بسم الله الرحمن الرحيم

Sudan University of Science and Technology

Collage of Veterinary Medicine

Prevalence of Bovine Brucellosis in the Farm of Sudan University of Science and Technology (Hilat Kuku)

معدل الانتشار لداء البروسيلا في الابقار بمزرعة جامعة السودان للعلوم والتكنولوجيا- حلة كوكو

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إذا كان الإهداء يعبر ولو بجزء من الوفاء فالإهداء إلي

معلم البشرية ومنبع العلم نبينا محمد صل الله علية وسلم

إلي اللائي نثرن دعواتهن في طريقنا حتي بلغنا ما نحن علية.....أمهاتنا

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ABSTRACT

The main objective of the present study was to monitor prevalence of brucellosis in cattle in the farm of Sudan University of Science and Technology at Hilt Kuku in Khartoum North. A total numbers of 25 (24 females and one male) bovine sera were examined using Rose Bengal Plate test. Results revealed that all animals (100%) were negative to brucellosis. This results showed that the farm free from bovine brucellosis.

Key words: Brucellosis, Rose Bengal Plate test, serum, prevalence.

مستخلـص الأطروحة

الهدف الاساسي لهذه الدراسة هو اجراء مسح مصلي لمرض الاجهاض المعدي في الابقارفي مزرعة جامعة السودان للعلوم والتكنلوجيا بمدينة حلة كوكو في شمال الخرطوم .فحصت عدد 25 عينة سيرم من قطيع أبقارالهجين (عدد 24 من الاناث وذكر واحد) بواسطة اختبار الروزبنقال. اظهرت النتائج عدم وجود الاجهاض المعدي بالمزرعة.

INTRODUCTION

Brucellosis is a zoonotic bacterial disease which has a great effect on public and animal health in many countries of the world. It affects a variety of domestic and wild animals and man. It is caused by any one of the members of the genus *Brucella* (Amel , 2005). According to Krieg and Holt (1984), *Brucella* is a groups of bacteria which are morphologically and antigentically similar. It has 10 species according to the primary host, *Brucella abortus* (*B. abortus*) cattle , (*B.melitensis)* sheep and goats,(*B. suis)* pigs, (*B. ovis)* sheep, (*B. canis)* dogs, and(*B. neotomae)*desert wood rat. (Stonner and Lackman, 1957). Recently, *B. pinnipedialis and B. cetia* marine strain of *Brucella* (Foster *et al*., 2007), *B. inopinata* has been isolated from a breast implant infection (Scholz *et al*., 2009) and *B.microti* has been isolated from systemically infected common voles (*Microtusarvalis*) in South Moravia. (Scholz*et al*., 2008a). Later on, *B. microti* was isolated from mandibular lymph nodes of wild red foxes (*Vulpesvalpes*) hunted in Austria (Scholz*etal*., 2008b). Furthermore, specific *B. microti* DNA sequences were recently detected in soil, but whether soil is the primary habitat of *B . microti* remains to investigated (Scholez*et al*., 2008c) . The first isolation of *Brucella* organisms from animals was made by Bang (1897). *Brucella melitensis* was the first species reported as the cause of brucellosis due to consumption of raw infected goat milk (Bruce, 1887). Brucellosis in bovine exhibit one principle symptom i.e. abortion, the first abortion can occur when cow reache five months of pregnancy. The two majority of abortions are seen around the seventh month, a cow usually aborts once and then becomes a carrier. Some cows may abort a second and occasionally even a third time. The other manifestations occur such as hygroma, orchitis, retention of placenta, weakness of stillbirth, long calving intervals, infertility, bursitis and arthritis. These

symptoms occur variably and to a lesser extent in other animal species (Musa *et al*., 1990b). Infected cows must be culled if eradication is needed, but this causes economical losses. Milk from infected animals can be treated by pasteurization following international standard efficient methods, so that *Brucella* organisms will be destroyed. To avoid transmission of *Brucella* organisms through the ingestion of infected milk or by the conjunctiva or inhalation or direct skin contact to foetal contents, farmers must be cautious to isolate brucellosis positive animals and also those with symptoms of early delivery or the latent carriers. *Brucella* can cross react with *Yersinia enterocolletica* and this can give false positive result, so the antigen was modified by addition of EDTA to make the test more specific (Garin and Trap, 1985). Brucellosis has a major economic impact due to abortion, consequent decrease in milk yield, death of infected animals and rejection of exported consignments containing infected animals. Also countries incurs cost generated by prophylactic activities, control and eradication programmes, hospitalization of human patients, loss of work or income and failure of financial investment (Chukwu, 1987). The disease must be controlled by testing, isolation of reactors and vaccination using full doses of *B. abortus* strain-19 vaccine for calves and reduced dose for adults.

In Sudan cattle brucellosis was reported in all parts of the country and prevlance rate was found to be higher compared to other animal species. The first incidence of bovine brucellosis was reported from dairy herd in Khartoum where *brucella abortus* was isolated from an aborted cow (Bennett,1943). Biter (1986) examined 948 camels from different herds in Estern Sudan and the prevlance rate that ranged between 16.5 and 32.3%. *Brucella abortus* was isolated from camel in this area. The prevalence rates in Kassala in sheep 2.1%, goats 30.5%, in camels 17.1% and in cattle 10.9% (Omer *et al*, 2007).

Objectives of the study

1. To examine the presence of Brucellosis in cattle in farm of Sudan University of Science and Technology (Hilt kuku).
2. To estimate the prevalence of Brucellosis in farm of Sudan University of Science and Technology.

CHAPTER ONE

LITERATURE REVIEW

1.1. Brucellosis

1.1.1 Definition

Brucellosis is a wide spread bacterial disease of animals and man caused by any one of the members of the genus *Brucella* (Corbel and Hendry, 1983). It was named brucellosis after Bruce (1887) who was the first one to isolate the organism and recognized the disease. In animals, the disease is characterized by bacteraemia followed by localization of organisms in the reproductive organs, reticuloendothelial tissues and sometimes joints (Gillespie and Timoney, 1981).The disease in man is known as Malta fever and is characterized by undulant fever, chills, headache, pain in legs, large joints and lumber regions, profuse neutral sweating, insomnia, sometimes laryngitis and bronchitis (Van Der Hoeden, 1964).

1.1.2 History of Brucellosis

Bruce isolated *Brucella melitensis* (*Micrococcus melitensis* at that time) in 1887 from the spleen of a British soldier who died from a febrile illness (Malta fever) common among military personnel stationed on Malta. For almost 20 years after isolation of *B. melitensis*, Malta fever remained a mystery and was thought to be a vector-borne disease until The mistocles Zammit accidentally demonstrated the zoonotic nature of the disease in 1905 by isolating *B. melitensis* from goat's milk (Wyatt, 2005; Sriranganathan *et al.*, 2009). It was believed that goats were not the source of infection since they did not become ill when inoculated with *Brucella* cultures. The discovery that healthy goats could be carriers of the disease has been termed one of the greatest advances ever made in the study of epidemiology (Wyatt, 2005; Sriranganathan *et al.*, 2009).

In 1897, a Danish veterinarian, L. F. Benhard Bang, discovered Bang's bacillus or bacillus of cattle abortion (*B. abortus*) to be the causative agent of Bang's disease (Sriranganathan *et al.,* 2009). Alice Evans, an American scientist who did a landmark work on pathogenic bacteria in dairy products, confirmed the relationship between Bang's disease and Malta fever and renamed the genus *Brucella* to honor David Bruce. Her work on *Brucella* was central in gaining acceptance of the pasteurization process to prevent human brucellosis in USA. The discovery of the *Brucella* in marine mammals in early 1990 has changed the concept of a land-based distribution of brucellosis and associated control measures (Sriranganathan *etal.,* 2009).

1.2. Economic Importance

Losses in animal production due to this disease can be of major importance, primarily because of decreased milk production in aborting cows. The common sequel of infertility increases the period between lactations and in an infected herd the average inter-calving period may be prolonged by several months. In addition to the loss of milk production, there is loss of calves and interference with the breeding program. This is of greatest importance in beef herds, where the calves represent the sole source of income. A high incidence of temporary and permanent infertility results in heavy culling of valuable cows and some deaths occur as a result of acute metritis following retention placenta (Radostits *et al*, 2007).

1. 3 Geographic Distribution

Brucellosis is found worldwide but it is well controlled in most developed countries. Clinical disease is still common in the Middle East, Asia, Africa, South and Central America, the Mediterranean Basin and the Caribbean (OIE, 2004). *Brucella* species vary

in their geographic distribution. *Brucella abortus* is found worldwide in cattle-rasing regions except Japan, Canada, some European countries, Australia, New Zealand and Israel, where it has been eradicated (Ozekicit *et al*., 2003). Eradication from domesticated herds is nearly complete in the U.S. *B. abortus* persists in wildlife hosts in some regions, including the greater Yellow stone area of North America. *Brucella melitensis* is particularly common in the Mediterranean. It also occurs in the Middle East and Central Asia around the Arabian Gulf and in some countries of Central America. The organism has been reported from Africa and India, but it does not seem to be endemic in Northern Europe, Northern America (except Mexico), South East Asia, and Australia. *B. ovis* probably occurs in most sheep raising regions of the world. It has been reported from Australia, New Zealand, North and South America, South Africa and many countries in Europe. In the past, *B. suis* was found worldwide in swine-raising regions. This organism has been eradicated from domesticated pigs in the U.S, Canada, and many European countries. *Brucella canis* probably occurs of the world; however, New Zealand and Australia appear to be free of this organism. *Brucella* species also seem to be widespread in marine mammal.

1.4 Transmission

Brucella abortus, B. melitesis, 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 infective 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 mucus membranes, broken skin and possibly intact skin. *Brucella pinnipedialis*, transmission may occurs by

direct contact through mucosa and injured skin, oral route due to ingestion of other infected marine mammals (Foster *et al*., 2002).

Most or all *Brucella* species are also found in semen. Males can shed these organism for blong periods or lifelong. The importance of venereal transmission varies with the species, it is the primary route of transmission for *B. ovis*, *B. suis, and B. canis. Brucella* species can be spread by fomities, including feed and water. In condition of high humidity, low temperature and no sunlight, these organisms can remain viable for several months in water, aborted fetuses ,manure, wool, hay, equipment and clothes. They can withstand drying ,particularly when organic material is present, and can survive in dust and soil, and survive longer when the temperature is low, particularly when it is below freezing (OIE;2004) .

1.5. Incidence of Brucellosis

In cattle, sheep, goat and other ruminants the initial phase following infection is often not apparent (Roop *et al*., 2009). In sexually mature animals the infection localizes in the reproductive system and typically produces placentitis followed by abortion in the pregnant female, usually during the last third of pregnancy and epididymitis and orchitis in the male. Clinical signs are not pathognomonic and diagnosis is dependent upon demonstration of the presence of Brucella species either by isolation of the bacteria or detection of their antigens or genetic material or by demonstration of specific antibody or cell-mediated immune responses (OIE, 2006). Brucellosis is a disease of many animal species but especially of those that produce food: sheep (especially milk-producing), goats, cattle and pigs and, on a more localized scale, camels, buffaloes, yaks and reindeer. Brucella species are somewhat host-specific but cross-species infections occur, especially with *B. melitensis* (OIE, 2006). Infections in sheep and goats are highly contagious because of the pathogenicity

of *B. melitensis* and because of close contact caused by the density of the flocks or herds, the commingling of those of different owners and heavy exposure in housings (OIE, 2006). Animal-to-animal transmission occurs as a result of the large number of organisms shed in the environment in some parts of Africa, hygromas and abscesses are the major clinical signs usually observed in nomadic or semi-nomadic cattle herds infected with *B. abortus* biovar (OIE, 2006). There is lowered milk production due to premature births. Interference with fertility is usually temporary and most infected animals will abort only once and some are unaffected. The udder is often permanently infected, especially in the case of cows and goats. Shedding of organisms in milk is frequent. Localized infections in sheep result in orchitis or epididymitis in the case of *B. melitensis* and *B. ovis*. In goats, cattle, swine and dogs similar complications may follow infection with *B. melitensis*, *B. abortus*, *B. suis* and *B. canis* ,respectively Arthritis may also be a rare sign in *B. melitensis*-infected sheep and goats. In horses, local abscess formation in bursae may be the only clinical sign and infection in this species is often asymptomatic. Camels infected with *B. melitensis* shed the organisms in milk and in some countries this is a serious public health problem. Clinical signs of brucellosis in camels appear to be very rare (OIE, 2006).

The severity of the disease depends upon many factors such as previous vaccination, age, sex and management such as herd or flock size and density. Abortions are more prevalent in unvaccinated animals and numbers of organisms shed are much greater. The bacteria are found in tissues and fluids associated with pregnancy, the udder and the lymph nodes which drain the relevant areas. Most infections result from ingestion of bacteria either from diseased animals or contaminated feedstuffs. However, infection may also be acquired by respiratory exposure and by contamination of abraded skin and mucosal surfaces. Natural

breeding transmits infection in swine and dogs and, to a lesser extent, sheep and goats (OIE, 2006).

1.6 Incubation period:

The incubation period varies with the species and stage of gestation, and often cannot be accurately determined. The length of incubation period was inversely proportional to the stage of fetal development at time of exposure (Thomsen, 1950). The incubation period in brucellosis is affected by several factors such as gestation, exposure, dose, age, vaccination and other unknown host resistance influences (Nicoletti, 1980). 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, abortion 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 (OIE; 2004).

1.7. Causes and pathogenesis

Brucellosis is caused by members of the genus Brucella, it is an important zoonosis and a significant cause of reproductive losses in animals (Sriranganathan *et al*., 2009). Brucellosis is usually caused by *Brucella abortus* in cattle, *B. melitensis* or *B. ovis* in small ruminants, *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 economic; deaths are rare except in the fetus and neonate. Some Brucella species are also maintained in wildlife populations. Wildlife reservoirs including feral pigs, bison, elk and European hares complicate eradication efforts for *B. abortus* and *B. suis*. Marine mammal isolates of Brucella have recently been recognized in many species of

pinnipeds and cetaceans, and there are concerns that these organisms might have a deterimental impact on some species. Most species of Brucella can infect animals other than their preferred hosts, when they come in close contact (Godfroid *et al.*, 2005; CFSPH, 2009).

The ability of Brucella species to successfully survive and replicate within different host cells explains their pathogenicity. Extensive replication of Brucella species in placental trophoblasts is associated with abortion in their animal preferential hosts and persistence in macrophages leads to chronic infections that are a hallmark of brucellosis in both natural animal hosts and humans (Roop *et al*., 2009).

Brucella species are facultative intracellular pathogens and establish infection by invading macrophages and evading macrophage-induced host protection mechanisms. These characteristics contribute to clinical signs and therapeutic considerations, including the difficulty in both diagnosis and treatment. Following exposure in humans, the organisms travel along the lymphatic pathways; focal disease is most commonly identified in the reticuloendothelial tissues such as the liver and spleen. In chronic infections, organisms typically localize in joints, especially large joints such as the sacroiliac or lumbar vertebral joints (Glynn and lynn, 2008). Pulmonary disease is a less common form of brucellosis. In most animals, after ingestion of the organism, the bacteria travel through the oral mucosa to the regional lymph nodes. Infection leads to bacteremia, which is usually transient; the organisms ultimately settle in the reproductive tissues or musculoskeletal system (Glynn and lynn, 2008). In dogs and rams, venereal transmitted organisms establish chronic infections in the testes and epididymides and infection of the reproductive tissues of females of these species may occur (more commonly in bitches and uncommonly in ewes), the pathogenesis being similar to that in large animals (Glynn and lynn, 2008).

1.8 Clinical signs

Brucellosis affects many different organs in animals and consequently the signs of the disease will be influenced by the nature and extent of the infection and the species involved. Some infected animals may not show signs (Bishop *et al.,* 1994).

1.8.1 Bovine brucellosis (*B. abortus*)

In cattle, *B. abortus* causes abortion, stillbirths, and weak calves; abortion usually occurs 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, orchitis, seminal vesiculitis and testicular abscesses are sometimes seen in bulls. Infertility occurs occasionally in both sexes, due to metritis , orchitis or epidiymitis. Hygromas, particularly on the leg joints, are common symptoms 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 pregnant females are usually asymptomatic.

1.8.2 Ovine and caprine brucellosis (*B. melitensis*)

Brucella melitensis mainly causes abortion, stillbirths and the birth of weak offspring. Animals that abort may retain the placenta, and milk yield 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.

1.8.3 Ovine epididymitis (*B. ovis*)

Brucella ovis affect sheep but not goat . This organism can cause epididymitis, orchitis and impaired fertility in rams. Epididymitis may be unilateral or occasionally bilateral. Some rams shed *B. ovis* for long periods without clinically apparent lesions. Abortions, placentitis and prenatal mortality can be seen in ewes but are uncommon.

1.8.4 Canine brucellosis

Brucella canis can cause abortions and stillbirth in pregnant dogs. Most abortion occurs late, particularly during the seventh to ninth week of gestation. Usually subclinical although can be severe. Mild fever, emaciation, abortions, arthritis and anestrus (OIE; 2004).

1.8.5 Porcine brucellosis (*B. suis*)

In pigs, the most common symptom is abortion, which can occur at any time during gestation, and weak or stillbirth piglets. Swollen joints and tendon sheaths, accompanied by lameness and in coordination.

1.8.6 Brucellosis in horses

In horses, *B. abortus* and occasionally *B. suis* can cause inflammation of the supra spinous or supra-atlnatal bursa, these syndromes are known, respectively, as fistulous withers or poil evil. In chronic cases, nearby ligaments and the dorsal vertebral spines may become necrotic. Brucella associated abortion are rare in horses.

1.8.7 Brucellosis in marine mammals

Since 1990, Brucella strains have been isolated from a variety of marine mammal species, including seal, dolphins, whale, and other species (Ewalt *et al.,* 1994; Ross *et al*., 1996; Foster et al., 1996; Clavareau *et al*., 1998; Wyatt, 1999). These isolates have been

classified as *B. ceti* and *B.pinnipedialis*, referring to isolate from cetaceans and seals, respectively (Foster *et al.,* 2007).

1.9 Epidemiology

Epidemiology of brucellosis varies with the host species affected. For cattle, infection is usually caused by *B .abortus*. However *B. melitensis* and rarely *B.suis* can also establish themselves in cattle. These species are particularly dangerous to humans. Because of the high virulence of most *B.melitensis* and *B.suis* strains and of the large numbers of bacteria that are excreted by infected animals, Brucella is usually transmitted from animal to animal by contact following an abortion. Pasture may be contaminated and the organisms are probably most frequently acquired by ingestion but inhalation, conjunctival inoculation, skin contamination and udder inoculation from infected milking cups are other possibilities. The use of pooled colostrums for feeding newborn calves may also transmit infection. Sexual transmission usually plays a little role in the epidemiology of bovine brucellosis. However, artificial insemination can transmit the disease and semen must only be collected from animals known to be free of infection. In sheep and goats, *B. melitensis* is nearly always the infecting species. *B.ovis* can also infect sheep but is of little significance in relation to human disease. The mode of transmission of *B. melitensis* in sheep and goats is similar to that in cattle but sexual transmission probably plays greater role. The transmission of the disease is facilitated by purchasing animals from unscreened sources. Swine brucellosis is transmitted by contact with recently aborted sows, by ingestion of contaminated food or exposure to contaminated environment. However, sexual transmission is particularly important. For all species, embryo transfer is safe provided that recommended procedures are followed. For *B.canis,* sexual transmission is also an important means of spread and males can excrete the organism in large numbers in their semen. Urinary excretion also occurs and is a potential

hazard to humans. It should be remembered that dogs can acquire infection with *B. abortus* , *B. melitensis* or *B. suis* from aborted ruminants or swine, usually by ingesting fetal or placental material. In cattle, sheep, goats and swine, susceptibility to brucellosis is greatest in sexually mature animals; young animals are often resistant. Breed may also affect susceptibility, particularly in sheep. The milking breeds seem to be the most susceptible to *B. melitensis.* Latent or in apparent infections can occur in all farm animal species .These usually result from infection in utero or in the early post- natal period. Such animals can retain the infection for life and may remain serologically negative until after the first abortion or parturition(WHO,2006). The Brucella is a facultative intracellular parasite, so it has protection from the innate host defenses and from therapeutic agents. Natural or artificial infections usually persist indefinitely although about 10-15% recover spontaneously (Nicoletti, 1980).

1.10 Diagnosis

Diagnosis of the disease in animals must be carried out on a herd basis. There may be very long incubation period in some infected animals and individuals may remain serologically negative for a considerable period following infection. The identification of one or more infected animals is sufficient evidence that infection is present in the herd, and that other serologically negative animals may be incubating the disease and present a risk (WHO, 2006). Recently, polymerase chain reaction (PCR) has been shown to be available method for detecting DNA from different fastidious and non cultivated agents (Meyer and Mushuhwar, 1991). There are many methods which are used for the diagnosis of brucellosis:

1.10.1 Bacteriological methods

The isolation and identification of Brucella offers a definitive diagnosis of brucellosis and may be useful for epidemiological purposes and to monitor the progress of vaccination programme. It should be noted that all infected materials present a serious hazard and they must be handled with adequate precautions during collection, transport and processing.

1.10.1.1 Microscopic examination

Smears of placental cotyledon, vaginal discharge or fetal stomach contents may be stained using modified Zihel-Neelsen (Stamp's) or koster methods (Christofferson and Ottosen, 1941). The presence of large aggregation of intracellular, weakly acid–fast organisms of Brucella morphology is presumptive evidence of brucellosis. Care must be taken as other infectious agents such as *Coxiella burnetii* or *Chlamydia* may superficially resemble *Brucella.*

1.10.1.2 Culturing of samples for isolation

Brucella most readily to be isolated in the period following an infected abortion or calving, but isolation can also be attempted using postmortem. Brucella can be excreted in large numbers at parturition and can be cultured from a range of material including vaginal mucus, placenta, fetal stomach contents and milk using suitable selective culture media.

1.10.2 Guinea pig inoculation

This method is more successful than the direct culture especially from contaminated material. Injections are made intramuscularly inside the thigh, the guinea pig is killed 4-5 weeks after inoculation and its sera is subjected to five tube agglutination test.

Recovery of the organism from the spleen of the guinea pigs or positive Serum Agglutination Test at 1/10 or over is taken as evidence of infection (Brinely and Mccullough, 1978).

1.10.3 Serological methods

Recently, there are two types of serological tests available; very sensitive ones which are used for screening and definitive ones used for confirmation of infection. As a result, usually more than one type of tests are used for the diagnosis of brucellosis because there is no single test which is both sensitive and specific, has the ability to discriminate between vaccinated and non vaccinated animals and could distinguish between antibodies due to infection and those due to cross reaction. Many serological tests were developed for diagnosis of brucellosis using body fluids such as serum, hygroma fluid, milk, vaginal mucus, semen, bursa and muscle juices from suspected cattle; these fluids may contain different quantities of antibodies of the IgG, G1, G2 and other types directed against Brucella (Beh,1974). These tests are Rose Bengal Plate Test (RBPT), Serum and Tube Agglutination Test (SAT or TAT), Complement Fixation Test (CFT), Card, Plate Agglutination Test, Buffered Agglutination Plate Test (BPAT), Modified Serum Agglutination Test, Anti Globulin Test (AGT)Or Coomb's Test, Indirect Haemolysis Test (IHT), Haemolysis In Gel Test (HIGT), Enzyme Linked Immunosorbent Assay (ELISA),Milk Ring Test (MRT), Whey Agglutination Test and Allergic Skin Test (AST) (WHO, 1992).

The RBPT, MRT, ELISA and CFT are the conventional serological diagnostic methods and should continue in use for brucellosis surveillance. The important serological tests which are used in diagnosis of brucellosis are:

1.10.3.1 Rose Bengal Plate Test

This test is widely used as a screening test to detect the presence of B.abortus infection in cattle (Morgan *et al.*, 1969, Alton *et al.*, 1975). It can also be used as a definitive test (Nicoletti, 1967). Using antigen stained with Rose Bengal buffered at 3.65 PH to inhibit non-specific agglutinin, but not those of Brucella (Rose and Roepke, 1957). Test is a spot agglutination technique, because the test does not need special laboratory facilities and is simple and easy to perform. The test detects specific antibodies of the IgM and IgG types and is more effective in detecting antibodies of the IgG1 type than IgM and IgG2 types (Levieux, 1974). The test may yield negative result in infected cattle that give positive result with the CFT (Rose and Roepke, 1957).The low PH (+3.6) of the antigen enhances the specificity of the test. The temperature at which the reaction takes place may influence the sensitivity and specificity of the RBPT (MacMillan, 1990).

1.10.3.2 Milk Ring test (MRT)

The MRT is cheap, easy, simple and quick to perform; it detects lacteal anti Brucella IgM and IgA bound to milk fat globules. However, it gives false positive when milk contains colostrum, it is at the end of the lactation period, or from cows suffering from a hormonal disorder or from cows with mastitis (Bercovich and Moerman, 1979). Milk that contains low concentrations of lacteal IgM and IgA or which is lacking the fat – clustering factors tests false negative (Keer *et al.*,1959, Tanwani and Pathak, 1971, Patterson and Deyoe, 1978). According to WHO (1992) the MRT is not suitable for sheep and goats as ring formation does not readily occur. The results are influenced by factors such as mastitis, mechanical agitation and vaccination with B. abortus strain 19 vaccine. The test is used to

detect brucellosis in dairy cattle but, is not sensitive enough to detect brucellosis in goats (Shimi and Tabatabai, 1981).

1.10.3.3 Serum Agglutination test (SAT)

This test is widely used in some countries and its positive result is subjected to the definite CFT. The antigen used in the test is a Brucella whole cell and the antibodies detected are those directed against the surface molecules. SAT unlike other tests, detects antibodies of other isotypes (MacMillan, 1990). It can be performed in tubes or micro titre plates and plate was found to be more sensitive (Heer *et al*., 1982). Serum Agglutination test has international standardization; it is used for control programmes and import and export policies (MacMillan and Cockrem, 1985). According to reports of FAO/WHO Export committee on brucellosis (1994), the result of this test in cattle with antibody level less than 30 I.U should be considered negative in non- vaccinated animals or in those with unknown vaccination history.Where as in the vaccinated over 30 months of age, the level should be more than 30 I.U.

1.10.3.4 Complement Fixation Test (CFT)

This test is used for confirming the result of the RBPT and SAT. The test was found to be more accurate for bovine brucellosis (Morgan *et al.,* 1973). The CFT detects specific antibodies of the IgM and IgG types that fix complement (Hill, 1963 and Levieux, 1974). Meyer (1979) stated that the test was superior to other test in sensitivity and specificity, and it was found to have the highest specificity in both non- vaccinated and vaccinated cattle when compared with SAT, haemolysis in gel, indirect enzyme immunoassay and buffered plate antigen tests, but is laborious and requires highly trained personnel as well as laboratory facilities. This makes the CFT less suitable for use in

developing countries. Although (Corbel, 1972) stated that RBPT and CFT reactions are probable due to the same antibody which is IgG1. Although its specificity is very important for control and eradication of brucellosis it may test false negative when antibodies of the IgG2 type hinder complement fixation (MacMillan, 1990). The CFT measures more antibodies of the IgG1 type than antibodies of the IgM type, as the later are partially destroyed during inactivation. Since antibodies of IgG1 type usually appear after antibodies of the IgM type control and surveillance for brucellosis is best done with SAT and CFT (Levieux, 1979, Blasco *et al.*,1994a), found that the CFT was less sensitive than RBPT. Buxton and Fratser (1977) reported that the test useful in detecting chronically infected animals in which the complement fixing antibodies disappear more slowly than agglutinins.

1.10.3.5 Anti- globulin (Coomb's) Test

The antiglobulin (coomb's) test detects antibodies of the IgG2 type and use to confirm SAT results (Hill, 1963). The coomb's test, although laborious, is particularly important when the SAT is positive and CFT results are negative or conclusive (Kiss, 1971). However Coomb's test results are indicative for infection only when it titres are at least two times than titres of the SAT (Hill, 1963). This test's main limitation, as not all infected cattle show this ratio. The 2-mercatoethanol and the revanol tests detect specific IgG (Rossi and Cantini, 1969), and are usually used to differentiate between infected and vaccinated cattle.

1.10.3. 6 Enzyme –Linked ImmunoSorbent Assay (ELISA)

The Enzyme-linked immunosorbent Assay (ELISA) is a highly sensitive method used for serological diagnosis (Sutherland, 1985). The ELISA has proven to be specific and as sensitive as the MRT and SAT in detecting Brucella antibodies in milk and semen (Nielsen *et al.*, 1981).

ELISA results are usually in agreement with CFT results (Ruppanner *et al*., 1980, Bercovich and Taaijke, 1990).

The test can be used for screening and confirmation of brucellosis in both milk and semen. However, depending on the presence of traces of colostrums in the milk, or the presence of low concentration of lacteal immunoglobulin, ELISA may test false positive or false negative (Bercovich and Taaijke, (1990), Kerkhofs *et al*., 1990). It seems that the ELISA is less sensitive than the CFT, as some infected cattle that test positive with CFT may test negative with the ELISA (Cargill, and Clark, 1985; Sutherland, 1984). Some researchers imply that the main advantages of the ELISA when compared with CFT lies its relative simple test procedure (Sutherland *et al*., 1986). The assay is very costly when a few samples are tested, therefore, it is unsuitable for testing individual animals but it's the ideal test for screening purposes.

1.10.3.7 Indirect Haemagglutination Test (IHAT)

The test was found useful for the diagnosis of brucellosis in animal and man. It uses LPS of *B. Abortus* or intracellular antigen and could be carried out as a tube or micro titre plate test (Corbel and Dan, 1973). The IHAT is highly sensitive but it is specificity was offset by difficulty of interpreting reactions produce at low dilution of sera.

1.10.3.8 Allergic Skin test (AST)

It is routinely and officially used for the diagnosis of brucellosis in east European countries (Kolar, 1990). Kolar (1990) mentioned that the test could be used in farm animals but it was mainly intended for sheep, goats and pigs. In cattle the test could be used to confirm or current the result of serological test in cattle (Jerabek, 1962). Allergic Skin test is performed strictly into the skin. The side of injection depends on the animal species. The

test is specific and does not react to cross reacting organism (Kolar, 1990). Some workers believe that the AST is more sensitive than the serological test (Kolar and Kolarova, 1955).

1.10.4 Molecular methods

1.10.4.1 Polymerase Chain Reaction (PCR)

The technique is a very useful tool for the diagnosis of brucellosis because of its simplicity, high degree of sensitivity and specificity together with its speed, virility in sample handling and risk reduction for laboratory personnel (Mortata *et al*., 2001). Serum sample should be used preferentially over whole blood for the molecular diagnosis of brucellosis (Zerva *et al*., 2001). The test was used to diagnose brucellosis in goats and it was shown to be more sensitive than the RBPT and culture techniques (Leal-Klevezas *et al*., 2000). Recently, Amel (2005) examined 160 bovine milk samples using PCR. She was able to detect Brucella DNA from 20 milk samples (12.5%).

1.11. Treatment of Brucellosis

There is no practical treatment for infected cattle or pigs, but long-term antibiotic treatment is sometimes successful in infected dogs. Some dogs relapse after treatment. 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. In horses with fistulous withers or poll evil, the infected bursa may need to be surgically removed (OIE, 2009).

Due to intracellular localization of brucella and its ability to adapt to the environmental conditions encountered in its replicative niche e.g. macrophage (Seleem et al., 2008), treatment failure and relapse rates are high and depend on the drug combination and patient compliance. The optimal treatment for brucellosis is a combination regimen using two

antibiotics since mono-therapies with single an antibiotic has been associated with high relapse rates (Solera *et al*., 1997 ;Pappas *et al*., 2005; Seleem et al., 2009). The combination of Doxycycline with Streptomycin (DS) is currently the best therapeutic option with less side effects and less relapses, especially in cases of acute and localized forms of brucellosis (Solera *et al*., 1995; Ersoyet al., 2005; Alp *et al.*, 2006; Falagas and Bliziotis, 2006; Seleem *et al.*, 2009). Neither Streptomycin nor Doxycycline alone can prevent multiplication of intracellular brucella (Shasha *et al*., 1994). Although the DS regimen is considered as the gold standard treatment, it is less practical because the Streptomycin must be administered parenterally for 3 weeks. A combination of Doxycycline treatment (6 weeks duration) with parenterally administered Gentamycin (5 mg/kg) for 7 days is considered an acceptable alternate regimen (Glynn and Lynn, 2008).

Although DS combinations had been considered by the WHO to be the standard therapy against brucellosis for years, in 1986 the Joint FAO/ WHO Expert Committee on Brucellosis changed their recommendations for treatment of adult acute brucellosis to Rifampicin (600–900 mg/day orally) plus Doxycycline (200 mg/day orally) DR for 6 weeks as the regimen of choice. However, the studies that compared the effectiveness of DR regimen with the traditional DS combination concluded that DR regimen is less effective than the DS regimen especially in patients with acute brucellosis (Solera *et al*., 1995).

1.12. Control and prevention of Brucellosis

Three general methods of control of brucellosis in animals are often given. These are:

(1) test and slaughter (2) hygienic measures and (3) vaccination (Nicoletti, 2010). These are most effective when they are combined. Test and slaughter of sero-positive animals are usually a part of organized governmental programmes where the goal is eradication. The purpose of hygienic practices such as isolation of animals which have aborted is to reduce or

prevent exposure of susceptible animals. Pre-movement tests at local or international levels are parts of control efforts. These procedures are often difficult to administer and to gain acceptance. Livestock owners are reluctant to accept controls for long periods and usually they do only for emergency diseases (Nicoletti, 2010). Contamination of areas requiring disinfection is a factor which may have limited impact on reducing exposure. Many studies have shown variables in survival rates of Brucella species. There is a wide agreement that vaccination is the most effective and practical method for reducing the incidence of many diseases including brucellosis in livestock (Nicoletti, 2010).

Vaccination against diseases is widely accepted since it is commonly used. The live vaccines *B. abortus* strain 19 and the *B. melitensis* Rev 1 have proved to be the most effective agents in cattle and in sheep and goats, respectively. Strain RB51 has replaced S19 in some countries. There is some controversy about its effectiveness. S19 and Rev 1 are relatively inexpensive to produce and are highly immunogenic. They may sometimes cause abortions but this may be practically eliminated by reducing the dose of the vaccines. It is necessary to keep the vaccine refrigerated and post-vaccination antibodies may interfere with the interpretation of diagnostic test results. Although immunity may not be complete in some animals, vaccination practically eliminates clinical brucellosis and, in cattle, the herd immunity exceeds 90 % (Nicoletti, 2010). It is nearly always more economical and practical to prevent diseases than to attempt to control or eliminate them (OIE, 2006).

For brucellosis, measures of prevention include: (1) careful selection of replacement animals. These, whether purchased or produced from existing stock, should originate from Brucella-free herds or flocks. Pre-purchase tests are necessary unless the replacements are from populations in geographically circumscribed areas that are known to be free of the disease, (2) isolation of purchased replacements for at least 30 days. In addition, a serological

test prior to commingling is necessary, (3) prevention of contacts and commingling with herds of flocks of unknown status or those with brucellosis, (4) if possible, laboratory assistance should be utilized to diagnose causation of abortions, premature births, or other clinical signs. Suspect animals should be isolated until a diagnosis can be made, (5) herds and flocks should be included in surveillance measures such as periodic milk ring tests in cattle (at least four times per year), and testing of slaughtered animals with simple screening serological procedures such as the RBT, (6) proper disposal (burial or burning) of placentas and non-viable fetuses. Disinfection of contaminated areas should be performed thoroughly and (7) cooperation with public health authorities to investigate human cases. Animal brucellosis, especially when caused by B. melitensis, can often be identified through investigations of cases in humans (OIE, 2006).

CHAPTER TWO

MATERIALS AND METHODS

2.1 Study area

The study was conducted in the farm of Sudan University of Science and Technology, which located at Hilt Kuku, Khartoum North, which is situated between 15º 38` N and longitudinal 32º -26` E. The total area extends over approximately 21.000 square kilometer. The climate of Khartoum is an arid type which is characterized by a wide range in daily and seasonal temperatures. During cool season between Decembers to February, the weather is cool and dry with minimum daily temperature of 24º C. The season is characterized by low humidity. A hot dry weather prevails between March to October, a temperature of 45º C may occur during the day. The maximum rainfall is during the period from mid-July to September, in this season there is an increase in relative humidity with maximum 68% in August. It is more convenient to divide the year into a cool dry season, hot dry season and hot wet season.

2.2. Husbandry and management

2.2.1. Housing

The yard of barn was designed for at least 80 cows (16 dry cows and 64 milking cows) based on about 10 square meters per cow. The designed total area is 1200 square meters (60 × 20), which was shaded. The shade area laid along the whole western lengths and 400 square meters (267 square meters for milking cows and 133 square meters for the dry cows).The roof made up from hay (traditional Sudanese roof). The margins will be from steel Scuttle butt. The depth of margins was 50 cm and is situated along the outside of the yard so that feeding can be done without entering the yard. The length of feeding area was 8

meters long, therefore allowing at least space of 0.85 meters per cow. The watering more than the 20 liters barrel plastics fixed to the ground by steel frame and supplied by pipes which was connected to the water system (figure 1).

2.2.2. Feeding:

The fodder was cut and fed either green or dry to the animals in their yards. The concentrate portion of the milking cows' diet was feed at milking time. The dairy nutrient requirements of various classes of the dairy cattle. The dairy cows were fed on the forage produced in the farm. The forage production was allowed feeding the cereal fodder (Maize–Abu70) and leguminous fodder (Lubia- Alfa Alfa). The remaining nutrients were met by feeding a concentrate diet (figure 2).

Figure.1: **Housing of cattle in Sudan University Farm -Hilt Kuku**

Figure .2: **Feeding area of the cattle in Sudan University Farm -Hilt Kuku**

2.3 Sampling

2.3.1 Sources of samples

A total of 25 serum sample were collected from Cattle (24 crossbreed cows+1 bull) ,during the period from February to April 2016 ,the Average animal age between three to eight year, and Body weight between 300-550 kg.

2.3.2 Collection of blood samples

Five ml of blood were collected from the jugular vein of each cow in sterile tubes using disposable syringes. The collected samples were placed in a thermo- flask and transported to the laboratory and left to clot. The clots were separated and the tubes were kept overnight at 4°C to separate the serum, then the separated serum were placed in sterile tubes and stored at -20° C till used.

2.4. Rose Bengal Test

This test is a simple spot agglutination test using antigen stained with Rose Bengal and buffered to a low PH, usually 3.65±0.05, this antigen was obtained from Central Veterinary Research Laboratory (CVRL), Soba. The test was performed according to the OIE manual, (2004).

Test procedure:

- The serum samples and the antigen were brought at room temperature (22±4°C); only sufficient antigen for the day's tests was removed from the refrigerator.
- An amount of 25-30 µl of each serum sample was placed on a white tile, enamel or plastic plate.
- The antigen bottle was shacked well, but gently, and an equal volume of the antigen was placed near each serum spot.

- Immediately after the last drop of antigen has been added to the plate, both the serum and antigen were mixed thoroughly (using a clean glass or plastic rod for each test) to produce a circular or oval zone approximately 2cm in diameter.
- The mixture was rocked gently for 4 minutes at the ambient temperature on a rocker or three directional agitators (if the reaction zone is oval or round, respectively).
- Agglutination was immediately read after the 4 minutes period had completed. Any visible reaction was considered positive. A control serum that gives a minimum positive reaction should be tested before each day's tests are begun to verify the sensitivity of test conditions.

CHAPTER THREE

RESULTS

All examined cattle (24 females +1 male) were negative for brucellosis depended on the Rose Bengal Plate Test (table 1).

Table 1: **Result of Rose Bengal plate test of cattle in Sudan University Farm -Hilt Kuku.**

Sample No	Cow No	Result
1.	463	-ve
2.	907	-ve
3.	413	-ve
4.	349	-ve
5.	432	-ve
6.	917	-ve
7.	910	-ve
8.	914	-ve
9.	927	-ve
10.	426	-ve
11.	412	-ve
12.	415	-ve
13.	923	-ve
14.	928	-ve
15.	927	-ve
16.	911	-ve

Sample No	Cow No	Result
17.	918	-ve
18.	908	-ve
19.	412	-ve
20.	912	-ve
21.	925	-ve
22.	Bull	-ve
23.	Marrow	-ve
24.	Soba	-ve
25.	Hwida	-ve

DISCUSSION

In the present study, the prevalence of bovine brucellosis in the farm of Sudan University of Science and Technology,(Hilt kuku) using Rose Bengal Plate Test were showed 100% negative for brucellosis. This due to vaccination and good management, provided by the farm managers. This finding is disagreement with that reported by Angara et al. (2004) who recorded 93.3% of samples were positive for Rose Bengal Plate Test. This deference between two results may be due to large size of samples and confirmatory test (c-ELISA) that were used in previous study.

Also, Hamid *et al*. (2014) found that the prevalence rate of brucellosis using Rose Bengal Plate Test in cattle in Bahari province was 35.2% (207 samples). The previous results were in accordance with our results.

In Ibadan, Nigeria, Cadmus *et al*. (2006) reported that the prevalence of brucellosis in 1210 cattle was only 5.8% of samples were positive for Rose Bengal Plate Test. While, in the present study the number of examined samples were collected from 25 cattle.

CONCLUSION:

It could be concluded that the farm of Sudan University of Science and Technology is free from bovine brucellosis according to Rose Bengal Plate Test

RECOMMENDATIONS:

It is recommended that:

1- Further study must be needed for isolation and identification of *Brucella* species and biovars which affect animals in kuku farm.

2- Control programmes should be planned to prevent spread of brucellosis in animals and man.

3- The healthy cattle must be vaccinated to prevent the incidence of disease in the study area.

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