2006) who reported a result of 4.11% prevalence rate of bovine brucellosis in the Gokwe Smallholder Dairy Project Herd of Zimbabwe. It was on line also with results reported in the Gambia (1.1%), Senegal (0.6%) and the District of Labe in Guinea where the disease was absent (Unger *et al*., 2003). It was noted that in this study and those three studies in West Africa, all serum samples examined for the estimation of brucellosis prevalence were subjected to screening test.

The study showed the results obtained by four different serological methods of diagnostic techniques RBPT-mRBPT-BAPA and SAT, which applied on 100 cows selected randomly from 20 farms, from each farm 5 blood samples (Table 2), also explained that three approaches mRBPT-BAPA and SAT provided an approximately similar result 16-17%, but RBPT gave a result 13% with a highly significant difference. This implies that there was a false negative results with RBPTs, may be due to the cows status (in the last stage of pregnancy, had a recent abortion or during the incubation period of the disease), this may explain the miss detection of RBPT for the positive results. However, we should rely on the other three techniques results in this study because they were more sensitive and reliable to be adopted than

RBPT. But it should be noted that application of mRBPT in cattle is only preferred to enhance the clarity of test reading as any visible agglutination is considered to be positive (OlE, 2009). This observation support the report of Omer *et al.* (2010) who reported that mRBPT facilitated the reading of agglutination and was recommended for screening test for brucellosis.

Tests of brucella applied by RBPT showed that the percentage of brucella infection was 13%, which considered as moderate range, while the tests of brucella applied by mRBPT, BAPA and SAT showed that the percentage of brucella infection was 16%, 16% and 17% respectively which consider as a highly range of brucellosis according to the criteria of Thimm and Wundt, (1976).

The present study showed that the seroprevalence of brucellosis in milking dairy cattle in Khartoum North is high 16-17% (Table 2). An approximately similar prevalence rate 16.9% was reported recently in cattle in Kassala state (Omer *et al*., 2010).

It was found that Cross-breeds recorded (95%), local breeds (5%) and no foreign breed. This may be due to the low milk production of local breed, and the high cost of foreign breed, and cross breed has a higher milk production than local breed and more adaptation to environmental condition than foreign breed (Table 3).

From the observation, all farms were using manual (hand) milking. Despite of the hygienic problems which cause milk contamination (personal hygiene, dust, manure and barn insects), and the probability of catching brucella or any other zoonosis disease, all farms prefer hand milking from the economical point of view (Table 3).

Usually, milking performs inside the pen (90%). Most of the milkers prefer indoor milking to decrease heat stress from cows while milking by providing shade to protect the milk and the cows from direct sun light (Table 3).

The percentage of private milkers was (80%), and the other (20%) worked in more than one farm. and this is due to poor management, which can lead to the transmission of brucella and other diseases from the other farms (Table 3).

Owners who did not know if they have brucella infection in their farms or not were about (75%), and owners who did know were (25%), and unfortunately many of them didn't do anything about it. All of this may be due to ignorance, negligence or lack of attention to the serious cows and human health problems caused by brucella, and devastating economic impact of brucella infection (Table 3).

Most of the farms do not make brucella test periodically (85%). This may be due bad management or financial problem (Table 3).

Milkers and owners usually detect brucella by observation (85%). Usually owners try to reduce costs, and rely on the experience of themselves and the farm milkers instead of brucella diagnostic techniques (Table 3).

All milkers stated that they were checking for cow's udders health during dry period (100%). The dry period is the most important phase of a dairy cow's lactation cycle. During this phase, the cow and the udder are prepared for the next lactation, hence any abnormalities during the dry period will have a negative effect on the cow's health and milk production after calving (Table 3).

Most of the farms clean the cow's pen once a week (90%). It's may be to reduce labour costs, because manure buyers come once a week, and that makes the farm subjected to internal infections problems (Table 3).

The farms that received an annual vaccinations against the infectious diseases (Anthrax, Black leg, Contagious bovine pleuropneumonia and hemorrhagic septicemia) were about (45%), and who didn't receive, or even knew that annual vaccination must be taken were about (55%). That may be due to poor management and lack of awareness of owners about the threat of those contagious fatal diseases, and if the farm owners didn’t know about those threats, the most probably, they didn’t know about of brucella either (Table 3).

The most common disease in the area was mastitis (40%). Poor management and control efforts on milking technique and hygiene, dirty and wet bedding, dirty and wet udders at the time of milking, lack of concern about teat-end lesions and not culling the severely infected cow that can transmit mastitis, all of that may increase mastitis infection (Table 3).

The spread of the disease in the area of study is expected to increase as long as the previous mentioned factors exist. This was concluded in review of previous data reported from Kassala state that showed seroprevalence of brucellosis was progressively increased from 5.1% to 17.1 % during the period 2004-2006 (Ahmed *et al*., 2007).

Conclusion and Recommendations

Conclusion:

The present study on brucella in milking dairy cattle in Khartoum North, Sudan recorded high occurrence of brucellosis which can pose a considerable potential risk to the animal welfare, public health and animal production. Application of bio-security is very week, the absence or lack of control and hygiene measures, and inadequate application of vaccination program had played a major role in spread of the disease.

The study concluded that Khartoum North area should be considered as endemic with bovine brucellosis. Brucella infection in the area of study might have been accompanied by other infections.

Brucellosis situation in Khartoum North, Sudan should be tackled seriously considering its negative impact and the zoonotic nature of the disease, the heavily populated wide area (The capital city) supplied with milk products from those farms located inside the area, especially cheese which made from unpasteurized milk which can transmit the disease.

Recommendation:

- The study recommended a formulation of long term plan to control brucella disease in Sudan.
- There is a need to plan a national eradication strategy for brucellosis in the country based on epidemiological reality.
- Effective combating of brucellosis in Khartoum state by application of vaccination program to protect dairy cows against brucella infection
- Control over this zoonotic disease could be achieved by using S19 vaccine against brucella.
- More surveys are required to investigate brucellosis in milking dairy cattle in Khartoum State.

- Raising farm owner’s awareness about brucella threat.
- Culling of infected cows immediately.
- Impose that farm workers must work in one farm.
- Proper bio-security practices in farms (proper cleaning and disinfection and other hygienic measures in dairy farms, including personal hygiene of workers).
- Pasteurization of milk and milk products for human consumption.
- ELISA technique is recommended as a sero-surveillance technique, as it gives an accurate and rapid result, because the system is computerized.

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Appendix 1

Study area

Professional Management			
No.	**Farms**	**Number of samples**	**Location**
01	Sudan University of Science & Technology farm	5	Shambat
02	Khartoum university farm	5	Shambat
03	Sudan University of Science & Technology farm	5	Kuku
04	Albatihani farm	5	Kuku
05	Ali Saad Alrayh farm	5	Kuku
06	Mohammed Sakin farm	5	Alsilait
07	Abdalraheem farm	5	Alsilait
08	Mamoon farm	5	Alsilait
09	Ahmed Alsideeg farm	5	Alsilait
10	Yonis Omer farm	5	Alsilait
Non-Professional Management			
11	Mostafa Ibraheem farm	5	Kuku
12	Abomidian farm	5	Kuku
13	Mohammed Ali farm	5	Kuku
14	Almerafabi farm	5	Kuku
15	Almahjoob farm	5	Kuku
16	Alajab farm	5	Alsilait
17	Mohammed Alnaeem farm	5	Alsilait
18	Hasan Alarabi farm	5	Alsilait
19	Futihalrahman farm	5	Alsilait
20	Wad alfaw farm	5	Alsilait

Appendix 2

The study questionnaire form

جامعة السودان للعلوم والتكنولوجيا
كلية الدراسات العليا
كلية الدراسات الزراعية
قسم إنتاج الحيواني
تقييم لإنتشار مرض البروسيلا المعدي في أبقار اللبن بمحافظة الخرطوم بحري

استمارة استبيان

التأريخ:.....

مزرعة رقم:.....

اسم صاحب المزرعة:.....

عدد الأبقار في المزرعة:.....

01	مانوع السلالة المرباة ؟	محلية	هجين	أجنبية
02	ماهي طريقة الحلب المتبعة ؟	آلي	يدوي	
03	أين يتم الحلب ؟	داخل الحظيرة	خارج الحظيرة	محلب خاص
04	هل الحلابين خاصين بالمزرعة ؟	نعم	لا	
05	كم عدد حالات البروسيلا بالمزرعة ؟			
06	هل يتم الكشف علي البروسيلا دوريا	نعم	لا	
07	مانوع الإختبار المستخدم للكشف عن البروسيلا ؟			
08	هل يتم الإهتمام بالضرع في فترة التجفيف ؟	نعم	لا	
09	متي تتم نظافة الحظائر ؟	يوميا	اسبوعيا	شهريا
10	ماهي أكثر الأمراض السائدة في المزرعة ؟			
11	هل تخضع الأبقار للتطعيم السنوي ؟	نعم	لا	

		بقرة 1		بقرة 2		بقرة 3		بقرة 4		بقرة 5	
12	عدد الولدات										
13	حدوث اجهاض	نعم	لا	نعم	لا	نعم	لا	نعم	لا	نعم	لا
14	تأخر الحمل	نعم	لا	نعم	لا	نعم	لا	نعم	لا	نعم	لا