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The Prevalence of Brucellosis for Exported Sheep In Alkadaru Quarantine Khartoum State –Sudan

إنتشار الإجهاض المعدي في اغنام الصادر بمحجر الكدرو ولاية الخرطوم –السودان

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Dedication

To my parents who surrounded me with care and to whom I am always indebted.

To my teachers , to my uncle , brothers and sisters , to my friend,to all whom i love i dedicate this work .

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Abstract

In the Sudan sheep are very important source for meat, milk, export and source of wealth for the foreign currency. Sheep brucellosis impairs fertility of rams, causes abortion in ewes and is considered potential risk to human, especially veterinary practitioners, animal owners and animal products consumers.This study was designed to determine the occurrence of brucellosis in sheep for export form November (2016) to February (2017). The study was conducted in Alkadaru quarantine on 500 heads(300 form Kardofan State and 200 form Darfur State) of ready to export sheep . Serum samples, were used for the test and then the collected sera were divided in-to 10 groups.Seven groups form Kardofan State and three form Darfur State.Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT) and Buffered Acidified Plate Antigen(BAPA) were used for examination of brucellosis .The overall prevalence of seropositive was 0.6 %(3samples out of500), when the positive result was confirmed by SAT and (BAPA)only1 sample was found positive(form Kardofan State).Among the risk factors studied only age had a significant relationship with brucellosis (chi square test .5.8, p value 0.05%).

This study indicated that the presence of sheep brucellosis was in low rate in the sheep ready to export. Even this low incidence requires more attention and effort to implement procedures and regulation to eradicate the disease in the Sudan.

مستخلص البحث

تعتبر الاغنامفي السودان مهمه لإنتاج اللحم واللبن والتصدير بغرض الحصول علي عملات اجنبيه. يضعف مرض إلاجهاض المعدي خصوبة الحملان كما يؤدي الي إجهاض النعاج الُحمل كما يعتبر خطرا حقيقيا للانسان وخاصة الممارسين البيطرين ومالكي الحيوانات ومستهلكي المنتجات الحيوانية. أعدت هذه الدراسة لمعرفة نسبه الاصابه بمرض الاجهاض المعدي في الضأن المعد للصادر في الفتره ما بين نوفمبر(2016)حتي فبراير2017 إجريت الدراسة علي 500 رأس من الضأن الصادر مقسمه الي 10قطعان بمحجر الكدرو من ولايتي كردفان ودافور باستخدامإختبار الروز بنغال الصحنيوإختبارالتلازن (التراص) المصلي وإختبارالبابا Buffered Acidified plate Antigen نسبة الاصابة كانت0.6% باستخدام Rose Bengal plate Testو0.2% باستخدام Serum Agglutination Test, and Buffered Acidified Plate Antigen Test.وقد وجد أن العمر له علاقه معنويه مع مرض البروسيلا باختبار (chi-square test 5.8, p.value0.05).

هذه الدراسة تؤكد وجود مرض البروسيلا في الضأن بمعدل منخفض ويتطلب منا بذل مجهود اكبر لتطبيق إلاجراءات واللوائح بغرض إستئصال المرض من السودان .

Table of contents

List of tables

List of Figures

Introduction

Ovine brucellosis which is characterized by abortions in ewes and epididymitis, orchitis and reduced fertility in rams is due to the infection of *Brucella ovis,* a gram-negative bacterium which is non-spore forming and non-motile cocco-bacilli. Brucella organisms get colonize in the udder and supra mammary lymph nodes in non-pregnant animals and invade placenta when the animal becomes pregnant, causing lesions in the organ walls leading to endometriosis, ulcers in the inter-cotyledonary space and distraction of villi resulting in death and expulsion of the fetus . The main route of entry for brucella is oral, by the ingestion of feed and water, which is contaminated with secretions or aborted foetal remains from infected sheep or by licking the vaginal secretions, genitals, aborted fetus or new born lambs of infected ewes (Nagendra*et.al,*2015).

Brucella is facultative gram negative bacteria which are survivors in both extracellular and intracellular environments. The main domestic animals that are affected are cattle, sheep, camel, goats and pigs (Ashraf *et.al*, 2014).

Brucellosis has a considerable impact on human and animal health as well as a socioeconomic impact, especially in rural areas that largely rely on livestock. In developing countries, brucellosis is still considered the most serious and devastating zoonotic disease (Osman Gani*et .al* ,2016) .Brucellosis is a contagious disease of animals which is transmissible to man and is caused by main six species of the genus brucella. These species are further subdivided in to biovars that are useful in epidemiologic studies. 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. (Mohamed and

Atif ,2012).Brucellosis is one of the most important bacterial zoonosis with a cosmopolitan. It is an infectious disease, almost invariably transmitted by direct or indirect contact with infected animals or their products. The disease is caused by gram-negative coccobacilli bacteria which belong to the genus Brucella which includes *Brucella melitensis* and *B. ovis* as well as many other species. The natural reservoirs of the species *B.melitensis* are basically goats and sheep but also cattle and swine. However *B. ovis* is primarily affecting sheep. The significance of the disease is due to its zoonotic and economic impact it can be transmitted to people in contact with infected animals or consuming their products. However, the causative agent has a very low infectious dose; only 10 organisms of *B. melitensis* are sufficient to cause an infection in man. Furthermore, in animals, brucellosis causes severe economic losses as result of stormy abortions or reproductive failure, sterility and reduced milk production rates, beside that, it adds to the burden shouldered by the farmers; the costs of control and management. Also brucellosis of animals reduces the Foreign Exchange Earnings (FEE) by denying exportation of sheep to international markets. Pre-requisition of good knowledge on risk factors associated with the occurrence of infections such as brucellosis in sheep is imperative for the correct design and effective and efficient implementation of disease control strategies too. Nonetheless, important factors that contribute to the spread of brucellosis in sheep include: farming system and practices, farm sanitation, livestock movement, mixing and trading of animals and sharing of grazing grounds and watering points .Further complications arise through wild animal reservoirs which may also carry and transmit the disease . Abortion materials characteristically contain high numbers of brucella and consequently pose significant infection risks if not properly handled and disposed off Similarly, environmental contamination contributes to additional spread among animals. (Ahmed*et .al,* 2015) .

Justifications:

Justifications for prevention of the introduction of brucellosis into populations of animals are the same as those for the control of the disease in population which are already infected, economic benefits and the protection of public health.

Objectives:

1-To use serological test (RBPT, SAT and BAPA) for the detection of Brucella antibodies in collected sera form sheep.

2-To examine the risk factors associated with presence of Brucellosis in sheep ready to export.

3- To contribute to the knowledge of the status of Brucellosis in Alkadaru quarantine.

Chapter One

Literature Review

Ovine brucellosis:(OB)

Brucellosis is a contagious disease that infects animals and can be transmitted to humans. This zoonotic disease is caused by different species belonging to the genus Brucella, *Brucella melitensis* was first isolated by David Bruce from the spleen of a hospitalised soldier in Malta and since then brucellosis has been an emerging disease. Today, the genus brucella includes ten species *Brucella melitensis, B. abortus, B. suis, B ovis, B canis, B.neotomae, B.ceti, B. pinnipedialis, B. microti and B. inopinata*(Gumaa*et.al*,2014).

Brucellosis in sheep is primarily caused by *B. melitensis* though *B. abortus* and *B. suis* cause sporadic infections. Transmission of brucellosis in sheep occurs via oral,inhalation, conjunctival and venereal routes of exposure, as well as in utero. The main sources of contagious material are placenta, fetal fluids and vaginal discharge expelled by infected ewes after abortion or full-term parturition. Persistent infection of mammary glands and supra-mammary lymph nodes leads to intermittent or constant shedding of the organism in milk. The transmission of a given Brucella species to a non-preferential host (e.g. *B. menselitis* to cattle or *B. abortus* to small ruminants) is facilitated by mixing of herds and flocks, purchasing of animals from unscreened sources and sharing of bulls and rams for breeding. Such practices promote the transfer of pathogens between herds and flock. Although sexually mature animals of both genders are equally susceptible to the disease, the predominant signs of acute infection are reproductive failure with abortion in the last trimester and birth of weak offspring. *Melitensis Brucella* infection in sheep is most commonly encountered in countries around the Mediterranean sea and in the Middle East, Central Asia

and parts of South America. The economic impact of brucellosis results from abortions, infertility, drop in milk yield, veterinary care and the cost of replacing infected animals. In addition, brucellosis is an important public health concern in the Sudan, brucellosis in sheep was first reported in 1990 and the brucellosis epidemiology in livestock has been reviewed in different parts of the country (Gamaa*et.al*,2014).

1.1Causative agent:

Brucellosis is caused by Gram-negative bacilli of the genus Brucella. The genus encompasses 10 recognized species including three species that are of major public health and economic importance These are *B. melitensis* which predominantly infects sheep and goats, *B. abortus* which affects cattle, and *B. suis*, which affects swine (Krishna *et.al*,2016).

Sheep brucellosis can be divided into classical brucellosis and ram epididymitis. Ram epididymitis is caused by non-zoonotic agent *B. ovis*, while classical brucellosis is caused by *B. melitensis* and remains a major public health threat equal to goat brucellosis(Acha and Szyfres, 2003).

1.2 Clinical signs:

1.2.1Clinical signs in ewes: The main clinical manifestations of brucellosis in sheep and goats are, as in all female ruminants, reproductive failure, i.e. abortion and birth of weak offspring. Abortion generally occurs during the last 2 months of pregnancy and is followed in some cases by retention of fotal membranes.

1.2.2Clinical signs in rams:

In the male, localization in the testis, epididymis and accessory sex organs is common, and bacteria may be shed in the semen. This may result in acute orchitis and epididymitis and later in infertility. Arthritis is also observed occasionally in both sexes (EC, 2001) .*Brucella ovis*, a gram negative coccobacillus infecting domestic sheep, occurs in most sheep-raising areas of the world. In domestic sheep, *B. ovis* produces male reproductive tract lesions including epididymitis, testicular atrophy, adhesions of the tunica vaginalis, and decrease in semen quality. Infection in ewe is less severe, causing occasional abortions and increased perinatal mortality; however, infection rarely extends from pregnancy to the next natural transmission in domestic sheep primarily results from homosexual behavior between rams and passive venereal transmission from ram to ram via infected semen in the ewe's vagina.Mating by infected rams causes a low percentage of ewes to become infected and although infected females shed *B. ovis* vaginally, they have not been shown to transmit to naïve males Lambs born to infected dams of 10 have maternal antibody but are rarely infected among wildlife. Natural infection with *B. ovis* resulting in lowered semen quality has been detected in red deer New Zealand (Matt *et.al*,2013).

In New South Wales OB occurs in all districts, in any sheep breed and causes considerable economic loss in many flocks through ram wastage, low lamb - marking percentages and extended lambing periods. Infection causes inflammation of the male reproductive organs, in particular the epididymis in rams, resulting in infertility and sterility in some affected rams. In some flocks over 50 per cent of rams are affected. OB has occasionally been associated with abortion in ewes, and increased perinatal mortality .OB infection can be overlooked in a ram mob because infected rams generally do not show any

signs of ill health. Abnormalities will only be detected by scrotal palpation of a group of rams. Suspicion of infection can be confirmed with blood testing done by veterinarians there is no vaccine or other preventive treatment available and infected rams cannot be cured. Eradication of the disease from infected flocks requires identification of infected rams and culling them the first clue may be a reduction in lambing percentages, or a longer lambing period, depending on how reproduction is managed in the flock. Ram libido is not affected. There may also be other significant factors causing lamb mortality. The picture on each infected farm will be different OB should be suspected in any case where lambing performance is below par, and can be easily investigated with an examination of the ram mob. Scrotal palpation is a good screening test and can be followed up with blood testing if abnormalities are found. (Samantha, 2013).

Ovine epididymitis is clinical or sub clinical disease, where *Brucella ovis* is a causative agent. This disease negatively influences reproduction in sheep breeding. The course of the disease is mostly chronic and is manifested by changes in the genital organs in rams (epididymitis, orchitis) and by inflammation of the placenta in ewes (Placentitis) .Ewes may also abort. The disease can result in reduced fertility of rams and in increased perinatal mortality. Asymptomatic course of the disease is frequent as well. It is not transmissible to people. Infection is most frequently transmitted by contact of infected rams with healthy ones or during sexual intercourse. The main source of infection is an infected rams that spreads *Brucella ovis* with semen. Brucella penetrates to the animal mainly orally and through genitalia. The pathogen causes inflammatory necrotic changes in sexual organs, chiefly in the epididymis. The infection may have a latent course as well. Infection is spread in flocks with higher number of rams in different age categories. Young rams more sensitive at the time of sexual maturation additionally, ewes fertilised by infected rams help to spread the infection. Sheep are in hazard only during one

mating period, they recover after finishing it. The presence of genital changes (epididymitis occurring at one side and occasionally at both sides), diagnosed by palpation, can indicate *Brucella ovis* infection in a herd. Clinical examination discloses only about50%of rams infected by *Brucellaovis* (DANkA*etal*, 2007).Clinical diagnostics is also non-specific, because clinical epididymitis can be caused also by other microorganisms*: Actinobacillusseminis, Histophilusovis, Haemophilu spp., Corynebacterium pseudo- tuberculosis, Brucellamelitensis, and Chlamydia psittaci*. The disease is rarely clinically manifested by swelling, painfulness and increased temperature of the reproductive organs, especially the epididymis. The formation of cysts and various adhesions, and gravidity disorders with following complications in ewes are observed during a chronic course. The following clinical signs have been exceptionally found at auction markets of breeding rams: asymmetry of the scrotum palpable thickening of the deferent ducts and enlargement of the epididymis head, but mainly the epididymis tail with a size of walnut up to the size of chicken egg frequently, only one epididymis is affected .The testis in this case is enlarged or, in the case of excessive enlargement of the epididymis, atrophied. Latent course of the infection without clinical signs is the most frequent at present (DANKA *et.al*,2007).

Passive venereal transmission via the ewe appears to be a frequent route of infection but ram-to-ram transmission is also common infected ewes may excrete *B. ovis* in vaginal discharges and milk and, accordingly, ewe-to-ram and lactating ewe-to-lamb transmission could also be determinant mechanisms of infection. Accordingly, the ewes are as relevant as rams in the epidemiology of infection, and control or eradication of *B.ovis* is feasible only if females are included in the corresponding program me. (N;B adopted byOIE, 2009).

1.3 Economic importance of brucellosis:

Brucellosis causes severe economic losses as result of stormy abortions or reproductive failure, sterility and reduced milk production rates, besides to that, it adds to the burden shouldered by the farmers; the costs of control and management. Also brucellosis of animals reduces the Foreign Exchange Earnings (FEE) by denying exportation of sheep to international markets. (Ahmed *et.al*,2015).

Food and Agriculture Organization of the United Nations (FAO) and the Organization of Animal Health (OIE) consider the importance of this disease Brucellosis has not only direct public health implications but also poses a Potential barrier to international trade of animals and animal products. Such a barrier could seriously impair socio-economic development, especially in rural populations (WHO, 1997). The disease has a considerable impact on animal and human health , as well as socio economic impacts, especially in areas where the rural Income depends on live stockbreeding and dairy products. It is one of the most serious diseases in developing countries. The rates of infection vary greatly from one country to another and between regions within a country the economic loss from brucellosis in developed countries arises from the slaughter of cattle herds that are infected with brucellosis and all the cost of eradication and control program. Farmers in developing countries suffer from the actual abortion of cows and the decreased in milk yield, birth of weak calves that die soon after birth, retention of placenta, impaired fertility and sometimes arthritis or bursitis and all the cost of tests and samples. Death may occur as a result of acute metritis (Radostits *et. al*, 2000) .The loss in a developing country is due to prophylactic activities, control and eradication program, hospitalization of human patients, cost of research , loss of work or income and failure in financial investment (Chukwu ,1987). Also the

restriction of international trade in animals and their products constitute a major economic loss (Corbel,1973).The main point in quantification of the financial effects of animal diseases is to make decision to know the best way of disease control measures based on costs and benefits (Chilonda and Huylenbroeck,2001). The quantification of the losses due to individual animal diseases depends on the disease investigation work undertaken. Once the actual disease prevalence, the nature and magnitude of the losses tested in infected herds at the regional and national levels have been defined, the economic portion of the analysis can be accomplished by:

1- Organize and classify the information on disease losses.

2-Quantify the losses, choosing prices that reflect the economical of the analysis being undertaken. Depending on the information available the estimation of the annual level of losses associated with the disease can be made by estimating the value of the animal and the effect of disease on the final output .

3-Attempt to quantify the indirect losses attributable to a disease (Put *et.al*, 1988).To estimate the financial loss caused by brucellosis, it depends mainly on the type of cattle farming, herd size, and loss in reproduction in meat and milk due to abortion .The infected non aborting dairy cows produce 10% below potential and aborting 20% (Crawford *et.al*,1978). The percentage of abortion in infected cows annually is 10-35% (Shepherd *et.al*,1979).

The disease causes heavy economic losses in small animal production resulting from abortions, abortion rated up to 50% in sheep and goats have been reported by Nicolette (1982).Sterility, decreased milk production, the costs of replacer animals and the effect of the disease on ram fertility can influence the number of rams required in a flock. Lambing percentage is reduced by30% in flock recently infected and by 15-20% in

endemic infection (Arizaet.al,1992 and Radostitet.al,2000). In addition the disease is an impediment to free animal movement and export.

1.4 Mode of transmission:

Brucella spp. are usually transmitted between animals by direct contact with the placenta, fetus, fetal fluids and vaginal discharges from 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 sub sequent pregnancies. Entry of Brucella into the animal body occurs by ingestion and/or through the mucous membranes of animal(broken skin and possibly intact skin). (Quinn *et. al,*1994). In vertical transmission *Brucella abortus* infection in cattle and, *Brucella melitensis* can be transmitted from the mother to their newborns or kids. The majority of infections are probably acquired by consumption of colostrum or milk. These newborns orkids may have infections in the lymph nodes draining the gastro-intestinal tract and may shed these organisms for long periods or lifelong. The importance of venereal transmission varies with the species and is apparently important only in swine *Brucellasuis* and *Brucellaovis* infection in sheep. Sharing of breeding stock males between farms seem to promote transfer of infection between farms(Alton,1985;Mikolon *et.al,*1998) . The infected bulls used in natural service do not play a major rule in spreading the infection However in artificial insemination because the infected semen is ejaculated in-to the uterus it may spread the disease (Bendixin and Blood,1947).Brucella species have also been detected in other secretions and excretions including urine, feces, hygroma fluids, saliva, and nasal and ocular secretions. In most cases, these sources seem to be relatively unimportant in transmission . Brucella can be spread on fomites including feed and water in conditions of high humidity, low

temperatures, and no sunlight. Wild carnivores and dogs present special risk to intensively managed livestock and their human owners as they carry the aborted material to clean areas (Nicoletti,1980). Human to human transmission is rare, but has been reported examples of human-to -human transmissions by tissue transplantation or sexual contact are occasionally reported but are insignificant (Mantur*et.al*,1996). Common sources of infection for people include contact with animal abortion products ingestion of unpasteurized dairy products from cows, small ruminants or camels, or other uncooked meat products. Also contact with laboratory and tissue samples or cultures can aid in the transmission (Schnurrenberger*et.al*,1975Stableforth and Galloway 1959).

1.5Survival of Brucella species:

Brucella species are intracellular organism. The external membrane component of Brucella, which is lipopolysaccharide (LPS), has a unique structure that afford it with a very low endotoxicity, hence resist the host immune response and confers resistance to antimicrobial activity and acts as virulence factor for survival and intracellular replication (Lapaque*et.al*,2005).In the environment the ability of Brucella to persist outside mammalian hosts is relatively high compared with most other non-sporing pathogenic bacteria, under suitable conditions. Brucella can survive drying, particularly when organic material is present, and survive in dust and soil. Survival is longer when the temperature is low. Brucella is sensitive to direct sunlight disinfectants and pasteurization. The organism is killed by pasteurization or complete exposure to Ultra violet (UV) or Gamma rays (King,1951) Environment is not considered an important source of infection(Wray,1975)

1.6 Risk factors for transmission:

The factors affecting the transmission of Brucella could be classified to tow:

1-Factors affecting transmission between herd like movement of the animal from an area to another so the disease can spread from infected herd to non-infected herd.(Radostits*et.al*,1994). Also sharing the same pastures is a way of infection where infected animals mix with uninfected ones or get in touch with contaminated premises or manure. Introducing new infected animal to uninfected herd can also aid in the transmission of disease.

2- Factors affecting transmission within the herd and these include density of animal populations, the herd size, the type and breed of animal (dairy or beef) the type of husbandry system and other environmental factors are thought to be important determinants of the infection (Salman and Meyer,1984).

The herd level important risk factors for small ruminants brucellosis identified are large flock size addition of new animals from unscreened sources, intensive system of management, history of abortion in grazing communal pasture and keeping sheep and goat together (Kabagambe*et.al*2001).

1.7Pathogenesis of brucellosis :

Infection by Brucella varies and affected by the size of the infective dose and virulence of the bacteria. A fully virulent Brucella are highly invasive and capable of penetrating the mucosa of the nose, throat, conjunctiva urogenital tract, teat canal, and abraded skin.(Davis *et.al*,1990). The resistance of the animal varies according to age, sex and the reproductive status of the animal (Nicoletti,1980). The normal route of infection is through the oral route by licking aborted fetus, infected placentas and vaginal discharges or by ingestion

of contaminated feed and water. The bacteria enter the body through penetration of the mucous membranes of the alimentary tract, survive and multiply in cells of the mononuclear phagocytic system (Herr1994, Godfroid *et.al*,2004a). After penetration, the organisms are phago-cytosed by the neutrophils and macrophages and carried to the regional lymph nodes ,the organisms multiplies leading to lymph-adenitis and these may be followed by bacteraemia (Radostits*et.al*,1994,Godfroid *et.al*,2004b).

During bacteraemia the organisms are carried intra cellularlly or free in the plasma then will be localized in organs like pregnant uterus. In acute cases, up to 85% of the bacteria are in cotyledons, placental membranes, and allantoic fluid, udder, supramammary lymph node and the spleen (Radostits*et.al*,2000). In non-pregnant cows the organisms localized in the udder and in cases where the animal becomes pregnant bacteremic phases occur in the udder. Infected udders are clinically normal but they are important as a source of infection of the uterus and also a source of infection to calves and humans by drinking the infected milk (Corbel, 2006). In male the testes and male accessory sex glands are infected (Godfroid *et.al*, 2004a).Abortion is typically the first clinical signs of the pregnant female, and orchitis and epididymitis are typical clinical signs of the male(Corbel and Macmillan,1998).Infection by *Brucellamelitensis* in sheep and goats resembles infection by *Brucella abortus* in cattle however, the udder is an important predilection site for *Brucella melitensis* .Greatly reduced milk yield follows abortion, and infection of the udder following a normal birth also leads to a considerable reduction in yield. In spite of this, clinical signs of mastitis are seldom detectable in naturally infected goats as well sheep that abort often excrete the bacteria in the milk (Alton,1990).

1.8 Epidemiology of brucellosis:

1.8.1 Geographic Distribution:

The global distribution of the disease worldwide, the distribution of the different species of Brucella and their biovars varies with geographic areas. *B.abortus* is the most widespread; *B. melitensis*and *B.suis*are irregularly distributed; *B.neotomae*was isolated from desert rats (Neotomaelepida) in Utah (USA), and its distribution is limited to natural foci, as the infection has never been confirmed in man or domestic animals. Infection by *B. canis* has been confirmed in many countries on several continents and its worldwide distribution can be asserted. *B. ovis* seems to be found in all countries where sheep raising is an important activity (PAHO, 2001). It is well established in the Middle-East and that it affects both cattle (*B.abortus*) and small ruminants (*B. melitensis*) (WHO/MZCP,1998).

Sheep and goats and their products remain the main source of infection, but *B.melitensis* in cattle has emerged as an important problem in some southern European countries, Israel, Kuwait, and Saudi Arabia.

1.9The distribution of the disease in the Sudan:

Brucellosis caused by *B. abortus* was first reported in the Sudan a dairy farm in Khartoum. The prevalence of the disease was 160 (80%) of 200 Friesian and 49 (38%) of 130 local zebu cattle(Bennent,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)above25% according to the criteria of Thimm and Wundit (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 tow 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 (Musa *et.al*,2008)

1.10 Diagnosis of Sheep Brucellosis:

The clinical signs of the disease are not pathognomonic, although the herd history may be helpful and hence, laboratory diagnosis is required for identification and elimination of infected animals . There is no single test by which a bacterium can be identified as Brucella. A combination of growth characteristics, serological, a bacteriological and/or molecular method is usually needed, (OIE, 2009). However, the most accurate diagnosis of the disease can be made only by the isolation and identification of Brucella from abortion material. Uddersecretionor from tissues removed at postmortem(OIE,2009).But in situations where bacteriological examination is not practicable, diagnosis of Brucella infection must based on serological methods (Alton *et. al*,1988).Diagnosis and control of the disease in animals must be carried out on a herd basis there may be a very long incubation period in some infected animals andindividuals 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. Diagnostic tests fall into two categories: those that demonstrate the presence of the organisms and those that detect an immune

response to its antigens. The isolation of Brucella is definitive proof that the animal is infected, but not all infected animals give a positive culture and the methods and facilities that must be employed are not always readily available. The detection of antibody or a hyper sensitivity reaction provides only a provisional diagnosis, but in practice is the most feasible and economic means of diagnosis. False positive reactions to serological tests can occur through a number of factors, including vaccination, and this must be borne in mind when interpreting results. Similarly, dermal hypersensitivity only indicates previous exposure to the organism, not necessarily active infection, and may also result from vaccination (Corbel,2006).Abortion of an infective nature may be suspected on the basis of history and clinical examination, especially when several ewes are involved. However only bacteriological and serological tests may confirm the presence of *B.melitensis* (Aitken,2007). The existence of clinical lesions unilateral or occasionally, bilateral (epididymitis) in rams may be indicative of the existence of infection (OIE,2009c). Positive blood culture soon after the infection occurs, or isolation of the organism from the aborted fetus, vaginal mucus, or milk, is the common laboratory procedures used in diagnosis. The organism is moderately acid-fast and staining smears from the placenta and fetus with a modified Ziehl-Neelsen method may give a tentative diagnosis; however this does not distinguish this infection from *B.ovis* or the agent of enzootic abortion and culture is required The rose Bengal test has excellent specificity and high sensitivity, is easy to perform, and is suitable for herd and flock testing (Radostits, 2006). CFT and a gar gel immune diffusion (AGID) test can be used (OIE,2009c). The organism can be detected by PCR in the abomasal fluid of aborted fetuses and, compared with culture; PCR has a sensitivity and specificity of 97.4% and 100%, respectively PCR can also be used to detect the

organism in semen (Radostits,2006). The SAT must be used only as a screening test and, in cases in which a low titer is found additional methods are necessary (Aitken, 2007). Other tests that have been developed include ELISAtests,radialimmunodiffusion,andcounterimmunoelectrophoresis; the sensitivity and specificity of these appears to vary between laboratories. An ELISA test using purified antigen is described as being able to differentiate the seropositivity of *B. melitensis* from that of *B.ovis*. (Radostits,2006).

1.10.1 Stained Smears:

Smears of placental cotyledon, vaginal discharges or fetal stomach contentsmay be stained using modified Ziehl-Neelsens (Stamp) or Kosters' methods. This is the usual procedure for the examination of smears of organs or biological fluids that have been previously fixed with heat or ethanol. The presence of large aggregates of intracellular, weakly acid-fast organisms with Brucella morphology is presumptive evidence of brucellosis. Care must be taken as other infectious agents such as*(Coxiellaburnetii or Chlamydia psittaci)* may superficially resemble Brucella (Corbel, 2006 and OIE, 2009b).

1.10.2 Culture

Brucella may most readily be isolated in the period following an infected abortion or parturition, but isolation can also be attempted post-mortem. Brucella are 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. It is of the most importance that fecal andenvironmental contamination of the material is kept to a minimum to give thegreatest chance of successfully isolating Brucella. If other material is unavailable orgrossly contaminated,

the contents of the fetal stomach will usually be otherwise sterile and are an excellent source of Brucella some circumstances it may be appropriate to attempt the isolation of Brucella at post-mortem. Suitable material includes supramammary, internal iliac and retropharyngeal lymph nodes, udder tissue, testes and gravid uterus. Milk samples should be allowed to stand overnight at 4 °C before lightly centrifuging. The cream and the deposit are spread on to the surface of at least three plates of solid selective medium. Placental samples should be prepared in the field by selecting the least contaminated portion and cutting off pieces of cotyledon. In the laboratory, the portions should be immersed in alcohol which should be flamed off before cutting with scissors or scalpel and smearing the cut surface on three plates of selective medium. Other solid tissues can be treated in a similar manner, or, ideally, they should be macerated mechanically following flaming before plating out. The tissues may be ground manually or homogenized in a blender or stomacher with opening the abdomen, by searing the surface of the stomach with a hot spatula andaspirating the liquid contents with a Pasteur pipette or syringe (Corbel, 2006).

1.10.3 Serological methods:

Serological tests can be divided broadly into two groups Screening tests: used in the field clinics or in regional laboratories, such as the Rose Bengal or buffered plate agglutination. The Rose Bengal test has a very high sensitivity to ensure that infected animals are not missed. . Indirect ELISA tests are also being used to screen milk and serum Confirmatory tests: used in a central or regional laboratory, such as competitive ELISA, immune diffusion or complement fixation tests. They are very useful indistinguishing vaccinal antibody responses from those induced by field infection.(FAO, 2003).

Serological results must be interpreted against the background of disease incidence, use of vaccination and the occurrence of false positive reactions due to infection with other organisms (Corbel, 2006).

1.10.3.1Rose Bengal plate test (RBPT) (Buffered Plate Antigen or Card Test):

The RBPT is one of a group of tests known as the buffered Brucella antigen tests which rely on the principle that the ability of IgM antibodies to bind to antigen is markedly reduced at a low ph. such tests play a major role in the serological diagnosis of brucellosis worldwide (Aitken, 2007).

1.10.3.2 Serum Agglutination Test (SAT)

The SAT has been used extensively for brucellosis diagnosis , because it'ssimple and cheap to perform, its lack of sensitivity and specificity mean that it should only be used in the absence of alternative techniques.SAT is generallyregarded as being unsatisfactory for the purposes of international trade (OIE.,2009b).

1.10.3.3Complement Fixation Test) (CFT): The sensitivity and specificity of the CFT is good, but it is a complex method to perform requiring good laboratory facilities and trained staff. If these are available and the test is carried out regularly and in a professional manner with good attention to quality assurance, then it can be very satisfactory. (Corbel, 2006). CFT is diagnostically more specific than the SAT, and also has a standardized system of unit age (OIE 2009). CFT remains the prescribed test for international trade because of the lack of standardized methods recognized at the international level for I-ELISA and Agar Gelimmune Diffusion (AGID) (OIE, 2009c)

1.10.3.4ELISA Tests: Two main types immune sorbent assay have been used: the indirect and competitive formats:

1.10. 3.4.1Indirect ELISA (I.ELISA):

A useful test during an eradication program, after vaccination the ELISAs are more sensitivethan the RBPT or CFT. (Corbel, 2006).it has ceased, for screening or as a supplementary test to CFT. The sensitivity and specificity of indirect ELISA has been excellent but it could not distinguish between the antibody response induced by vaccination with *B.abrtus* strain19 and natural infection with the organism.

1.10.3.4.2Competitive ELISA (C. ELISA):

Can differentiate between the induced antibody responses. An improved competitive enzyme immunoassay (ELISA) has a sensitivity of 100% and specificity of 99.7% beside that is considered a reasonable alternative as a single assay for serological diagnosis of brucellosis. Has ability to detect residual anti-*B.abortus* strain 19 anti-bodies in adult cows vaccinated with strain 19 vaccine between 3 and 8 months of age (Radostits .,2006). The ELISAs are more sensitive than the RBPT or CFT. (Corbel, 2006).

1.10.4 Supplementary Tests: Many other serological tests have been employed. Some, such as the Rivanol or 2-ME test, are variations of the SAT and, although more specific, share many of its disadvantages. At present, the use of such procedures in the place of the standard test is not advised (Corbel, 2006).

1.10.4.1 Fluorescence Polarization Assay) (FPA):

This test can be done outside the diagnostic laboratory, allowing for rapid and accurate diagnosis. The FPA technology has been developed and

validated for the serological diagnosis of brucellosis in cattle, pigs, sheep, goats and bison.

1.10.5Molecular Methods:

1.10.5.1 Polymerase Chain Reaction (PCR):

The PCR-based assays for Brucella have been developed and are simple The PCR has been applied to tissues such as aborted fetuses and associated maternal tissues, blood , nasal secretions, semen, and food products such as milk and soft cheeses. The detection of Brucella DNA from aborted bovine fetuses by PCR has been compared with microbiological techniques and the estimated concordance calculated by Kappa index was 0.73 which is considered sat is factory Brucella spp. Can be detected in the milk of naturally infected cattle, sheep, goats and camels using a PCR assay which is more sensitive than the culture method(Radostits e*t.al*,2006) .

1.11 Treatment of Brucellosis:

In vitro nearly all Brucella strains are sensitive to gentamicin tetracycline and rifampin (Corbel and Brinley,1984),but in fact because of the intracellular characteristics of Brucella which determine the chronic course of the disease and its tendency to relapse , antibiotic treatment of known infected animals, or of those which are potentially exposed to them has not been commonly practiced. Treatment should be ruled out as an option in the control of brucellosis and according to Corbel (2006) treatment has been used in animals of special breeding value, but because of the uncertain outcome it is not generally recommended. However, the course of the disease may be modified by tetracycline alone or in combination with streptomycin. A limited number of studies have shown rapid reductions in the incidence of brucellosis when the herd or flock was treated but this procedure is considered to be

restricted in practice. According to (Radwan, *et.al*,1987) a long term treatment with a high dose of ox tetracycline (1000mg/day for6weeks, I/P) had completely eliminated *Brucella melitensis* from naturally infected sheep.

1.12Prevention, Control of Brucellosis:

1.12.1 Prevention:

It is nearly always more economical and practical to prevent diseases than to attempt to control or eliminate them. For Brucellosis, the 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 test are necessary unless there placements 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 aperiodic milk ring tests in cattle (at least four times per year), and test slaughtered animals with simple screening serological procedures such as the RBPT

6- Proper disposal (burial or burning) of placentas and non-viable fetuses Disinfection of contaminated areas should be performed thoroughly. (Corbel, 2006).

1.12.2Control: Effective controls must be based on minimizing the infection by improve the sanitary methods; control the factors that help in the spread of the disease, and a vaccination program (Fensterbank,1976; Nicoletti and Milward,1985).

1.12.2.1 Test and Isolation/Slaughter:

There are no path gnomonic signs of brucellosis in animals at individual level; the occurrence of abortion storms in naive herds/flocks is usually a strong indicator of infection. Therefore, serological (and sometimes allergic) tests are the usual method of identifying possible infected animals. Bacteriological procedures are useful for confirming test results and for epidemiological studies. The decision about slaughter of test-positive animals is made after regulatory, economic and prevalence factors are considered.

1.12.2.2Hygiene:

The goal in the application of hygiene methods to the control of brucellosis is reduction of exposure of susceptible animals to those that are infected, or to their discharges and tissues. This is a classical procedure in disease control.

1.12.2 .3 Control of Animal Movement (Quarantine):

This is a period of time during which cattle movement is restricted and the cattle are tested. This will prevent inter herd transmission by infected cattle, especially those that are test-negative and incubating the disease. The quarantine period should be sufficiently long that all cattle have had sufficient time to develop brucellosis and insure that the remaining cattle will not be a source for

inter herd transmission. The time will usually range from 120 days to 1 year, or until all breeding animals have completed a gestation without test evidence of infection (Radostits, 2006).Control of animal movement may be regarded as an aspect of hygiene. However, it is essential in any program me to limit the spread of brucellosis. Animals should be individually identified by brand, tattoo or ear tag. Unauthorized sale or movement of animals from an infected area to other areas should be forbidden. Similarly, importations into clean areas must be restricted to animals that originate from brucellosis-free areas, that have a herd/flock history of freedom from the disease and that have given negative reactions to recently performed diagnostic tests. In practice, it is much more difficult to control the movement of camels and small ruminants kept under nomadic or semi-nomadic conditions than that of beef or dairy cattle kept under intensive conditions. The owners of herds and flocks may be accustomed to seasonal migrations which may cross national boundaries (Corbel, 2006).

1.12.2.4 Vaccination:

There is general agreement that the most successful method for prevention and control of brucellosis in animals is through vaccination. While the ideal vaccine does not exist, the attenuated strains of *B. melitensis* strain Rev.1 for sheep and goats and *B.abortus* strain 19 have proven to be superior to all others. The non-agglutinogenic *B.abortus* strain RB51 has been used in the USA and some Latin American countries, with encouraging results. The source and quality of the vaccines are critical. The dosages and methods of administration, especially with Rev.1, vary and these can affect the results. Consequently, whole herd or flock vaccination can only be recommended when all other control measures have failed. When applied, the vaccinated animals must be identified by indelible marking and continually monitored for abortions resulting from the vaccine. Positive serological reactors and secretors must be

removed from the herd on detection. Vaccination of animals usually results in elimination of clinical disease and the reduction in numbers of organisms excreted by animals which become infected. Furthermore, animal owners are more likely to accept vaccination as a method of control since they are accustomed to this form of disease control. In many countries, vaccination is the only practical and economical means of control of animal brucellosis (Corbel, 2006).

1.12.2.4.1*Brucellaabortus* strain 19 vaccines:

B.abortus strain 19 vaccine has been most widely used to prevent bovine brucellosis. The vaccine protects uninfected animals living in a contaminated environment, enabling infected animals to be disposed off gradually. This overcomes the main disadvantage of the test and disposal method of eradication, in which infected animals must be discarded immediately to avoid spread of infection. Strain19*B.abortus* has a low virulence and is incapable of causing abortion except in a proportion of cows vaccinated in late pregnancy, although it can cause undulant fever in humans. Its two other weaknesses are its failure to completely prevent infection, especially infection of the udder, and the persistence of vaccinal titers in some animals. The optimum age for vaccination is between 4 and 8 months and there is no some animals. The optimum age for vaccination is between 4 and 8 months and there is no significant difference between the immunity conferred at 4 and at 8 months of age .In calves vaccinated between these ages the serum agglutination test returns to negative by the time the animals are of breeding age, except in a small percentage (6%) of cases. The lipopolysaccharide with an O-chain on*B. abortus* strain19 explains the appearance and persistence of antibodies in serum following vaccination. These antibodies are detectable in the serological assays used for diagnosis of brucellosis and are the major problem with strain 19 vaccination, since they

prevent easy differentiation of vaccinated from infected cattle. Theappearance and persistence of these antibodies depends on age, dose, and route of vaccination. This situation makes the continued use of the vaccine incompatible with simultaneous application of test and slaughter procedures for the control of brucellosis. In most control programs, vaccination is usually permitted up to 12 months of age, but the proportion of persistent post vicinal serum and whey reactions increases with increasing age of the vaccinates. Such persistent reactors may have to be culled in an eradication program unless the reaction can be proved to be the result of vaccination and not due to virulent infection. Vaccination of adultcattle is usually not permitted if an eradication program is contemplated but it may be of value in reducing the effects of an abortion 'storm'. Vaccination of bulls is of no value in protecting them against infection and has resulted in the development of orchitis and the presence of*B. abortus*strain 19 in the semen. For these reasons the vaccination of bulls is discouraged (Radostits, 2006). The main objective of systematic and mandatory vaccination of calves in a given area or country is to reduce the infection rate and obtain herds resistant to brucellosis, so that eradication of the disease may then begin. It is estimated that 7to 10 years of systematic vaccination are necessary to achieve this objective. The recommended dose is one to three billion cells of strain 19 Brucella administered subcutaneously (PAHO, 2001).

1.12.2.4.2 *Brucella melitensis* Rev. 1 vaccine:

The live attenuated *B. melitensis* Rev.1 strain is presently recognized as the best available vaccine for the prophylaxis of brucellosis in sheep and goats.

Numerous independent field and controlled experiments confirm its value for this Purpose Moreover, “correctly standardized Elberg 101 strain Rev.1 vaccine should continue to be considered as the basis of brucellosis control in small ruminants where vaccination is applied, until new safer and effective versions of

B. abortus and*B.melitensis* vaccines, based on rough strains, are tested under controlled experimental and field conditions and shown to be at least equivalent to the Rev.1 vaccine." (WHO, 1997). The ability of the vaccine (Rev.1 strain) to produce a high level of immunity against both artificial and natural challenge has been convincingly demonstrated both for sheep and goats (Alton, 1990). It has been well established that a large proportion of vaccinated animals is protected against infection (Elberg,1959, quoted by Garrido,1992), and in those vaccinated animals where infection occurred, it is often transitory. Hence, the period of Brucella excretion from the udder or vagina is shorter, the degree of microbial contamination of the surroundings is reduced and, consequently, disease transmission within and between herds is significantly reduced (Garrido, 1992).As with all highly-contagious diseases, the effect of vaccination increases the greater the coverage of the animal population. Erratic administration of vaccines or their use without adequate quality control is not effective. Adequate protection is only possible if the vaccine quality is good and if the vaccines are administered to at least 80 % of the animals at risk (Garrido,1992).The duration of immunity conferred by vaccination with Rev.1 was investigated by vaccinating Maltese goats when they were 4 to 12 months of age and challenging some at 2 ½ years (Alton, 1966) and others at 4 ½ years (Alton, 1988) after vaccination. Those challenged at 4 ½ years were as resistant as thosechallenged at shorter intervals after vaccination, and it was concluded that immunity could be considered lifelong. Similar results were observed in sheep in Iran challenged 2 ½ years after vaccination (Biggi, 1956 and Alton, 1990). More recent work has demonstrated the efficacy of Rev.1 vaccine in sheepeither vaccinated as lambs (CJ or SC route) or challenged 9-10 months

(Fensterbank*et.al*,1985) or 7.5- 15.5 months later, respectively (Verger etal.1995). Or vaccinated as adults (CJ route) and challenged 2 ½ years after (Durán- Ferrer, 1998). Likewise, good results of protection were obtained when

young goats were vaccinated at 4months of age (CJ or SC route) and challenged 8.5-12.5 months after (Fensterbank,1987).Used exhaustively in whole flock vaccination programmes, the live *B. melitensis* Rev.1 vaccine greatly decreases the prevalence of brucellosis in both sheep and human population (Elberg, 1981b, 1996). Once the prevalence has been diminished, a more efficient control of the disease may be achieved through the implementation of a programme based on Rev.1 vaccination of lambs combinedwith the test-and-slaughter of adults. Finally, it may be possible to use a test-and-slaughter programme only (Garin-Bastuji*et. al*,1998)

Chapter Two

Material and Methods

2.1 The study area:

Alkadaru quarantine is located in the north of Khartoum State (bahry) about three km on the east direction of Alkadaru town. Located within free area of animal disease. It is located in semi desert zoon between latitude (15.8-16.69) north, longitude (31.36-34.25) east. The total area of Alkadaru quarantine is 100fadden. Since it was established in1973and till now a administrates initial health quarantine processes for exported animals.

It is considered the main medium quarantine for exporting different types of animals abroad through form trucks to the final quarantine.

Alkadaru quarantine provided a distinguish animal health care for exported animals by applying OIE [Office international des epizooties] criteria.

2.2Target populations:

The study population was sheep selected from herds which were prepared for export from the Alkadaru quarantine.

2.3Study type: Across sectional study was conducted to estimate the prevalence and risk factors of ovine brucellosis in export sheep.

2.4Sampling methods:

The sampling method used in this study was cluster sampling.

2.5 Sample the Size:

The study was conducted in Alkadaru quarantine, and the information was collected form (500) sheep samples, 300 sheep samples from North Kardofan State and 200 sheep samples form Darfur State. They were divided into 10 herds of different age groups namely; one herd less than 2 year, six herds between 2-3 years and the other three herds were more than 3 years. (Table 1).

Table (1) Herd, breed and age groups for examined sheep for brucellosis.

Area	Number of herd	Breed	Age	Number of samples
North kardofan	7	Hamari	≤ 2year 2-3 years ≥3years	0 100 100
		Kabshi	≤ 2year 2-3 years ≥ 3years	100 0 0
Darfur	3	Zaghawi	≤ 2year 2-3 years ≥ 3years	0 100 100

2.6Questionnaire execution:

A questionnaire was used for collection of data about the examined sheep**,** it included the following questions:

Sheep owners: occupation, education and knowledge about brucellosis.

Sheep: breed, age, and type of breeding.

Farms: flock size, type and origin of sheep.

General management factors: grazing, source of water, feeding, drinking equipment's, and other animals prevalent in the same farm.

Vaccination against brucellosis: whether applied or not.

The information obtained was used to reveal risk factors with the disease. (Appendix 1)

Samples collection:

Blood samples were taken, cleanly, by venipuncture. From the jugular vein. The skin at the site of venipuncture was shaved and swabbed with 70% alcohol and allowed to dry. 5 ml of blood were been taken by needle and plain vacuum tube. The blood tubes were placed in racks and left to stand at ambient temperature for 1–2hours in slanting position until the clot began to contract. The racks bottles placed in a refrigerator at 4°C. After overnight, sera were decanted or removed with a pipette in eppendorf tubes, labeled and preserved in an ice box which was there transported to laboratory. All sera samples were kept at -20ºCbefore serological tests.

2.8 Serological Tests:

2.8.1 (RBPT Rose Bengal Plate Test):

Brucella colored antigen used in this test was donated by Division of Brucella research in Veterinary Research Institute (VRI) Soba, the test method was done as described by Alton *et. al*, (1988). The antigen and the serum samples were removed from the refrigerator and warmed at 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 anticlockwise. The results were read immediately after 4 minutes. Interpretation of the result was done according to

the degree of agglutination. Definite agglutination was considered as positive reaction. Agglutination appeared as + (weak positive), ++ (positive), +++ (strong positive) or++++ (very strong).

2.8.2Serum 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. The test was done according to Buxton and Fraser (1977), the test was performed as follows:

1- Eight test tubes were placed in raw in a rack for each sample.

2- 0.8 ml of 5% NaCl solution was added to the first tube and 0.5 ml into eachof the remaining seven tubes using 1 ml graduated pipette.

3- 0.2 ml of serum was added to the first tube of each raw mixed well with the5% NaCl by sucking and expelling gently to avoid producing bubbles.

4- 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 soon.

5- Doubling the dilution was continued up to the 8th tube then 0.5 ml from the last tube was discarded.

6- 0.5 ml of the diluted antigen was added to each tube.

7- Control positive tubes containing equal amounts of antigen and known positive serum were included in the test.

8- Control negative tubes containing equal amounts of antigen and knownNegative serum were included in the test.

9- After shaking, the tubes were incubated at 37ºC 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).

2.8.3 Buffered Acidified Plate Antigen (B APA)

The test is prescribed by the OIE(2016a)for international trade, it is a quick easy presumptive test to start with in order exclude to negative samples from further serological testing, it is a secondary binding qualitative plate agglutination test that uses colored acidified antigen (PH 3.8 0.05) to inhibit non-specific reactions due to lgM and enhance the agglutination ability of specific lgG1.

Test steps:

1- The samples and antigen were allowed to come to room temperature.

2- 20, 40 and 80µl of sample were measured and placed on the center of glass plate of the Minnesota testing box .known high positive, controls were included in each day's work.

3- 30µl of BAPA antigen were added to each quantity of serum (the antigen bottle was mixed thoroughly by gentle shaking and inversion to ensure a homogenous suspension).

4- The sample and antigen were mixed thoroughly using stirrer enlarging the circle of the mixture to about 2cm in diameter.

5- The glass plates in a circular motion for 4 rotations and left for 4 minutes in the Minnesotabox.

6- Plates were rotated 4 times again, incubated for another 4minutes in the box and finally rotated 4 further rotations.

The reaction was read immediately against an illuminated back ground of the Minnesota box. Visible agglutination within 8minutes was considered positive .No agglutination within 8minutes was negative.

Result interpretation:

No agglutination (-ve), complete agglutination with very clear fluid (4+ve) nearly complete agglutination with clear fluid (3+ve), marked agglutination with less clear fluid (2+ve), and Slight agglutination with turbid fluid (1+ ve)

Chapter Three

Results

This study was planned to investigate the prevalence of brucellosis in sheep intended for export in Alkadaru quarantine. A total of 500 serum samples were collected from 10 different sheep herds in Alkadaru quarantine. Sheep herds were coming from North kardofan and Darfur. The lambs were divided in to age groups, namely; less than 2 years, between 2-3years and more than 3 years. Lambs were found to belong to Hamari, Kabashi and Zaghawi breeds (table 2)

Table (2): The source, breed and age groupsof positive sampleby(RBPT)screening test.

Source	Breed	number of positive	Remaks
North Kardofan	Hamari	1	2-3 years
		1	≥3 years
Darfur	Zaghawi	1	2-3 years

Form the 500serum samples, 3sample (0.6%) were seropositive for Brucellainfection by the screening test (RBPT) and 1 sample (0.2%) by the serum agglutination test (SAT) and buffered acidified plate antigen test (BAPA). When100seronegative samples by (RBPT) were examined using serum agglutination test and buffered acidified plate antigen test were also confirmed negative. Only 1sample (33.3%) from the 3 sera positive by RBPT was found to be positiveby serum agglutination test and buffered acidified plate antigen test. Only age group was found in association with brucellosis in male sheep (table 3).

Table (3): Chi-square analysis of brucellosis

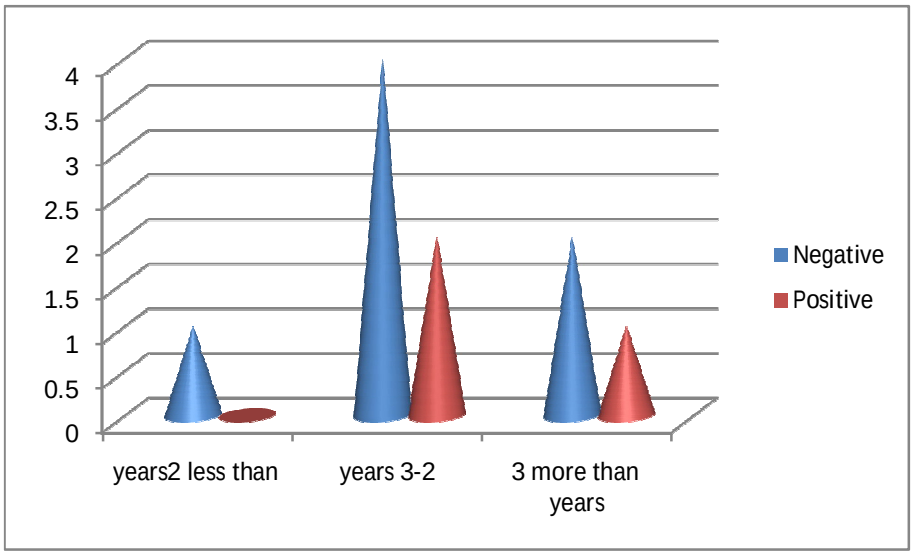
	Number Tested	*Positives cases (%)*	χ^2	*Df*	*P-value*
Region					
Darfur	3	2 (20)	1.0	1	0.30
North Kardofan	7				
Age(years)					
<2	1	2(20)	5.8	2	0.05
2 3	6	1 (10)			
>3	3				
Breed					
Hamary	6	2 (20)	1.6	2	0.43
Zagawi	3	1(10)			
Kabashi	1	_			
Herd size					
Small	4	2 (20)	1.6	1	0.19
Large	6				
Owner Education					
Educated	5	1(10)	0.0	1	1.00
No Educate	5	1(10)			
Owner Awareness					
Aware	6	1(10)	0.1	1	0.74
Not Aware	4	1(10)			

herd risk factorIn Redcolor explains the risk factors whichhave significant associations to Brucellosis at 95% CI.

This study revealed that other risk factors have no a significant association withbrucellosis these included:-

Sixteen risk factors; namely occupation, education for owner, awareness about brucellosis, flock size, type and origin of sheep, general management factors, grazing, source of water, feeding, drinking equipment's, other animals prevalent in the same farm, vaccination against brucellosis and castration.

Distribution of brucellosis among age groups in 10herds divided 1 herd less2 year , 6 herds 2-3 years and 3 herds more 3 years. figure (1).



. **figure (1). Distribution of brucellosis among age groups of sheep ready for export .**

Table (4) Distribution of positive samples according to source of animal

	Darfur	North kardofan
Negative	2	5
Positive	1	2

.

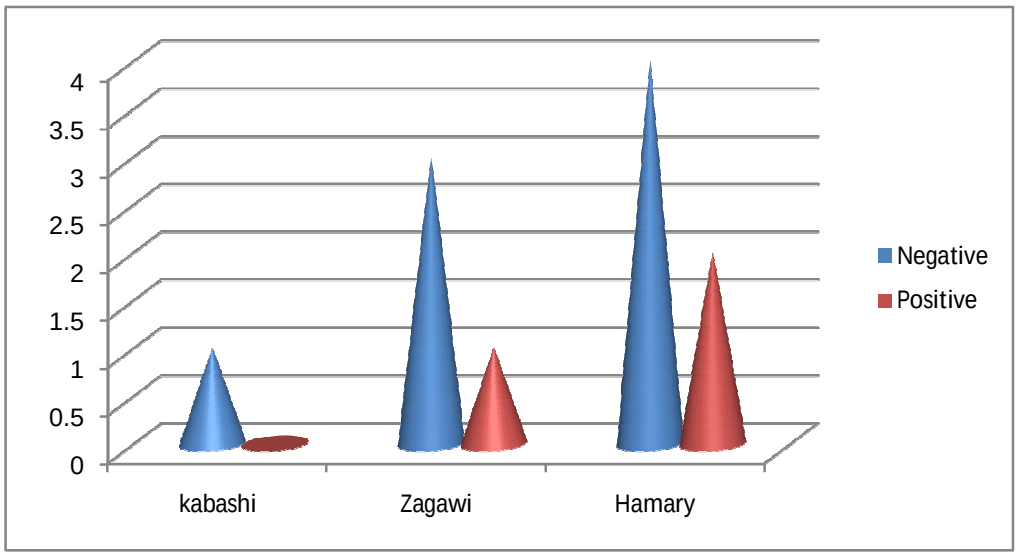
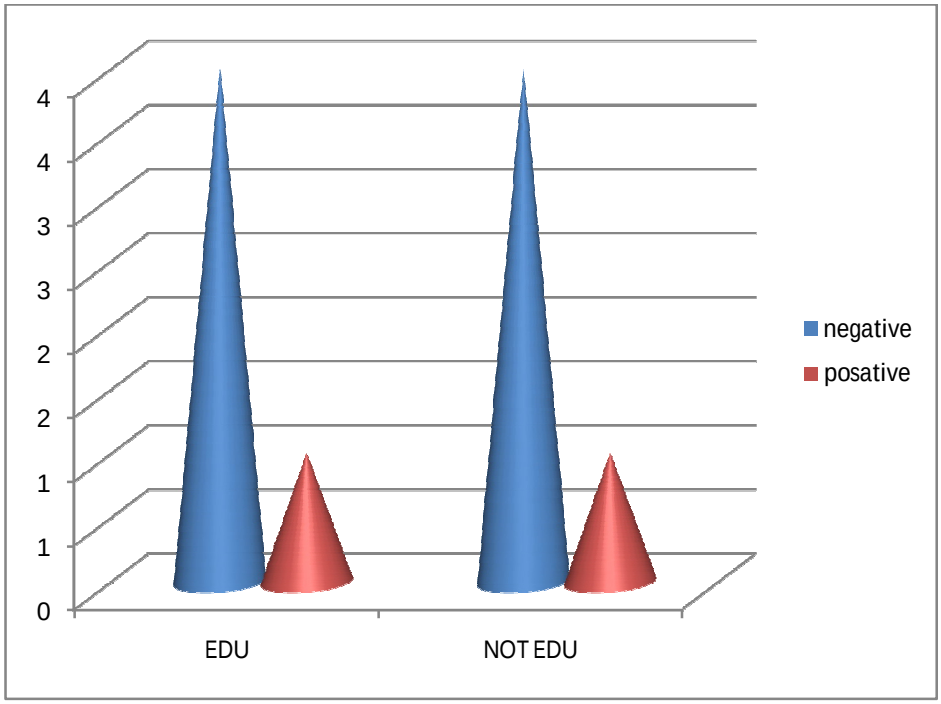


Figure (2) distribution of brucellosis in different breed .



Figure(3) association of owner education level and brucellosis

Table (5) shows owner awareness and occurrence of brucellosis

	Awareness	**Not Awareness**
Negative	5	3
Positive	1	1

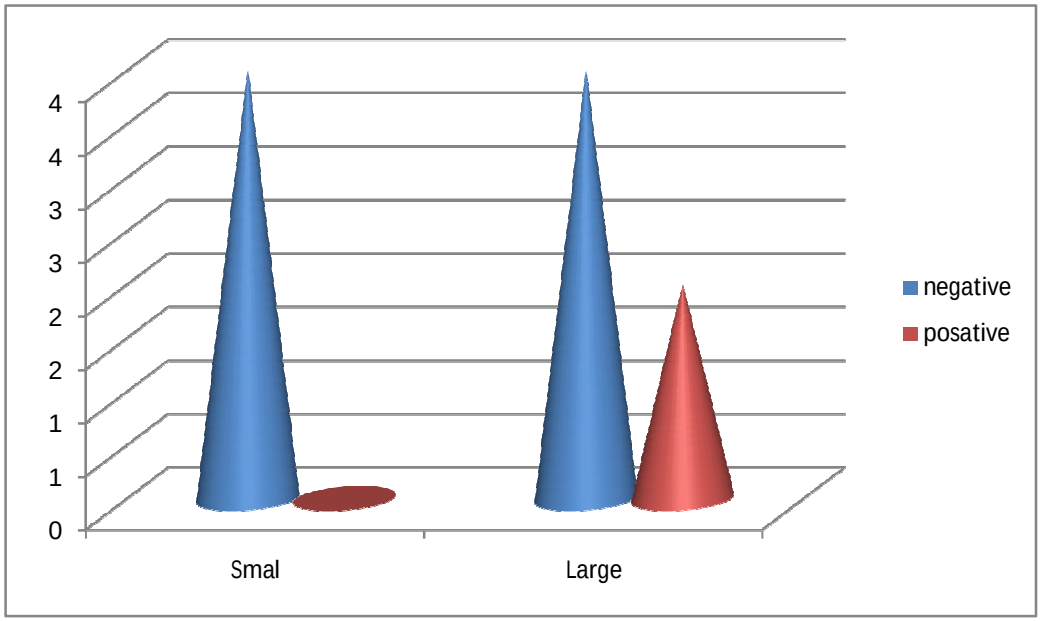


Figure (4) the association of flock size and brucellosis

Chapter Four

DISCUSSION

Sudan exportssheep, goat, camels and cattle to many countries especially to Saudi Arabia and other Arabic Gulf countries(Anon, 2011b).These exported numbers are influenced with epidemics that emerge spontaneously. (The number of tested animals, in different species, for brucellosis depends on this exportation movement).

This study revealed that the overall percentage of ovine brucellosis in exported sheep in Alkadaru quarantine was 0.6% by (RBPT) and 0.2%by the (SAT) and (BAPA)this could be due to cross-reaction between brucella and other bacteria.

Positive samples of brucellosis in the export animals were relatively low; these may be due to the good healthy status of these animals, high selectivity of them for export, awareness of owners with health and freeness of animals from the diseases. Castration at an early age before puberty, and collection of animals from markets and not from breeding area may also contribute to the freeness of exported animals from brucellosis and other diseases.

The low seroprevalence rate of ovine brucellosis in export sheep revealed in this studyis in agreement with ELSanousi (2012). The low prevalence rates of brucellosis was attributed to several factors which might reduce the spread of disease; these factors included the climatic conditions of the Sudan (persistence of the sun light at the most hours of the day, dry desert weather and low humidity) which may not favor survival of brucella organisms for long periods. In addition to that, the management breeding system of most sheep in the Sudan is nomadic pastoralist which prevents clustering of animals and herds. Moreover the harvest of the sheep herds in Sudan usually takes place early

before sexual maturation and so favors elimination of both infection and contamination of the pasture. (ELSanousi ,2012).

The low prevalence rate of ovine brucellosis obtained in this study is not in accordance With Ahmed (2012) who revealed that the overall prevalence of sheep brucellosis in Khartoum State was 0.74% by (RBPT) . However the prevalence in sheep that were kept for live export was 1% while there were no positive results in sheep brought for slaughter for local consumption .

This study revealed no association between as risk factors and ovine brucellosis this result dis agree with Omer *et .al,* (1989-1990) who Screened 33,591 castrated male lambs that were ready for export from Alkadaru quarantine, Khartoum State and (Port Sudan)quarantine, Red Sea State by (RBPT) and found the prevalence rate of sheep Brucellosis as 0.01%. This low prevalence may have relationship to the previous restricted regime castration of all export sheep males.

More over Omer *et. al,* (2007) reported low prevalence of ovine brucellosis in Kassala eastern Sudan during 2004-2006. The results were 0.1%, and 0.4% respectively. Which agree with our results .

However El-Ansary *et.al, (2001*) found the prevalence rate of sheep brucellosis as 1.0% using sera of sheep brought for slaughter to Kasslaabattoir.

Another study reported by Ahmed, (2004) in the Red Sea State who found the rate as 0.3% from 2,050 heads..Which agree with our results .

Conclusion:

1. Sheep brucellosis prevalence at a very low percentage (0.2%) in the exported sheep from Alkadaru quarantine.
2. Age was significantly associated with the brucellosis .
3. Other risk factors (namely; occupation, education for owner, awareness about brucellosis, flock size, type and origin of sheep, general management factors, grazing, source of water, feeding, drinking equipment's, other animals prevalent in the same farm, vaccination against brucellosis and castration, had no effect on the occurrence of brucellosis .

Recommendations:

1. There is need to plan, implement and monitor national eradication strategy for brucellosis in the country based epidemiological reality.
2. Movements of animals should be controlled by appropriate legislation and regulations.
3. Increase public awareness efforts should be made by the government and the other concerned parties to raise awareness of the disease which is transmissible to human and its impact on public health.
4. In addition of effective veterinary services, educational programmes to other stakeholders such farmers, effective enforcement of legislation with the animal disease control.
5. More investigation on infertility problems need to be carried.
6. The study should be applied in the production areas to determine the actual prevalence ratio.

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Questionnaire:

Questionnaire sheet for ovine brucellosis survey in Khartoum State (Alkadaru Quarantine)- Sudan

Date ……Serial No……

1- Governorate :

Alkadaru Quarantine

2-Locality :

Bahry

Personal data of the farm owner :

Name (farm owner):……..

Address: …….

3-Occupation :

(i) Farm (ii) Trader (iii) Governmentofficial

(v) Other (specify).

4-Education :

(i) Illiterate (ii)Khalwa (iii) Primary

(v) High secondary (vi) University (vii) Postgraduate**.**

5-Type of the size herd :

(i) Small (ii) Large

6- Sheep breed :

(i) Hamari (ii) Kabashi (iii) Zaghawi

7- Type of breeding:

(i) close system farm (ii) open farm

8- Age:

(i) $\leq$ 2years (ii) 2-3 years (iii) $\geq$ 3 years

9- Do you vaccinate your animals against brucellosis?

(i) Yes (ii)No

10-Do you knowledge about brucellosis ?

(i) Yes (ii)No

Herd Status;

1**1- Did you observe any signs of illness your animals (%age)**

(i) Yes (ii)No

Of yes ;

(i) Whate are their clinical signs .

(ii) Did some animal aborted form the original female, or males showing swollen testis.

Herd management data;

12- How do you feed and water your animals?

(i) in separate containers (ii) Commoncontaine

Whate is the source of the green fodder your provide animals? 13-

(i) Grazing land (II) Cut it from the farm (III) Buy it from other farm

(v) Buy it from market.

14- Whate is the source of water that you provide to your herd?

(I)Common canal (II) Wells (III) Tap water

15- Origin:

(I) North Khardofan (ii) Khartoum (III)Darfur state

16- Castration:

(I) Castrated (II) Not castrate