Sudan University of Sciences
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Serological Survey of Brucellosis in Camels

(Camelus Dromedarius) in Suakin quarantine - Sudan

المسح المصلي لمرض البروسيلا في الابل بمحجر سواكن - السودان

A thesis submitted to the College of Graduate Studies, Sudan University of Science and Technology in partial fulfillment for the requirements for the Degree of Master of Preventive Veterinary Medicine (MPVM)

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DEDICATION

TO MY

Father

Mother

SISTERS

BROTHERS

DEAR WIFE

AND TO MY KIDS

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Abstract

A cross-sectional study was conducted from October, 2017 to October, 2018, in Suakin quarantine, Red Sea State, Sudan to determine sero-prevalence of camel (*Camelus dromedaries*) brucellosis based on Modified Rose Bengal Plate Test (mRBPT) and Competitive Enzyme Linked Immuno-Sorbent Assay (cELISA).A total of 500 sera were collected from dromedary camels from different states which came to Suakin quarantine in the Red Sea State namely: Kassala, north kordofan, red sea and Khartoum. The overall sero-prevalence rate in the quarantine using modified Rose Bengal Plate Test (mRBPT) was 6% (No .of positive cases = 30). The Seroprevalence rate in the different states as follow: from Khartoum was 8.2% (No. of positive cases = 8) , Kassala was 6.6% (No. of positive cases = 13), 4.5% (No. of positive cases = 7) from north kordofan, 4.2% (No. of positive cases =2) from red sea and. Statistically the difference between the states was not significant *(Chi- square = 1.928 df = 3 P-value = 0.587 > 0.05).* Furthermore, sero-prevalence rate in females was 2.2% (No. of positive cases =1) and in males was 6.4% (No. of positive cases = 29). Also statistically the difference between the sex and brucellosis was not significant *(Chi- square = 1.315 df = 1 P-value = 0.252 > 0.005).*Also no association between age and purpose with the disease respectively,*(chi square =5.166 df =1p-value=0.23>.0.005 , chi square =1.453 df =1 1p-value=0.23>.0.005* .only one strong association was observed for breed and presence of the disease (Chi-square = 11.330 df = 1 P-value 0.001 < 0.005).Among the 500 serum samples collected from Suakin quarantine, only 30 samples that were positive with mRBPT were chosen for examination with cELISA as confirmatory test; the later test showed 24 sero-positives. Seroprevalence rate of camel brucellosis using mRBPT was relatively high in the quarantine, hence, comprehensive control programme which include serological diagnosis followed by vaccination are recommended.

ملخص البحث

أجريت دراسة مقطعية في الفترة من أكتوبر2017الي أكتوبر2018 لتحديد مدى انتشار مرض البروسيلا في الإبل في محجر بيطري سواكن ،ولاية البحر الأحمر،السودان. باستخدام اختباري الروز بنقال(RBPT) والمقايسة المناعية بالإنزيم المرتبط للتأكيد(CELISA) . تم جمع عينات دم لعدد 500 من الابل جمعت من ولايات مختلفة اتت لمحجر سواكن بولاية البحر الأحمر وهي كسلا ، شمال كرفان ، البحر الأحمر والخرطوم .

في البداية تم فحص جميع عينات المصل بواسطة الروز بنقال(RBPT) (30 عينة) 6% كانت إيجابية للإختبار .كانت نسب المسح المصلي في مختلف الولايات كالاتي:

من الخرطوم (8عينه) (8.2%)، كسلا (13عينه) 6.6% ، شمال كرفان (7عينه) 4.5 % و البحر الأحمر(عينتان)(4.2%) . تم كذلك اختبار جميع العينات الايجابية بالروز بنقال (30) عينة بواسطة إختبار المقايسة المناعية بالإنزيم المرتبط للتأكيد .(CELISA) أكد الإختبار 24 حالة.

اظهرت الدراسة عدم وجود فرق معنوي يذكر في نسبة انتشار مرض البروسيلا بين الولايات (البحر الأحمر ،كسلا ،شمال كردفان او الخرطوم) $P-value = 0.587 > 0.005$ وكذلك بين الإناث والذكور$P-value = 0.252 > 0.005$ وبين الابل المستخدمه للسباق والابل المستخدمه للحوم $p- value=0.23>.0.005$ والأعمار الصغيرة والكبيرة $p- value=0.23>.0.005$ يوجد فرق معنوي كبير جدا في نسبة انتشار مرض البروسيلا بين سلالات الابل البلدي والبشاري $p- value=0.001>.0.005$.

نتائج هذه الدراسة توفر حالة انتشار طفيفة لمرض البروسيلا في الابل في محجر سواكن وعوامل الخطر التي تسهم في انتشار المرض بين قطعان الابل, لذلك لا بد من وضع برامج قوية للسيطرة علي المرض تتضمن الفحص المصلي متبوعا بالتحصين ضد المرض.

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Introduction

Camel husbandry is vital for numerous pastoralist groups in Africa and Asia. The camels have ability to survive and produce under harsh environmental conditions has made it possible to use marginal and decertified ecosystems; and over the centuries, the camel constitute iconic feature of stability for the pastoralists in the arid zones of the world (Abbas and Agab, 2002).

Many pastoral groups and communities in diverse ecozones throughout the world are depending on camels for their livelihood. This dependence consists of utilization of camel meat, milk, leather and wool, exportation of live camels, used as an important sport and tourism resource in the Arabian Gulf countries and lastly the use of camels as animals for packing, transport and riding (Wilson, 1984; Rollefson, 2000).

Camels are not defined to be primary hosts of brucella organisms, but they are susceptible to both *B. abortus* and *B. melitensis* (Cooper, 1991). Brucellosis is a serious contagious disease of animals and its azoonotic disease, and has different names: Infectious or enzootic abortion and Bang's disease in animals; and Mediterranean or Malta fever, Crimean fever, Undulant fever and Rock fever in humans (Xavier and Paixão, 2010).

Sir David Bruce (1855-1931) provided the first description of brucellosis and succeeded to isolate *Micrococcus melitensis*, the causative agent of a disease among British army soldiers in the Mediterranean area. The organism was later renamed *Brucella melitensis* (Nielsen and Yu, 2010). The disease causes substantial economical losses in livestock and a severe or chronic debilitating disease in humans that needs long periods of therapy with a combination of antibiotics (Whatmore, 2009).

Brucella is facultative intracellular, gram negative coccobacilli that lack a capsule, flagella, and endospores (Cutler et al., 2005). The ability of *Brucella* to replicate and persist in host cells is directly associated with its capacity to cause persistent disease and to circumvent innate and adaptive immunity (Fichi, 2003). The genus includes 10 Nomo-species based on their different host specificity (Halling et al., 2005). The six classical species are *B. melitensis*,(sheep and goats); *B. abortus*, (cattle and buffaloes); *B. suis*, (pigs, reindeer and small ruminants); *B. canis* (dogs); *B. ovis* (sheep); and *B. neotomae* (desert wood rats) (Eschenbrenner et al.,2006). Recently, four new species have been described. Two are of marine origin (*B. pinnipedialis* (seals), and *B. ceti* (dolphins and whales). *B. microti* (common vole Microtus arvalis) (Wareth et al., 2015). Finally. *B.inopinata* was isolated from a breast implant wound of a female patient (Galińska et al., 2013).

Brucellosis is a worldwide distributed and can spread among camels and other farm animals through direct contact with blood, placenta, fetuses or uterine secretions, or through consumption of contaminated raw animal products. Consumption of unpasteurized milk and milk products from camels and other farm animals are considered to be the main source of infection as well as an occupational hazard in human (Almuneef et al., 2004). Cross transmission can occur between cattle, sheep, goat, camel and other species (Ghanem et al., 2009). More or less all domestic species can be affected with brucellosis except cats which are resistant to Brucella infection. Bearing in mind the damage done by the infection in animals in terms of decreased milk production, abortions, weak offspring's, weight loss, infertility and lameness, it is one of the most serious diseases of livestock. It is also a major obstacle for the trade. Death may occur as a result of acute metritis, followed by retained fetal membranes (Radostits et al., 2000).

Brucellosis has been virtually eliminated from the majority of the developed countries, but it is still endemic in Africa, the Middle East, Central and Southeast Asia, Central and South America and in most of the Southern European countries (Donev et al., 2010).

In humans, the disease, which is often referred to as ‘undulant fever’ or ‘Malta fever’ is a serious public health problem. Human brucellosis remains one of the most common zoonotic diseases worldwide, with more than 500,000 new cases annually (WHO and FAO, 1986). Infection prevalence in the animal reservoirs determines the incidence of human cases (Von Hieber ,2010).

Brucella melitensis and *B. abortus* are the two species most commonly found in human cases, and *B. melitensis* is responsible for the most serious infections. Human brucellosis is mainly an occupational disease, and the main modes of transmission are contact through skin with animal tissues, blood, urine, vaginal discharge, aborted fetuses especially, placentas, and by consuming raw milk and other unheated dairy products. Airborne infections occur in animal pens, stables, laboratories and abattoirs (Schulzezur et al., 2010). Some cases have also occurred from accidental self-inoculation with live vaccines (Saleem et al., 2010).

Symptoms in human brucellosis can be highly variable, ranging from non–specific, flu-like symptoms (acute form) to undulant fever, arthritis, orchitis and epididymitis (Gul and Khan, 2007).

Large amount of surveys for prevalence of brucellosis using standardized serological tests (Hesterberg et al., 2008). Rose Bengal test has been widely used (Cho et al., 2010) and shown to be of significant sensitivity compared to other tests however, some surveys apply more confirmatory tests in addition to demonstration of *Brucella* in culture.

The evidences of *Brucella* infections have been serologically demonstrated by different workers in sera of animals in Sudan.

Previous serological surveys showed prevalence rates in different camel rearing areas, which summarized as follows:

Prevalence of camel brucellosis reported in eastern Sudan, in Gash and Tocker was ranged from 0.1 to 5.5%, (Mustafa and Nur, 1968).

310 camels in Kassala and Butana were examine and the reported prevalence was 1.75 and 5.7%, respectively, (Mustafa and El Karim, 1971).

Prevalence of the disease in camels in central, western and eastern Sudan was reported of 2%, 3% and 7.5% respectively (AbuDamir et al., 1984).

948 camels from different herds in eastern Sudan were examed and reported aprevalence of 16.5- 32.3% (Bitter, 1986) .

An investigation of 238 camel's serum samples was carried out in Sudan using slide agglutination test, low prevalence of brucellosis was reported (3%) (Abbas et al., 1987).

1502 serum samples from one humped camels *(Camelus dromedaries*) were collected . The prevalence rate of *B. abortus* tested by RBT was 6.54, 5.79, 9.32, 5.03 and 8.06%, respectively from 1985 to 1989. (Yagoub et al., 1990) .

RBPT used to exam 38 serm samples, 32(84.2%) were found positive for *brucella*, and *B. abortus* biovar 3 from 3 samples when an isolation was done. (Agab et al., 1994).

Seroprevelance for brucellosis was 0% when 64 camel sera from 5 herds were randomly collected and screened for *Brucella* antibodies by the slide agglutination test. (EL-Ansary et al., 2001).

3303 camel sera in Nyala abattoir, Sudan were examined, of which 3274 camels were examined by conventional serological tests as RBT, SAT and CFT. 256 (7.82%) were positive. The remaining 29 sera were examined by RBT and competitive ELISA (cELISA). Four (13.8%) out of the 29 sera samples examined by cELISA were positive, while only 3 (10.3%) were positive by RBT. (Musa and Shigidi, 2001).

756 camel serum samples were examined. Only 12 (1.6%) showed high agglutination titres . (Yagoub, 2005).

14372 camel serum samples were collected to estimate the prevalence of brucellosis in camels in Kassala area during 2004 to 2006. RBPT used to investigate all sera. The percentage of the positive sera during 2004, 2005 and 2006 was found to be 12.3, 15.5 and 30.5% (mean 19.4%), respectively. (Omer et al., 2007).

83samples obtained from afield outbreak of brucellosis (21camels mixed with cattle, sheep and goats and 62 apparently healthy camels from the abattoir in Darfur) were examined. Out of 21 camels, 5 (23.8%)

were serologically positive and only three camels exhibited clinical signs of brucellosis. From the abattoir samples 6 (9.7%) were serologically positive for brucellosis. (Musa et al., 2008).

Prevalence in eastern Sudan was reported of 37.55% (Omer et al., 2010).

A total of 415 camels were screened from 39 herds in Khartoum state. Twenty four camels were positive to the RBPT giving an individual prevalence of 5.8%. (Mohamed et al., 2015).

The Objectives of the study:

The aims of this study were:

1- To investigate the prevalence of brucellosis in camel by using MRBPT in Swakin Quarantine .
2- To confirm the *brucella* positive with MRBPT by using cELISA test.
3- To evaluate the risk factors associated with brucellosis.

Chapter One
Literature Review

Chapter One
Literature Review
1.1 Taxonomy, History and Distribution of the camel
1.1.1 Taxonomy

Camelidae are classified into the order Artiodactyla and to the sub-order Tylopoda. Artiodactyla comprises three sub-orders:
The suiforms (notably Suidae family), the ruminantia (notably Bovidae family) and the tylopodes, which have a padded foot.
Camelidae is the only family in this suborder. Thus, camelids (family Camelidae) as ruminating animals are classified in proximity to ruminants but developed in parallel and are not part of the suborder Ruminantia. Some differences as foot anatomy, stomach system and the absence of horns underline this fact (Schwartz and Dioli, 1992; Fowler, 1998; Ji et al., 2009).

The family Camelidae is divided into three genera: The genus camelus, old-world genus, includes two species: Camelus dromedarius, the dromedary, one-humped or Arabian camel; and the Camelus bactrianus, the bactrian or the two-humped camel and the new world camels (genus Lama with the species L. glama, L. guanicoe, L. pacos and genus Vicugna with the species V. vicugna) (Wilson and Reeder, 2005).

Table 1:1: Genealogy of the dromedary camel

Order	Artiodactyla (even-toed ungulates
Suborder	Tylopoda (pad-footed animals)
Family	Camelidae
Subfamily	Camelinae
Genus	Camelus
Species	Camelus dromedaries

Source: (Yam and Morteza, 2015).

1.1.2 History

The appearance of the Camelidae family was probably in North America by the Oligocene period, 35 million years ago (Epstein, 1971).Two domesticated species of old world camels exist, the dromedary or one-humped camel (Camelus dromedarius) and The Bactrian or two-humped camel (*Camelus bactrianus*). The dromedary camel is the most important livestock animal in the semi-arid areas of Northern and Eastern Africa as well as in the Arabian Peninsula and Iran. The one-humped camel was domesticated about 3000 B.C.E. in southern Arabia mainly for its meat and milk (Epstein, 1971). The name of the dromedary derived from the Greek, “dromeus” which means runner or droma- running (Jassim and Naji, 2002).

The one-humped camel was probably domesticated in the region of today's Yemen and Oman about 3.000 to 4.000 years ago (Fowler, 1998). It is a multipurpose animal and used for milk, meat, hides and transports (Burgemeister, 1974).

The Bactrian or two-humped camel (*Camelus bactrianus*) exist in the cold deserts and dry steppes of Asia. The name of Bactrian camel comes from the area of Bactriana in Asia that was the old name of Iran (Bakhtar or Bactar). The two-humped camel, the Bactrian, was domesticated on the border of Iran and Turkmenistan and spread to an area bordered by the Crimea, southern Siberia, Mongolia and China. These animals are stockier than the dromedary and covered by thicker wool. Also in the desert Gobi there is still a population of wild Bactrian camels classified as Camelus ferus (Rao et al., 1970; Fowler, 1998). The wild Arabian camel became extinct (Lensch, 1999).

In 1848, US scientist Joseph Leidy was explored the Poebrotherium which is one of ancestor of camels inhabited the open-wood land areas of North Dakota about 37-24 million years ago. They were lightly built and were goat-sized, about 3 feet long. Their head, with a distinctive narrow snout, and long neck looked similar to a modern-day llama. From 24 to 5 million years ago, camels increased in size with lengthening necks and limbs, also developing and efficient pacing gait for traveling through expanding steppe and grassland habitat of the time. The modern camel's tribes Camelini and Lamini diverged one another by about 17 million years ago. The Camelini had reached Eurasia via the Bering Isthmus about 5-3 million years ago, whereas Lamini dispersed to South America via Panama's Isthmus about3 million years ago (Abdulaziz et al., 2010).

Para camelus, the likely ancestor of Camelus, is known from the fossil records of Asia, Europe and Africa about 7.5-6.5 million years ago. In there are some of hypothesis that Camelus originated from African continent related these fossil evidents. But most of fossil records were found from North America. In the Yukon of Canada, rare fossil remains of a giant camels such as proximal phalanx, ankle elements, partial long bones and teeth collected from Plio-Pleistocene (3.5 million years ago) deposits of the Old crow Basin at 67th parallel north which are considered Para camelus in the North (Rybczynski et al., 2013). But a research team led by the Canadian Museum of Nature has identified the first evidence for an extinct giant camel in Canada's High Arctic in 2010 (Rybczynski et al., 2013). The discovery is based on Ellesmere Island at 97th parallel north or1200 kilometers away from the Yukon early camel fossil remains place and its represents the most northerly record for early camels. They identified using collagen fingerprinting of the fossil limb bone compared with a database of genus-specific collagen peptide markers from 37 modern mammal species as well as that of a fossil camel found in Yukon. The

collagen profile of the High Arctic camel most closely matched those of modern dromedary camels as well as the Yukon giant camel, which is thought to be Para camelus-ancestor of modern camels. The collagen information, combined the anatomical data they to conclude that the Ellesmere camel and the giant Yukon camel are near relatives and is likely the same lineage as Para camelus which lived 3.5 million years ago.

The relative size of the Ellesmere camel tibia is in length about 30% larger than that of modern camels. From the size of the tibia, the Ellesmere camel was comparable in body size to other giant camels such as the Asian Para camelus gigas and the Yukon giant camel. By the palaeo-environmental reconstruction of upper portions of the Ellesmere camel fossil site was determined in the High Arctic at a time when global temperature were 20 to 30°C warmer than today and the area supported a larch dominated forest habitat. Based on the High Arctic camel fossil record the researchers concluded that camels originated in North America and dispersed to Eurasia via Bering Isthmus a land bridge linking Alaska and Siberia. The Para camelus lineage were living in the North American Arctic for less than7 million years ago the populations may have dispersed across the Bering Strait in cold winter via Arctic sea ice (Rybczynski et al., 2013).
Scientists have been reconstructed an evolutionary life tree of the Camelidae based on its genome sequences analysis. The complete mitochondrial genome sequence of wild Bactrian camels said that the divergence time for Camelini and Lamini was estimated to be 25 million years. In tribe Camelini, Bactrian camel and dromedary

Speciation may have begun 8 million years ago, in tribe Lamini, at first appears alpaca 10.4 million years ago, then vicuna speciation have begun 6.4 million years ago and at later time llama and guanaco have diverged 1.4 million years ago. In this study they concluded that the extant wild Bactrian camel and domestic Bactrian camel have separate maternal origins and that the two subspecies diverged some 0.7 million years ago (Burger et al., 2012; Jirimutu et al., 2009; Cui et al., 2007).

Recent results of camel's genetic analyses haven't shown the domestic Bactrian camel originated from extant two humped wild camel. Furthermore, comparative mitochondrial DNA analyses conducted in bone samples of C.bactrians from late Bronze and early Iron Age sites of Siberia and modern domestic Bactrian camels as well as wild camels. The comparative DNA analyses showed that are inconsistent with an ancestry of the wild Bactrian camel to both the pre-historic and the modern domestic camels whereas the extant wild two humped camel is not the progenitor of the domestic Bactrian camels. A Dromedary and Bactrian camels were domesticated in Near East for use as a draft and saddle animals, food source as milk, meat and even may be textile source about 2500-3000 years ago. Although many claim there is a consensus within archaeological circles, in

reality, scholars debate exactly when the camel was first domesticated in the Near-East for any purpose. In many Bible sources mentioned camels being used as beasts of burden animals in early 3rd millennium and late of 2nd millennium BC. Some researcher notes that not found any evident of domestication camels up to 1000 years of BC. In 1845, British archaeologists were discovered "The Black Obelisk of Shalmanester III"-black limestone monument in northern Iraq at Kalhu capital of ancient Assyrian. The monument is decorated with domestic Bactrian camels. The obelisk was erected in 825 BC for achievements of King Shalmaneser III (reigned 858-824 BC).This archaeological finding is one of ancient evidences for Bactrian camel domesticated in Near East, Assyrian kings often collected exotic animals as an expression of their power (Kennedy, 2010). Near East, Arabian regions and Iran empire regions were main localities for domestication of animals and crops. May be the Bactrian camels domesticated in this region after then imported to near areas. Also, in the Syrian cylinder seal dated 1800 BC. Ancient historical findings and remains also document that over 1000 years before century Bactrian camels were reared in western China; in 840s BC Bactrian camels were used by people in Turkmenistan (Indra et al., 1998). Ancient Romans used to call two humped camels as Bactrian camels. Bacteri was a middle Asian country within Macedonia in 4th millennium BC. Hunnu people who lived in the territory of Mongolia used to have feasts by having camel racing. Historic manuscripts reveal that camel caravans used to head China from Hunnu Empire, and also they mention that 700 carriages and 1000 camels were captured (Indra et al., 1998; Luvsan, 1975). Ancient petroglyphs of camels from 2-3 thousand years before century are found in many places in Mongolia, including various drawings of camels such as grazing camels, riding, and leading by people and trotting camels that is shown, it was one of motherland of wild and domestic Bactrian camels (Luvsan, 1975; Sanjmyatav, 1995).

1.1.3 Camel distribution

In 2000 the estimation of world Camel population was to be around 20394305 (with 10,000 in Europe, 16,603,147 in Africa and 3,781,158 in Asia), and in 2010 was 24,681,261 (with 7,243 in Europe, 20,735,087 in Africa and 3,938,931 in Asia) (Faye 2013; Mirzaei 2012). About 85 % of the camel population inhabits mainly eastern and northern Africa (with 60 % alone in Horn of Africa) and rest in Indian subcontinent and Middle East counties.

Table 1.2: Camel population in some selected countries

Country	Number (million)
Somalia	7.00
Sudan	4.25
Ethiopia	2.40
Niger	1.65
Mauritania	1.49
Chad	1.39
Mali	1.15
Pakistan	0.95
Kenya	0.94
India	0.51

Source: Adopted from (Gupta et al., 2014).

The countries having population less than India, in order of ranking, are Yemen, Algeria, Mongolia, UAE, Saudi Arabia, China, Tunisia, Afghanistan, Iran, Kazakhstan, Oman and Egypt. The majority of world's camel population is of dromedary type except small population of Bactrian camels in central Asia (Faye 2013; NRCC 2030). The dromedary lives in the hot arid lands of northern Africa and eastern Asia, and the Bactrian in the cold steppes and deserts in Central Asia. A new large camelid has been described a few times. It is a wild species living in very remote areas between Mongolia and China, and is called the Tartary camel (Camelus bactrianus ferus); it has been distinguished from the domestic double humped camel. (Wilson, 1984).

1.2 Potential Importance of Camels

Dromedaries are tolerating the drought, they can able to thrive in arid zones of many countries in the world and provide food, hides and transport. Although, other domesticated animals have difficulties to survive, dromedaries had developed an increasing interest in arid countries. Camels have ability to graze on low productive pastures on which the production of milk is possible and economically profitable. For this reason, camels may reduce the dependence of pastoralists on other livestock that is usually much more vulnerable to drought than camels (Farah and Fischer, 2004).

1.2.1Milk:

Camel milk is one of the main products with a high interest for local population in arid lands for at least three reasons: firstly the main part of the production is self consumed and thus, contributes to the food security of arid lands; secondly Camel milk has higher shelf life due to a reason for having higher protein contents that Performs an inhibitory action against certain bacteria. So it is easy to market it with basic hygienic conditions even in higher temperature (thirdly there is an inclination to the devolvement of dairy camel intensive system which could be profited for settled producers (Faye et al., 2002; Faye & Konuspayeva, 2012; Yaqoob and Nawaz, 2007). Camel milk is rich in fat, protein minerals and vitamins especially in vitamin C. It's rich in phosphorus, therefore in many aspects; camel milk is superior to the milk of other domestic species (Qureshi, 1986). Traditional preference for raw camel milk consumption must be considered for zoontic risks.

1.2.2Meat:

Informations are quite difficult to collect as the main part of the camel meat data comes from the informal market. Traditionally, camel meat consumption is not common in a subsistence system, the size of the carcass needing to share the meat between a wide numbers of people. However, the urbanization has increased the camel meat demand in most of the arid countries. (Hjort Af Ornäs, 1988). an important source of income of pastoralists is sold camels as slaughter males and infertile female by saling these animals for meat production to people and societies that do not breed camels for an increasing demand of camel meat, thus, leading to a higher number of camel abattoirs and butcheries in several countries that mainly slaughter young animals (Farah and Fischer, 2004 and Finke, 2005).

1.2.3Wool:

A mature camel produces 1-3 kg hair per year which is used for making ropes, mats, bags, carpets and blankets. It also produces some fine wool (especially of first shorn in new born calves) that is used for making blankets. While its hides are used for making saddles and shoes. (Khan et al., 2003).

1.2.4 Other purposes:

In spite of the rapid urbanization in the camel countries the interest of camel for cultural events is increasing. The camel race is still very popular in gulf countries especially. This activity has pushed much innovative research on genetic, biotechnology, physiology and contributes to a better understanding of camel biology (Faye, 2014).

1.3 Brucellosis

1.3.1 Definition

Definition of the disease

Brucellosis is an infectious disease caused by the bacteria of the genus *Brucella*. Various *Brucella* species affect sheep, goats, cattle, deer, elk, pigs, dogs and humans, (CDC, 2002). The disease was also reported in camels (Abbas and Agab, 2002; Hegazy et al., 2004; Teshome et al., 2003) and in marine mammals (seals, sea otters, dolphins, propoises) (Forbes et al., 2000).

In animals the disease is characterized by Losses due to abortion or stillbirths, irregular breeding and loss of milk production, and in human-beings, the disease is characterized by intermittent fever, chills, sweating, headache, myalgia, arthralgia, and a diversity of nonspecific symptoms (Young and Corbel, 1989; Nicoletti, 1982).

1.3.2 Synonyms

Alternate Synonyms for "brucellosis is Bang's disease, brucellosis, Bruce's septicemia, Chumble fever, continued fever, Crimean fever, Cyprus fever, febris melitensis, febris undulans, fist of mercy, Gibraltar fever, goat fever, melitensis septicemia, melitococcosis, Malta fever, Maltese fever, Mediterranean fever, milk sickness, mountain fever, Neapolitan fever, rock fever, Satan's fever, slow fever, undulant fever and undulating fever (Joseph,2019) .

1.3.3 Historical Prospective

Brucellosis is characterized by its type of fever, with its regular remissions or intermissions and its occurrence has been documented along the Mediterranean littoral since the time of Hippocrates in 450 B.C. In the 19th century, the disease was noted to have affected the British troops and the local population of Malta. In 1861, Marston, a British surgeon working in the Mediterranean described the symptoms of brucellosis as, "gastric remittent fever". In 1887 when Sir David Bruce a Scottish physician in 1887 the organism *(Micrococcus melitensis)* responsible for Maltese fever from a British soldier who died from the disease in Malta. In 1897 Hughes portrayed in a monograph the findings in people in greater detail, emphasizing "undulant fever" and suggested the name undulant fever. In 1897, Wright and Smith detected antibodies to *M. melitensis* in human and animal sera through agglutination test, and in 1905 Zammit isolated the organism from the milk and urine of goats. In 1914 in the United States of

America Traum isolate gram-negative rod bacillary in shape from the foetus of aborted swine (Hani, 2009)**.** In1956, Buddle and Boyce discovered *B. ovis.* In 1957, Stoenner and Lackman isolated *B. neotomae* from desert wood rat in Utah in the USA (Nidia et al., 2005). Two new *Brucella* species, provisionally called *B. pinnipe-diae* and *B.cetaceae* have been isolated from marine hosts within the past few years (Ewalt et al., 1994; Ross et al., 1996).

1.3.4 Zoonoses

Bacteria of the genus *Brucella* are responsible for one of the world's most widespread zoonotic infections, causing infectious abortion in animals and a febrile disease, known as Malta fever, in man (Samartino and Enright 1993; Corbel,1997). In human, most prevalent cause of Brucellosis is *Brucella melitensis* followed by *B. suis, B. abortus* and *B. canis.* However, other species of bacteria are also pathogenic to human (WHO and APHA, (2005) ; Sprague et al., 2012).. In addition, disease in marine mammals has resulted in the proposition of new species called *B. Maris.* Yet, phylogenetic differences have further led to dividing *B. Maris* into *B. pinnipediae* (seals and otters) and *B. cetaceae* (porpoise and whale) (Moreno et al., 2002; Corbel, 2006; Mantur et al., 2006).

Brucella spp, are also potential agents of bioterrorism , The Centers for Disease Control and Prevention (CDC) in the USA classified and listed *B.melitensis*, *B. suis* and *B. abortus* as potential bio-weapons in group B (second-highest priority agent), This is due to the highly infectious nature of all three species, as they can be readily aerosolized. Moreover, an outbreak of brucellosis would be difficult to detect because the initial symptoms are easily confused with those of influenza. The two species, *Brucella melitensis* and *B. abortus* are most commonly found in human cases, and *B. melitensis* are responsible for the most serious infections. Human brucellosis is mainly an occupational disease, and the main modes of transmission are contact through skin with animal tissues, blood, urine, vaginal discharge, aborted fetuses and especially placentas, and by consuming raw milk and other unheated dairy products. Airborne infections occur in animal pens, stables, laboratories and abattoirs (Schulzezur ,2010; Sriranganathan et al., 2010). Meat products are not considered high risk and the actual risk is likely negligible (International Commission of Microbiological Specifications for Foods, 1996).

Table 1. 3: Zoonotic potential and host preference of brucella species

Species	Colony type	Zoonotic Potential	Host Preference
B. melitensis	Smooth	High	Goat, sheep, camels, cows
B. abortus	Smooth	High	Cattle, buffalo, camels, bison, elk, yak
B. suis	Smooth	High	Pigs (biotypes 1-3), wild boar and European hares (biotype 2)
B. canis	Rough	Mild	Dog
B. ovis	Rough	No	Sheep
B. neotomae	Smooth	Unknown	Desert wood rat
B. ceti	Smooth	Mild	Dolphin, porpoise, whale
B. pinnipedialis	Smooth	Mild	Seals
B. microti	Smooth	Unknown	Vole, fox, (soil)
B. inopinata	Smooth	Mild	Unknown

Adapted from (Mulukken, 2016)

1.3.5 Economical Importance of Brucellosis

Brucellosis is characterized by epizootic abortions, chronic endometritis, infertility, arthritis, orchitis or chronic infections (Cutler, et al., 2005).

In camels Abortion is the major feature (Al-Khalaf and El-Khaladi, 1989).

In cattle *B.abortus* cause abortion which usually occurs during the second half of gestation, stillbirths and weak calves, retained placenta and decreased milk. Subsequent pregnancies are generally normal after the first abortion. However, cows may shed the organism in milk and uterine discharges. Occasionally Metritis or orchitis cause Infertility in both sexes. Systemic signs do not usually occur in uncomplicated infections, and deaths are rare except in the fetus or new-born. Infections in non-pregnant female are usually asymptomatic (OIE, 2009b).

In pig Abortion and other reproductive disorders may occur in sows. In boars, orchitis occurs and less commonly arthritis, spondylitis or abscesses in various organs may occur (Pappas et al., 2005).

In Canine brucellosis is characterized by abortion storms in females and testicular atrophy, epididymitis and infertility in males and generalized lymphadenitis in both males and females (Oncel, 2005).

In sheep and goats *Brucella melitensis* mainly causes abortion, stillbirths and the birth of weak offspring, animals that abort may retain the placenta, and milk yield is significantly reduced in animals that abort, as well as in animals whose udder becomes infected after a normal birth.

However, clinical signs of mastitis are uncommon. Acute orchitis and epididymitis can occur in males, and may result in infertility. Arthritis is seen occasionally in both sexes. Many non pregnant sheep and goats remain asymptomatic (Molhima, 2009).

1.3.6 Public Health Importance of Brucellosis

Brucellosis in human represents a major public health hazard, which affects social and economic development in various countries. A wide variety of symptoms and revealed by persons who acquired the disease in a slaughter plant, on a farm or ranch, or from the consumption of raw milk or cheese made from raw milk, many of which did not result in an initial diagnosis of brucellosis (Young, 1983).

In man, transmission occurs as a result of breaks in the skin, direct contact with tissues, blood, urine, vaginal discharges, aborted fetuses or placentas, ingestion of raw milk and other dairy products, but rarely from eating raw meat from infected animals. Occupational airborne infection in laboratories and abattoirs has also been documented. And transmission also occurs as closed contacts with animal during watering, grooming, riding, nursing sick ones and delivery assistance. Accidental inoculation of live vaccines (such as *B. abortus* Strain 19 and *B. melitensis* Rev.1) can also occur, resulting in human infections. There are also case reports of venereal and congenital infection in humans (Robinson et al., 2003; Abbas et al., 1987).

1.4. Epidemiology of Brucellosis

The disease has a worldwide distribution and affects cattle, pigs, sheep, goats, camelids, dogs and, occasionally, horses. *Brucella* infections have also been documented worldwide in a great variety of wildlife species and, more recently, in marine mammals. A spillover of infection from domestic animals to bisons, elks or African buffalos may also be possible (Saegermann et al., 2010).

1.4.1 Aetiological agents

Brucella, the causal organism of brucellosis is Gram negative cocci, coccobacilli or short rods measuring 0.5-0.7 µm by 0.6-1.5 um with straight or slightly convex sides and rounded ends, arranged singly and rarely in short chains;. They do not ferment carbohydrates in conventional media (Quinn et al., 1999; Chomel et al., 1994).

Brucella species is composed of eight terrestrial species and at least two marine species. Terrestrial *Brucella* species include *B. abortus, B. melitensis, B. suis, B. canis, B. ovis, B. neotomae, B. microti, and B. inopinata.* And *Brucella* isolated from marine mammals are, *B. ceti and B. pinnidialis* (Nielsen and Yu, 2010).

B.bortus, B. melitensis, B. suis, and *B. neotomae* generally occur in smooth form, while *B. ovis and B. canis* are invariable rough species (Nielsen et al., 2004). Theoretically, the three *Brucella* species known to cause brucellosis in camels (*B. abortus, B. melitensis, B. ovis*) can cause infection anywhere (Higgins, 1986). Abroad spectrum of smooth *Brucella* isolates have recently been described from a wide variety of cetacean and pinned marine mammals (Briker et al., 2000).

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

1.4.2 Transmission

Brucella transmitted among animals vertical and horizontal. Ingestion of contaminated feed, skin penetration, via conjunctiva, inhalation and udder contamination during milking are a horizontal transmission occurrence. Congenital infection that happens during parturition is frequently cleared and only few animals remained infected as adult (Radostits et al., 1994).

The possible means of acquisition of brucellosis include: infection from a contaminated environment, occupational exposure, and food-borne transmission. Occasional cases have been reported in which circumstantial evidence suggests close personal or sexual contact as the route of

transmission. More potential significance is transmission through blood donation or tissue transplantation. Certain occupations are associated with a high risk of infection with brucellosis. These include people who work with farm animals, especially cattle, sheep, goats and pigs. Farmers, farm labourers, animal attendants, stockmen, shepherds, sheep shearers, goatherds, pig keepers, veterinarians and inseminators are at risk through direct contact with infected animals or through exposure to a heavily contaminated environment (WHO, 2006).

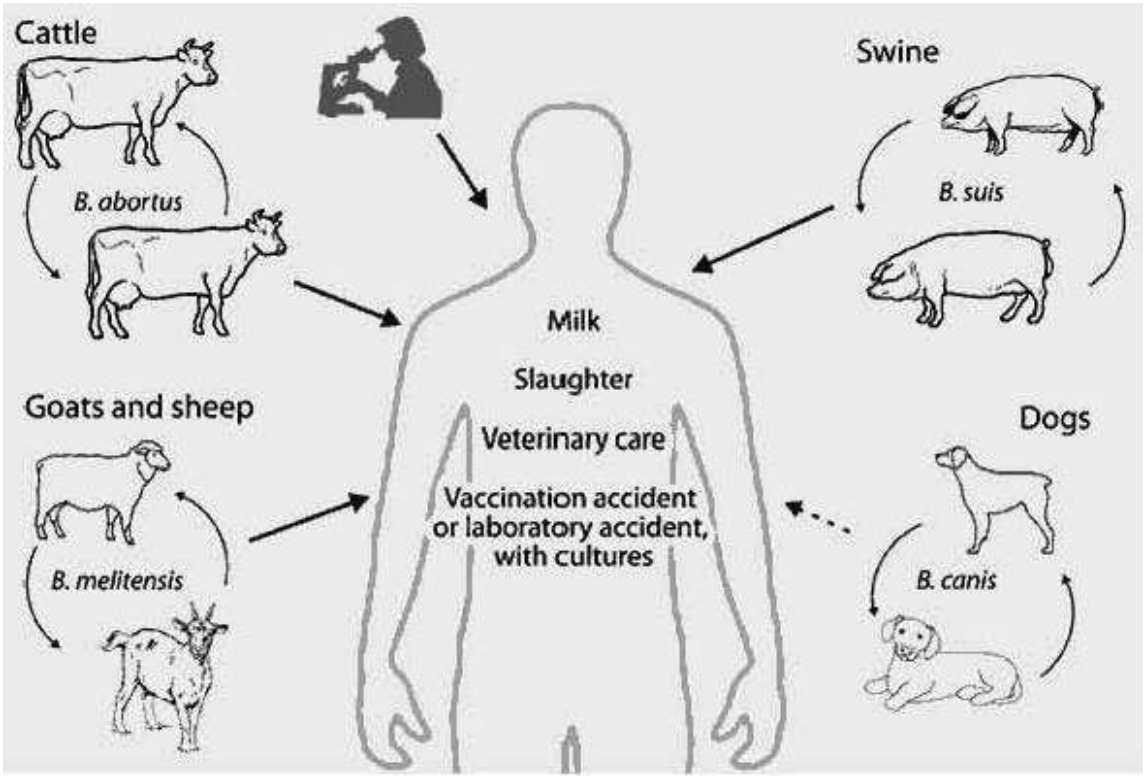
In man there is a direct relationship between the level of brucellosis in animal and the human infection. The source of human infections is consumption of unpasteurized raw milk and dairy products is a common method transmission and raw semi-cooked or pickled meat (OIE, 2009).

An urban populations usually acquired brucellosis by ingestion of fresh milk or dairy products prepared from unheated milk is the main source of infection for most populations. Cow, sheep, goat or camel milk contaminated with *B. melitensis* is particularly hazardous as it is drunk in fairly large volume and may contain large numbers of organisms (FAO, 2003).

In cattle and other bovidae, *Brucella* transmission is usually from animal to animal by contact following an abortion. Pasture or animal barn may be contaminated and probably acquired the organisms by ingestion but inhalation, conjunctival inoculation, skin contamination and udder inoculation from infected milking cups are other possibilities. The infection may also transmited by using of pooled colostrums for feeding newborn calves. Sexual transmission usually plays 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 (OIE, 2009b).

Suggested that small ruminants act as extensive reservoir of *B. melitensis*, which constitutes a threat of infection to large ruminants including camels and man due to prolonged contact. The chance of transmission is higher during parturition and abortion when most of the *Brucella* contamination occurs (Abbas and Agab, 2002).

Figure: 1.1: Transmission of Brucella to humans



Source: http _pcp/storage/images/media/transmission-bovine-tuberculosis-and-brucellosis/32598-1-eng-GB/transmission-bovine-tuberculosis-and-brucellosis.jpg.

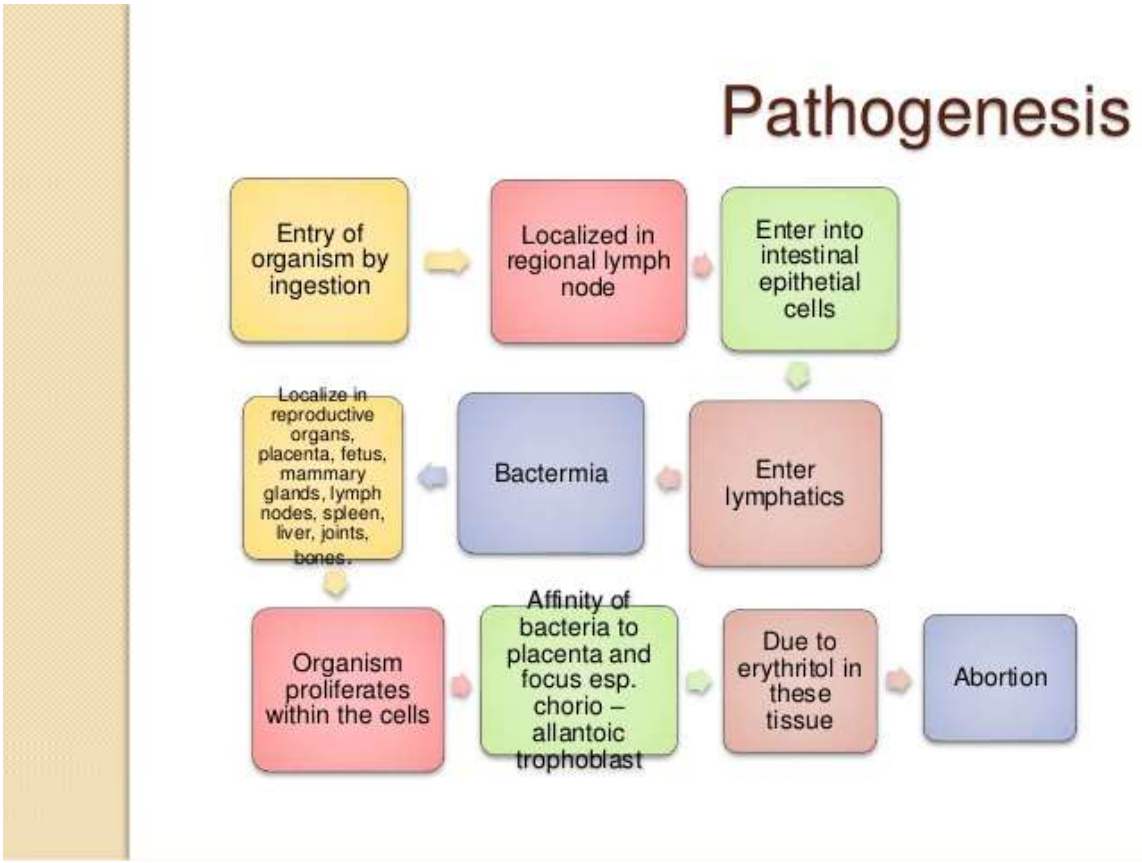
1.5 Pathogenesis and immune response

Pathogenically, *B.melitensis* infection is sheep and goats is similar to *B.abortus* in cattle, differences are significant, and each species of brucella causes a different disease (OIE, 1996). There are no differences of pathogenesis of brucellosis among livestock species and humans. *Brucellae* are facultative intracellular parasites of the reticulo-endothelial system. Infection occurs mainly through the mucous membranes of the oropharynx, upper respiratory tract and conjunctiva. Other potential routes of infection are through the mucous membranes of the male and female genital tract.

First, the bacteria invade the mucosa or break in the skin, after gaining entrance to the body, some of the *Brucella* organisms are able to evade or hinder the phagolysosomal action of the neutrophils and macrophages by redirecting the intracellular trafficking of the phagolysosomal action the organism succeed in arriving via the lymph channel at the nearest lymph node (Enright, 1990; Dornand et al., 2002; Gorvel and Moreno, 2002; Franco et al., 2007). When the bacteria prevail over the body defense, a primary bacterimia is generally established which is preceded by multiplication of the micro-organisms at the site of entry

followed by localization in the lymph nodes, the udder and the uterus and mild systemic reaction. In pregnant animals the uterus is invaded resulting in abortion. The udder is an important predilection site for *brucella*. The infection also becomes established in various lymph nodes and organs (Scientific Committee on Animal Health and Animal Welfare, 2001; Radostits et al., 1994). The virulence of *brucella* varies between species and strains. Small knowledge of the pathological changes in camels. Gross lesion may be found in the predilection sites uterus, udder, testicles, lymph nodes, joint bursa and placenta. Hydro bursitis was often observed in brucellosis positive dromedaries causing swelling of the bursa (Werney and Kaaden, 2002). The probable possibilities for the abortion in farm animals may be due to placentitis, direct effect of endotoxins or inflammatory response in fetal tissue (Walker, 1999).
Humoral as well as cell mediated immune mechanisms are activated in infected animal. The serological response is transient and sometimes missing in young sexually immature animals.

Figure1.2: Pathogenesis of brucellosis



Source https://image.slidesharecdn.com/brucellosis-15100607 3213-lva1-app6892/95/brucellosis-17-638.jpg?cb=1444116844.

1.6 Clinical Manifestation of Brucellosis

Brucellosis could be suspected in any herd with history of abortion during the last stage of abortion, hygroma, orchitis, arthritis, epididymitis, metritis, retention of placenta, weak or still births, neonatal mortality reduced fertility and lowered milk production (Blood and Radostits, 1989; Musa et al., 1990; Poester et al., 2010; Corbel et al., 2006), while the disease in camels generally is not accompanied by clear-cut symptoms, A retained placenta is rare in Camelidae. This may be a result of the difference in the placental attachment. Camelids possess a placenta diffusa like the horse and not a cotyledonary placenta. (Mustafa, 1987; Fowler, 2010), and occur without obvious signs. Generally infection does not persist more than four years (Solonitsuin, 1949). Correct diagnosis is reliant on isolation of the bacteria or detection of; genetic material, antigen, antibodies or cell-mediated immune responses since the clinical signs are not patho gnomonic (Corbel et al., 2006).

In human the disease is caused by direct or indirect contact with infected animals and the infection usually causes severe or chronic illness (Raga, 2000).Clinical manifestation among humans is an acute or sub-acute febrile illness usually marked by an intermittent or remittent fever accompanied by malaise, anorexia , joint pain, headache, inappetance, hepatomegaly, splenomegaly and prostration, and which, in the absence of specific treatment, may persist for weeks or months (Cutler et al., 2005; WHO, 2006; International Commission of Microbiological Specifications for Foods, 1996; Corbel, 2006; Mantur et al., 2006; Franco et al., 2007). In addition, pregnant women can abort including abortion during early trimesters (Corbel, 2006).

In chronic case of the disease, the untreated acute disease develops into chronic infections of organs, especially the spleen and liver, result in the formation of granulomas around infected phagocytes and cells (International Commission of Microbiological Specifications for Foods, 1996; Franco et al., 2007). Granuloma formation is seen as a result of complications. These complications include endocarditic, osteoarticular disease, meningitis, hepatic dysfunction and impacts on any body system where granulomas are affecting function (Corbel, 2006; Mantur et al., 2006; Franco et al., 2007).

1.7 Brucellosis worldwide

Brucellosis occurs worldwide, except in countries where it has been eradicated, including Britain, Norway, Sweden, Finland, Denmark, Germany, Belgium, the Netherlands, Switzerland, Austria, Czech Republic, Slovakia, New Zealand, Canada, France and Italy (Pappas et al., 2009).
Eradication was done through implementation of stringent disease control strategies that included test and slaughter policies in most countries that are free of *Brucella* (Pappas et al., 2009). However, the disease is important in

developing countries, with Brucella abortus strains being the most common occurring particularly in the tropical countries (OIE, 2004; Kunda et al., 2007).

Camel's brucellosis is common in Arabian region, Latin America, Africa, and some parts of Asia like Iran (Hadush and Pal, 2013).

Bovine brucellosis is reported to occur in most countries in Africa (Chukwu, 1985; Faye et al., 2005).

The prevalence of the disease varies between countries, regions and farming sectors due to vast differences in terrain, climate, social customs, resources, livestock management and attitude towards disease control (Nicolette, 1984; McDermott and Arimi, 2002; Bishop et al., 1994).

Caprine and ovine brucellosis are common in Mediterranean and Middle East region and other parts of the world such as Africa, Central America and Mexico where the incidence is very high and the disease is known to be enzootic (Herr, 1994; Banai et al., 2002; OIE, 2004; Leyla et al., 2003).

There is substantial amount of information on brucellosis in most parts of Africa particularly for ruminants and wildlife (Muma et al., 2006). However, the extent of distribution of equine brucellosis is not really known (Gous et al., 2005).

It is believed that the distribution of equine brucellosis follows that of cattle and to some extent swine brucellosis (Radostits et al., 1994). Horses kept together with infected cattle are at a higher risk of exposure to *Brucella* infections (Quinn et al., 1999).

1. 8 Diagnostic Methods

Great care should be employed during handling any material containing *Brucella* organisms. Generally, precautions to be taken include use of safety cabinet in laboratory; wearing gloves, protective cloth and facemask, autoclaving materials in contact with the organism and disinfecting contaminated surfaces (Alton et al., 1975).

The morphology of the Brucella bacterial colonies is associated with the presence of lipopolysaccharides (LPS) in the external membrane of the bacterium. Smooth (S-LPS) and rough (R-LPS) phenotypes are differentiated. The S-LPS phenotype is found in most Brucella species, only *B. canis* and *B. ovis* possess the R-LPS. Some proteins of Brucella are responsible for serological cross-reactions between Brucella spp. And other bacterial species (Emmerzaal et al., 2002). Cross-reactivity exists to:

– *Yersinia enterocolitica O: 9*
– *Escherichia hermannii*
– *E. coli O: 157*
– *Francisella tularensis*
– *Stenotrophomonas maltophilia*
– *Vibrio cholera O: 1*

– *Salmonella serotypes group N*

Therefore, difficulties may arise in the diagnosis of brucellosis. Abortion and reduced fertility in the camel frequently have other causes, such as salmonellosis, trypanosomosis, or infections with Campylobacter or Tritrichomonas fetus (Wernery and Ali,1989; Wernery ,1991; Wernery and Wernery ,1992), making laboratory testing essential. An incorrect diagnosis of brucellosis may occur when based on serology alone.

Many workers used serological tests for diagnosis of the disease. A definitive diagnosis of brucellosis requires the isolation and identification of the etiological agent (Davis et al., 1980; Volk, 1982). Several methods are used for diagnosis of brucellosis and include:

1.8.1 Bacteriological Methods:

1.8.1.1 Direct smear microscopic examination

A presumptive bacteriological diagnosis of Brucella can be made by means of the microscopic examination of smears from vaginal swabs, placentas or aborted fetuses, stained with the Stamp modification of the Ziehl-Neelsen staining method. However, morphologically-related microorganisms, such as *Chlamydophila abortus*, *Chlamydia psittaci* and *Coxiella burnetti* can mislead the diagnosis because of their superficial similarity (Marin et al., 1996; Poiester et al., 2010).

1.8.1.2 Cultural isolation of Brucella organism

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

Brucellosis is usually diagnosed in the laboratory by the culture of blood, milk or tissue or the detection of antibodies in sera. Brucella organisms can be recovered from the placenta, but, more conveniently, in pure culture from the stomach and lungs of aborted fetuses. For isolation, the recommended medium is Farrell's medium, which contains six antibiotics. But other selective Brucella media are also in use for the growth of this pathogen from fresh camel milk and camel tissue samples (Radwan et al., 1995).

Samples of choice in slaughterhouses include mammary, iliac, pharyngeal, parotids and cervical lymph nodes, and spleen. Samples must be immediately sent to the laboratory, preferentially frozen at -20°C, and they must be identified as suspect of Brucella spp. Infection (Poester et al., 2010).

Vaginal swabs, semen and seminal fluid have low numbers of viable organisms, and therefore isolation is more difficult, often resulting in false negative results. Enrichment media containing selected antibiotics can improve the sensitivity in these cases (De Miguel et al., 2011; Her et al., 2010).

Brucella spp. colonies are elevated, transparent, convex, with intact borders, smooth, and a brilliant surface. The colonies have a honey color under transmitted light. Optimal temperature for culture is 37°C, but the organism can grow under temperatures ranging from 20°C to 40°C, whereas optimal pH ranges from 6.6 to 7.4. Some *Brucella* spp. requires CO2 for growth. Typical colonies appears after 2 to 30 days of incubation, but a culture can only be considered negative when there are no colonies after 2 to 3 weeks of incubation (Carmichael and Greene ,1990).

False negative results should be considered in the absence of bacterial growth since the sensitivity of culture is low (Poester et al., 2010). Usually, solid media such as dextrose agar, tryptose agar, and trypticase soy agar, are recommended for primary isolation of *Brucella*, but some species, i.e., *B. ovis* and *B. canis* require addition of 5-10% of sterile bovine or equine serum to the culture media. In the case of blood or milk, biphasic media such as Castaneda's medium is recommended for improving sensitivity (Poester et al., 2010).

1.8.1.3 Laboratory animal inoculation

Mice have been reported to be the animal model most frequently used in brucellosis research (Mense et al., 2001; Silva et al., 2011). Nevertheless, it has been reported that guinea pigs are also susceptible and can be used (Avong, 2000; Ocholi, 2005; OIE, 2009). Animal inoculation may be either subcutaneously or through abraded skin in guinea pigs or, preferably, intravenously, intraperitoneally, or through the digestive tract or nasal (aerosol) routes in mice (OIE, 2009; Silva et al., 2011). The spleen of mice is cultured 7 days after inoculation, while serum samples of guinea pigs are subjected to specific tests 3 and 6 weeks after inoculation (OIE, 2009). It is noteworthy however, that gastric acid can interfere with the infectivity of Brucella in laboratory animals (Silva et al., 2011).

1.8.2 Serological methods:

The detection of specific antibody in serum or milk remains the most practical diagnosis of brucellosis (WHO, 2006). There are several common serological tests available for detecting antibody response in animals and human, thus used for screening purposes (Minga and Balemba, 1990). The tests include Serum agglutination test (SAT), Complement Fixation Test (CFT), indirect enzyme linked Immunosorbent assay (I-ELISA), Competitive ELISA (c-ELISA) and Rose Bengal Plate Precipitation Test (RBPT).

1.8.2.1 Rose Bengal plate test (RBPT)

The Rose Bengal Plate Test (RBT) is a screening test with high sensitivity (90%) but low specificity (75%). As such it does not discriminate between S19 vaccinations and natural infections (Nielsen et al., 1995; 1996). 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 agglutinins, 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 temperature at which the reaction takes place may influence the sensitivity and specificity of the RBPT (MacMillan, 1990).

1.8.2.2 Complement fixation test (CFT)

The CFT detects mainly the IgG1isotype antibody, as the IgM isotypes are partially destroyed during the inactivation process. Since antibodies of the IgG1 type usually appear after antibodies of the IgM type, control and surveillance of this disease is best done with SAT and CFT (WHO/MZCP, 1998).

The test shows good correlations with the recovery of *Brucella* organisms from artificial recovery or naturally-infected animals (Madsen, 1994).

Although the test is fast and accurate, it does not allow for discrimination between antibodies due to infection from vaccinal antibodies (Nielsen, 2002; Poiester et al., 2010).

Other problems include large number of reagents and controls needed to carry out the test. Furthermore, each time the assay is set up, a large number of titrations are needed, and interpretation of the results is subjective due to differences in techniques (Madsen, 1994).

Occasionally, there is direct activation of complement by serum (anti-complementary activity) and the inability of the test to be amenable for use with haemolysed serum samples. The laborious nature of this test and the requirement of highly- trained personnel and suitable laboratory facilities make the CFT less suitable for use in developing countries (FAO, 2005).

The CFT may also test false negative, when antibodies of the IgG2 type hinder complement fixation (Nielsen et al., 1988; MacMillan et al., 1990). Despite these inherent problems, the CFT is a widely used test, and has been regarded as the most specific and accepted serological test for diagnosis of brucellosis. Thus, it is a recommended test for international trade (OIE, 2009). Complement Fixation test detects predominately IgG antibodies as most of IgM ones are destroyed during serum deactivation; it is thus so used as a confirmatory test (FAO, 2003). The test distinguishes

reaction caused by other factors like vaccines and other bacterial infections. *Escherichia coli O157, Yersinia enterocolitica O:9, Vibrio cholerae, Pseudomonas mallophilia* and *Salmonella* serotypes which share common chain of lipopolysaccharides (LPS) antigen with smooth *Brucella* strains and therefore cross react. Francisella tularensis also cross reacts for unknown reason (Wrathall et al., 1983). Rough *Brucella* strains also cross-react with *Actinobacilus equuli*, *Pasteurella multocida* and *Pseudomonas aeruginosa* (Corbel, 1990; Cloeckaert et al, 1992; Garin-Bastuji et al, 1999). These organisms contribute to false positive reactors for brucellosis in animal herds. Thus, the use of highly specific test such as monoclonal antibody-based competitive - Enzyme linked Immunosorbent Assay (c-ELISA) and CFT minimizes the risk of cross-serological reactions between *Brucella* and these groups of bacteria (Vizcaino et al, 1991; OIE, 2004).

1.8.2.3Serum Agglutination Test (SAT)

This has been used extensively for brucellosis diagnosis and, although simple and cheap to perform, its low sensitivity and specificity mean that it should only be used in the absence of alternative techniques (OIE, 2004; Quinn et al., 1999). This test is positive 7 - 10 days after infection (Godfroid et al., 2002).

During this stage of the disease the level of agglutinins associated with both immunoglobulin M (IgM) and IgG continue to rise. Sensitivity is rather low ranging from 61– 69%. High titre serum samples may not cause agglutination in low dilution (the prozone effect) (Quinn et al., 1999).

Therefore a range of serum dilutions from 1 to 10 to over 1000 should be made (Herr et al, 1991; Herr, 1994).

1.8.2.4Enzyme-Linked Immunosorbent Assay (ELISA)

It is known under a variety of names such as enzyme immunoassay (EIA) (Van-Weem and Schuurs, 1971), enzyme labeled assay (ELA) (Saunders and Wilder, 1974) competitive enzyme linked immunoassay (CELIA) (Yarde etal., 1976) and enzyme-linked Immunosorbent assay (ELISA) (Engvall and perlmann, 1971).

The Enzyme-Linked Immunosorbent Assay (ELISA) is a technique used to detect antibodies or infectious agents in a sample. Antibodies are made in response to infection and so an antibody ELISA can indicate whether or not an animal has been in contact with a certain virus. An antigen ELISA can tell whether an animal is infected with a virus by detecting it directly (WHO, 2006).

Although the ELISA is not a cheap test, several authors have highlighted several advantages in using this assay. Firstly, it has high sensitivity and specificity (Saunders and Clinard, 1976; Cargill et al, 1985; Sutherland et al, 1986). Secondly, and unlike the CFT, the ELISA is not affected by haemolysis, prozone and anti complimentary effects (Reynolds,

1987) and finally the technique is not complicated and is commercially available.

Among the ELISA methods the competitive ELISA (c-ELISA) was found to be more robust and easy to perform compared to others. The c-ELISA has several diagnostic merits and these include high sensitivity and specificity, ability to differentiate vaccinated animals from naturally infected ones, or those infected with cross-reacting or ganisms and its use in areas where disease prevalence is low (Nielsen et al., 1996). Indirect ELISA is used to test antibodies High sensitivity: More than one labeled antibody is bound per antigen molecule Flexible: Different primary detection antibodies can be used with asingle labeled secondary antibody.

1.8.3 Molecular methods:

Polymerase chain reaction

The isolation of *Brucella* organisms is still the preferred method of diagnosis. This method also allows typing of the isolated strains. However, new PCR techniques are now being implemented for both identification and phenotypic biotyping (Saegermann etal., 2010).

These PCRs can discriminate between *Brucella* species, and between wild and vaccine strains, but do not discriminate between *Brucella* biovars. So far, only monoclonal antibodies against different epitopes of the *Brucella* LPS can be used for biovar differentiation. PCR-based assays have been developed for brucellosis diagnosis and are based on the detection of specific sequences of the pathogen, such as genes of the locus 16S – 23S, the IS711 insertion sequence or bcsp 31 gene encoding for a protein of 31kDa. PCR assay designed with hybridization probes and primers targeting the insertion sequence of IS711 of the BMEI 1162 gene, has shown reliable results in the amplification of pure target DNA in bacterial dilutions, but the assay was less sensitive when tissue samples were tested. (Von Hieber, 2010).

The reasons for this may be explained by the extraction method used the intracellular presence of the pathogen and the distribution pattern of Brucella organisms.

1.9 Control and Prevention

There are a number of approaches in the brucellosis control and eradication programmes which include vaccination of animals, surveillance, testing, quarantine and culling (Godfroid, 1992; Madkour, 2001).

Control of camel brucellosis should suite conditions in particular countries where camels are raised. In most of the developing countries where camels are raised by pastoralists, brucellosis prevalence is low. Thus control by herd immunization and vaccination of calves at 4 to 8 months of age is helpful. On the other hand, test and slaughter policy can be followed in counties where intensification is practiced (Abbas and Agab, 2002).

1.9.1 Vaccination

Because of the serious medical and economic consequences of brucellosis, serious efforts have been undertaken to prevent the infection through the use of vaccines, both inactivated and attenuated *Brucella* vaccines have been used successfully.

Dromedaries were vaccinated with *B. abortus* strain S19 (Chichibabin, 1971) and with *B. melitensis* Rev 1 (Radwan etal., 1995).

The non-smooth strains of *B. abortus* RB51 and *B. melitensis* M111 have recently been introduced into some countries. These

Vaccines are said to be safe and do not interfere with serological tests (Wernery and Kaaden, 2002).

Young (three months) dromedaries received a full dose of the vaccine and adults (10 years) a reduced dosage. Both groups developed *Brucella* antibodies with titres of between 1:25 and 1:200 using the standard USDA BPAT, two to four weeks after vaccination. They receded after eight months in young stock and after three months in adult camels. (Agab et al., 1995)

1.9.2 Treatment

No practical effective treatment for brucellosis in livestock is known, and efforts are directed at control and prevention (Animal Health Australia, 2005). Treatment trials that have been undertaken have shown only partial success in eliminating the infection (Radostitis et al., 2000). An attempt to use antibiotic such as penicillin and oxytetracycline causes L-transformation on the bacterial cell wall thereby possibly creating carrier animals, and thus affecting future serological detection (Bishop et al., 1994).

No vaccine has been approved for the prevention of human brucellosis. Therefore, human brucellosis is usually prevented by controlling the infection in animals. Pasteurization of dairy products is an important safety measure where this disease is endemic. Treatment regimes for human brucellosis require combination of antibiotics like rifampicin or gentamicin and doxycycline twice daily is the combination most often used, and appears to be efficacious (Yohannes et al., 2013). The combination of doxycycline with streptomycin is currently the best therapeutic option with less side effects and less relapses, especially in cases of acute and localized forms of brucellosis (Seleem et al., 2010).

1.10: Risk factors prevalence and of Camel Brucellosis in Different Countries:Table 1.4: prevalence of camel brucellosis from different countries :-

country	Number tested	+ve	Prevalence %	Test used	References
Saudi	146	3	1.4	RBT	Hashim et al. (1987)
Arabia	2630	210	8	RBT, SPA	Radwan et al. (1992)
	236	19	8	RBT	Radwan et al. (1995)
	98	7	7.1	RBT, SAT	Hegazy et al. (2004)
	859	16	1.86	RBT	Alshaikh et al. (2007)
		27	3.03	cELISA	
Nigeria	232	3	1	RBT & SAT	Okoh (1979)
	329	38	11.4	(RBT, SAT, cELISA)	Junaidu et al. (2006)
	480	36	7.5	MSAT	Kudi et al. (1997)
Libya	967	40	4.1	RBT, SAT, CFT	Gameel et al. (1993)
	520	8	1.4	RBT	Azwai et al. (2001)
		7	1.2	SAT	
		16	3.0	cELISA	
Jordan	412	50	12.1	RBT, CFT	Al-Majali et al. (2008)
	640	91	14.2	RBT	Dawood (2008)
Kuwait	698	104	14.8	RBT, CFT	AL-Khalaf and EL-Khaladi (1989)
Abu	392	4	1.0	RBT	Afzal and Sakkir (1994)
Dhabi		6	1.5	SA	
	1794	105	5.8?(1990–1991)	RBT	Moustafa et al. (1998)
	7899	8	0.1?(1995–1996)	RBT	Moustafa et al. (1998)
Yemen	105	0	0.0	ELISA	AL-Shamahy (1999)
Pakistan	81	3	2.5	STA	Ajmal et al. (1989)
	71	6	8.0		Straten et al. (1997)
Iran	953	77	8.0	RBT,SAT, CFT , 2MET	Zowghi and Ebadi (1988)
	258	5	1.9	RBT, SAT, 2MET	Khadjeh et al. (1999)
	112	12	10.5	RBT	Ahmad and Nemat (2007)
Egypt	200	40	20.0	SAT	Zaki 1948
	175	18	10.3	SAT	Hamada et al. (1963)
	780	182	23.3	TAT	Salem et al. (1990)
		108	13.9	CFT	
		105	13.5	2 MET	
		64	8.2	RBT	
	360	41	11.5	TAT, RBT	Nada and Ahmed (1993)
	500	35	7.0	RBT	El-Sawalhy et al. (1996)
		12	2.3	cELISA	
	592	6	1.0	STAT	Abou-Eisha (2000)
		9	1.7	Card test	
	766	67	8.7	RBT	Abdel Moghney (2004)
		71	9.3	ELISA	
	340	25	7.4	CFT	EL-Boshy et al. (2009)
Eritrea	98	4	3.1	CFT	Omer etal., 2002)
Ethiopia	415	24/14	5.8/3.37	RBPT/CFT	
	1152	58/47	5.0/4.1	RBPT/CFT	Habtamu et al.,2015
	573	11/9	2.0/1.6	RBPT/CFT	Angesom et al.,2013
	461	25	5.4	RBPT/CFT	Omer et al.,2010
	1100	26/21	2.36/1.91	RBPT/CFT	
	768	94/58	11.9/7.6	RBPT/CFT	Sisay and Mekonnen,2012
Kenya	384	59	15.36	MRT	Wanjohi et al.,2012
	2000	750	37.5	SAT	
Sudan	2000	797/809	39.9/40.5	RBPT/cELISA	Omer et al.,2010
	3274	256	7.82	cELISA	Musa and Shigidi,2001
Somalia	1246	48/39	3.9/3.1	RBPT/ELISA	Ghanem et al.,2009

SPA (standard plate agglutination test)
MSAT (microtitre serum agglutination test)
SA (standard agglutination)
SAT (serum agglutination test)

TAT (tube agglutination test)

CFT (complement fixation test)

2 MET (mercaptoethanol test)

RBT (rose Bengal test)

cELISA (competitive enzyme linkedimmunosorbent assay)

STAT (standard tube agglutination test)

ELISA (enzyme linked Immunosorbent assay)

Chapter Two
Materials and Methods

Chapter Two
Materials and Methods

2.1 Study Area

A cross-sectional study was carried out from October, 2017 to October, 2018 in Swakin quarantine approximately (60) km, south of Port Sudan and (7) km, west of Swakin in Red Sea State. Red Sea state is one of the 18 states of Sudan. (Sudan States). Red Sea State is located in the north eastern part of Sudan (latitude 17○ to 22○ north, longitude 33○ to 38○ in the east) with the land area of 210.410 km2. Red Sea State constitutes approximately 10%.of the total area of Sudan and 63% of the Eastern region. It is delimited by Kassala State and Eritrea in the south, River Nile State in the west, Egypt in the north and the Red Sea in the east. It is divided into 10 localities: Port Sudan, Suakin, Sinkat, Tokar, Halayib, Ageeg, Alganab and Alawlieb, Haya, Derodieb and Gebiet. The principal types of livestock found in the state are cattle, sheep, goats and camels. Camels represent 6.06% of the ruminants in the Red Sea State (Anon, 2010).

The importance of the Red Sea State comes from the anumbers of economic institutions, namely Port Sudan maritime port and Suakin veterinary quarantine.

Swakin veterinary quarantine exports Sudanese live stock such as sheep, goats, cattle, and camels to Arab markets such as the Gulf cooperation council and Egypt, and import animal products from the world countries.

2.2 Study Population

The study populations were camels that are ready for export to kingdom Saudi Arabia, Arabian Gulf countries and Egypt.

Table 2.1: Camel's number exported to kingdom Saudi Arabia and Arabian Gulf countries(1998 -2016).

year	Numbers of camel export
1998	7550
1999	22308
2000	39653
2001	42742
2002	41470
2003	29088
2004	50146
2005	57257
2006	43871
2007	9832
2008	52678
2009	79516

Table 2.1: Camel's number exported to kingdom Saudi Arabia and Arabian Gulf countries(1998 -2016)continued.

2010	89930
2011	45815
2012	27985
2013	16569
2014	14763
2015	17197
2016	13301

Source: information and reports office – Suakin quarantine

Table 2.2: Camel's number exported to Egypt(2012 -2018) .

year	Numbers of camel exported
2012	18836
2013	1715
2014	14944
2015	18635
2016	4482
2017	31426
2018	22076

Source: information and reports office – Suakin quarantine

2.3 Study Design

Data was collected as part of a study on the serosurvey of Brucella infection in camels herding in Swakin quarantine, across sectional study was carried out during (October 2017 – October 2018) to estimate the sero prevalence of camel's brucellosis and to investigate associated risk factors. Non probability (convenience sampling) for homogeneous sampling units was designed based on location, age, gender, breed and purpose. Individual animals based on simple random sampling. Four locations (centre) selected randomly during the study namely, Red sea, Kassala, Khartoum and North kordofan.

2.4 Sample Size

The sample size of the study animals was determined by using the formula given for simple random sampling method. The relevant formula for 95% confidence and 5% precision was:

$$N=1.96^2 \, Pexp \, (1-Pexp)/D^2$$

Where: n = required sample size

Pexp = expected prevalence

D = desired absolute precision (Thrusfield 2005)

according to (Omer etal .,2010)With 5% desired precision, at 95% confidence level and with expected prevalence of 40%, a total of 240 serum samples were supposed to be collected from Swakin quarantine.

2.5 Sampling Technique

Blood samples were collected under sterile hygienic condition from camels which are ready for export to kingdom Saudi Arabia and Arabian Gulf countries. Blood samples (5ml) were obtained by jugular venupuncture using sterile new syringe and vaccutainer test tubes from each animal. The samples were left at room temperature overnight to allow clotting for sera separation. They were centrifuged at 1000 rpm for 5 mints. Sera were then decanted into 5 ml plastic tubes and stored in the refrigerator at -20°C in a leak-proof container until laboratory test was performed by RBPT and ELISA.

2.6 Questionnaire survey

Information of each camel sampled was obtained; this included location, age, gender, breed and purpose.

2.7 Diagnostic Techniques

2.7.1: m RBPT (modified Rose Bengal Plate 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(Blasco, 1994).

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 25µl of each serum sample was placed on aplastic plate, or in WHO haemagglutination plate. The antigen bottle was shaked well, but gently, and an equal volume of the antigen75 µl was placed near each serum spot. Immediately after the last drop of antigen had been added to the plate, both the serum and antigen were mixed thoroughly (using a clean plastic rod for each test) to produce a circulator 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 were begun to verify the sensitivity of test conditions. . Results were considered positive when there was any degree of visible agglutination, were read and recorded as + + ++ (coarse clumping and clearing), + + + (clumping and some clearing), + + (visible fine agglutination), + (weak fine

agglutinations using magnifying glass) and in case of positive reactions, and 0 (no agglutinations) in negative reactions.

2.7.2 Competitive ELISA

The competitive enzyme- linked Immunosorbent assay kit was carried out using SVANOVIR, brucella –Ab- ELISA kit purchased from the company, Svnovia Bio tech AB Uppsala, Sweden.

Test Procedure:

Test serum was added per each well of the micro titer plate which had ninety six columns (wells).45 µL of Sample Dilution Buffer was added into each well that would be used for serum samples, serm controls and conjugate controls.5 µL of positive, weak positive and negative serum controls was added into each of the appropriate wells .50 µL of mAb solution was added into all used for controls and samples. The plate was sealed and mixed the reagents thoroughly for 5 minutes used plate shaker. The plate was incubated at room temperature 18-25c for 30 minutes. The plates / stripes were rinsed 4 times with PBS Tween Buffer. 100 µL of the prepared conjugate solution was then dispensed in all wells and incubate at room temperature for 30 minutes. After 30 minutes incubation the plate was rinsed 4 times. 100 µL of substrate solution was added to each well and incubated for 10 minutes at room temperature. The reaction was slowed by adding 50 µL of the stopping solution to each well. Control Setup.

Results: The lack of color development indicated that the sample tested was positive. A positive / negative cut-off can be calculated as 60% of the mean of optical density (OD) of the 4 conjugate control wells. Any test sample giving an OD equal to or below this value should be regarded as being positive.

2.8 Statistical Analysis

Statistical analysis was done using IBM SPSS Statistics version 20.0. Descriptive statistics was used as count and percent for the variables. While analytical statistics such as chi-square ($\chi 2$) was employed to demonstrate the association between some factors and occurrence of the camel brucellosis. For quantification of positive association, logistic regression model was not used. The relationship of associated risk factors with positive serological test was computed using odds ratio (OR) signified by 95% confidence intervals (Thrusfield, 2005).

Chapter Three
Results

Chapter Three
Results

3.1 Frequency

3.1.1 Overall Serological Prevalence

In this study, 500 camels were screened using RBPT (Rose Bengal Plate Test), this showed that 30 were seropositive reactors out of 500 serum samples (6%) (Table 3.1). The positive reactors with RBPT (30) were further confirmed using c-ELISA (Competitive Enzyme Linked immunosorbent Assay). Accordingly, twenty four (80%) seropositive camels were observed 24/30*100.

Table 3.1: Frequency of *brucella* in 500 camels in Suakin quarantine examined by RBPT.

RBPT Results	Frequency	Percent	Valid Percent	Cumulative Percent
Positive	30	6.0	6.0	6.0
Negative	470	94.0	94.0	100.0
Total	500	100.0	100.0	

3.1.2 Serological Prevalence in Relationship to Risk Factors

3.1.2.1 General Risk Factors

State (origin):

The study was carried out from four states where the centers of vaccination was found, this comprised Kassala ,North kordofan , Red sea and Khartoum . The number of camels were selected from Kassala were 39.6 %(n=198) , North kordofan31.4(n=157) , Red sea 9.6% (n=48) and Khartoum 19.4% (n=97) (Table 3.2).

Seroprevalence of *Brucella* in camels which came from North Kordofan were 4.5% (n=7), Red sea were 4.2 %(n=2) and from Khartoum were 8.2 %(n= 8) and Kassala was 6.6 % (n=13) (Table3.3).

There was no significant statistical difference between the prevalence in the Four states (P value= 0.587) (Table 3.4).

3.1.2.2 Individual Risk Factors

Sex:

All breeding male and female camels were considered in the analysis. From the total camels tested 9.2% (n=46) were females while 90.8 %(n=454) were male camels. (Table 3.2). Seroprevalence of *Brucella* in male camels was 6.4 % (n=29), relatively higher than that of the female animals which were 2.2 % (n=1) (Table3.3).

There was no significant difference observed in the analysis between female and male(P Value=0.252) (Table 3.4)

Age:

Categorization was based on the physiological maturity for breeding purpose where young group were considered below 4 years and adult group above 4 years old.

Out of the total camels, sampled 23.8 % (n=119) were young while 76.2% (n=381) were adult camels (Table3.2). In this observation Seroprevalence of *Brucella* was 7.3 % (n=28) in adult camels and young camels 1.7 % (n=2) in (Table3.3). There was no statistical significance between 2 age groups (P value=.023) (Table3.4).

Breed:

Individual camels selected in this study came from 2 breeds, Baladi and Bishari .From the total camels screened 26.2% (n=131) were Bishari while 73.8% (n=369) were Baladi (Table3.2). Of the Baladi 8.1% (n=30) were found seropositive in the study while of the Bishari 0.0% (n=0.0) (Table3.3) There was statistical significance difference between the 2 breeds (P value=.001) (Table3.4).

Purpose:

Out of all camels examined 22.2% (n=111) were racing camels and meat camels 77.8% (n=389) (Table3.2).The occurrence of the disease in meat was 6.7 %(n=26) this was higher than in racing camels 3.6% (n= 4) (Table3.3). There was no statistical significance difference between two purposes (P value=0.228) (Table3.4).

Table 3.2: Distribution of 500 camels examined for brucellosis in Suakin quarantine according to potential risk factors

Risk factors	Frequency	percent %	Valid percent%	Cumulative Frequency %
State				
Kassala	198	39.6	39.6	39.6
North	157	31.4	31.4	71.0
Kordofan	48	9.6	9.6	80.6
Red sea	97	19.4	19.4	100.0
Khartoum				
Sex				
Male	454	90.8	90.8	90.8
Female	46	9.2	9.2	100.0
Age				
<4 years	119	23.8	23.8	23.8
>4 years	381	76.2	76.2	100.0
Breed				
Bishari	131	26.2	26.2	26.2
Baladi	369	73.8	73.8	100.0
Purpose				
Race	111	22.2	22.2	22.2
Meat	389	77.8	77.8	100.0
Total	500	100.0	100.0	

Table 3.3: Cross tabulation for the prevalence of brucellosis and associated risk factors in 500 camels examined by RBPT in Suakin quarantine

Risk factors	No. tested	No. positive	Percentage (%)
State			
Kassala	198	13	6.6
North Kordofan	157	7	4.5
Red sea	48	2	4.2
Khartoum	97	8	8.2
Sex			
Male	454	29	6.4
Female	46	1	2.2
Age			
<4 years	119	2	1.7
>4 years	381	28	7.3

Table 3.3: Cross tabulation for the prevalence of brucellosis and associated risk factors in 500 camels examined by RBPT in Suakin quarantine continued

Breed			
Bishari	131	0.0	0.0
Baladi	369	30	8.1
Purpose			
Race	111	4	3.6
Meat	389	26	6.7

Table 3.4: Univariate analysis for the prevalence and risk factors of brucellosis diagnosed by RBPT in 500 camels in Suakin quarantine using the Chi-square ($\chi 2$)

Risk factors	**No. tested**	**No. positive**	**Percentage (%)**	**Degree of freedom**	**$\chi 2$**	**P-value**
State				3	1.928	.587
Kassala	198	13	6.6	-	-	-
North	157	7	4.5	-	-	-
Kordofan	48	2	4.2	-	-	-
Red sea	97	8	8.2	-	-	-
Khartoum						
Sex				1	1.315	.252
Male	454	29	6.4	-	-	-
Female	46	1	2.2	-	-	-
Age				1	5.166	.023
<4 years	119	2	1.7	-	-	-
>4 years	381	28	7.3	-	-	-
Breed				1	11.330	.001
Bishari	131	0.0	0.0	-	-	-
Baladi	369	30	8.1	-	-	-
Purpose				1	1.453	.228
Race	111	4	3.6	-	-	-
Meat	389	26	6.7	-	-	-

3.2 Logistic Regression

The Univariate analysis by Chi-square on camel risk factors revealed one variables with P<0.005 (Breed P=.001). The multivariate logistic model could not be done because of one risk factor revealed by univariate analysis

Chapter Four
Discussion

Chapter Four

Discussion

Brucellosis is a worldwide distributed and can spread among camels and other farm animals through direct contact with blood, placenta, fetuses or uterine secretions, or through consumption of contaminated raw animal products. Consumption of unpasteurized milk and milk products from camels and other farm animals are considered to be the main source of infection as well as an occupational hazard in human (Almuneef et al., 2004).

In animals the disease is characterized by losses due to abortion or stillbirths, irregular breeding, loss of milk production, and in human-beings, the disease is characterized by intermittent fever, chills, sweating, headache, myalgia, arthralgia, and a diversity of nonspecific symptoms (Young and Corbel, 1989; Nicoletti, 1982).

In production system where livestock diversification is practiced, the disease circulates in sheep, goats and cattle, and further spreads to dromedaries (Andreani et al., 1982; Radwan et al., 1992).

Brucella species can infect humans and the most pathogenic and invasive species for human is *B. melitensis, B. suis*, *B. abortus and B. canis* (Acha et al., 2003).The zoonotic nature of the marine Brucellae *(B. ceti)* has been documented (McDonald et al., 2006).

The present study conducted in Swakin quarantine based on the results of RBPT revealed 6% overall seroprevalence of camel brucellosis. This seroprevalence is in agreement with the previous reports of(Elamir, 2014), 5.8% in Khartoum state, (Mohamed et al., 2015) in Khartoum state, 5.8%, (Tag Elsir, 2002), 6% Kassala State and (Raga, 2000), 6.2% in Darfur State. Slightly higher seroprevalence of camel brucellosis has been recorded by(Osman and Adlan, 1987) in Eastern Sudan, 8%, (Yagoub et al., 1990) 6.95% in Eastern Sudan. However, relatively higher seroprevalence of camel brucellosis has been recorded by (Bitter 1986), in the Eastern Sudan, who reported 16.5% and 32.5%, and reported by(Fayza et al 1990) 15.04%, in Khartoum State. The low seroprevalence observed in the present study might be due to constrict procedures of veterinary services which are done to animals before coming to Swakin quarantine for establishing export procedures.

Camels are infected by lateral infection from the primary host of *Br. abortus* (cattle), and *Br. melitensis* (sheep and goats). So, the prevalence rate of brucellosis in camels increases when herded with these animals. Similarly, prevalence rate in area where camels were reared with cattle, 1.9% and 4.8% in herds newly introduced into such areas.

As seen from the results , risk factors such as location ,sex ,age and purpose was not associated with present of the disease, only the breed of camel showed significant association with the prevalence of *Brucella* infection (P=0.001). The sero-prevalence rate in males was (6.4%), and in females was (2.2%), this agree with that reported by (Raga, 2000) in Darfur States, she found prevalence rated 11.4%in males and 4.2% in females. In contrast, (Musa, 1995) reported 7.05% in males and 7.69% in females.

The prevalence was lower among the young animals screened in this study compared to the older ones (P=0.023). In this observation seroprevalence of Brucella was 1.7 % (n=2) in young and 7.3 % (n=28) in adult camels. The same results was recorded by (Musa and Shigidi ,2001; (Bati, 2004; AlMajali et al., 2008; Dawood , 2008; Omer et al., 2010 and Swai et al., 2011). Usually young animals are protected by maternal immunity until when the immunity disappears, thus susceptibility seems to be low among them. Also, older camels are more exposed. The presence of growth factors such as erythritol and hormones favor infection in mature animals. The high prevalence seen in the older animals was demonstrating the chronic nature of brucellosis.

The occurrence of the disease in meat camel 6.7 %(n=26) was higher than in racing camels 3.6% (n= 4). There was no statistical significance difference between two purposes (P value=0.228) this result is in agreement with that reported by (Elamir, 2014)(camel meat 6.2 % (n=9) and racing 4.8 % (n= 9)).

In Swakin quarantine breed in this study considered to be statistical significance difference at the occurrence of the disease of seroprevalence of Brucella was 8.1% (n=30) in Baladi ,while of the Bishari was 0.0% (n=0.0) (P value=.001) . This result disagrees with that reported by (Elamir, 2014).

The result appeared that the prevalence of the disease in Bishari was 0.0% this may be due to the care of exporters to make sure that the animal should be free of brucellosis before entering to quarantine because of its precious high cost.

Conclusion

The present study showed that seroprevalence of camel brucellosis was low. In univariate analysis age, sex, purpose and location (state) had no significant(%) association with Brucella seropositivity at animal level. However, at breed, Brucella seropositivity was significantly associated with the degree of the disease. Although seroprevalence of camel brucellosis is low, the seropositive animals may serve as future foci of infection, pose public health risk, leads to low productivity and market value of camels. According to the (OIE, 2009) only samples positive with mRBPT should be confirmed by the cELISA as more false positive samples by the mRBPT. Results of the present study clarified the status of camel brucellosis in Swakin quarantine and the risk factors that contribute to the occurrence of the disease in dromedaries.

Recommendations :-

1. Further research on vaccination and suggestion for proper program for eradication of *Brucella* infection.
2. Further epidemiological studies leading to improvement of health and management of camels and education of pastoralists are imperative to fully exploit the camel resources of the areas.
3. Other preventive measures by government such as control of animal's movement across the borders should be followed.
4. A routine vaccination for cattle, sheep and goats should be considered in areas where camels are kept together with these animals.

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APPENDIX 1

Distribution of 500 camels examined for brucellosis in Suakin quarantine according to potential risk factors

Table 1: Distribution of Camels in states according to statu

State	Frequency	Percent	Valid percent	Cumulative percent
Kassala	198	39.6	39.6	39.6
North kordofan	157	31.4	31.4	71.0
Red sea	48	9.6	9.6	80.6
Khartoum	97	19.4	19.4	100.0
Total	500	100.0	100.0	

Table 2: Distribution of Sex among Tested Camels

Sex	Frequency	Percent	Valid percent	Cumulative percent
Male	454	90.8	90.8	90.8
Female	46	9.2	9.2	100.0
Total	500	100.0	100.0	

Table 3: Distribution of Age among Tested Camels

Age	Frequency	Percent	Valid percent	Cumulative percent
<4	119	23.8	23.8	23.8
>4	381	76.2	76.2	100.0
Total	500	100.0	100.0	

Table 4: Distribution of Breed among Tested Camels

Breed	Frequency	Percent	Valid percent	Cumulative percent
Bishari	131	26.2	26.2	26.2
Baladi	369	73.8	73.8	100.0
Total	500	100.0	100.0	

Table 5: Distribution of purposes among Tested Camels

purpose	Frequency	Percent	Valid percent	Cumulative percent
Race	111	22.2	22.2	22.2
Meat	389	77.8	77.8	100.0
Total	500	100.0	100.0	

APPENDIX 2

Cross tabulation for the prevalence of brucellosis and associated risk factors in 500 camels examined by RBPT in Suakin quarantine

Table 1: Prevalence of *Brucella* in the states by RBPT

state or origin			Result of Rose Bengal Test		Total
			positive	negative	
	Kassala	Count	13	185	198
		% within state or origin	6.6%	93.4%	100.0%
		% within result of result of Rose Bengal Test	43.3%	39.4%	39.6%
	North kordofan	Count	7	150	157
		% within state or origin	4.5%	95.5%	100.0%
		% within result of result of Rose Bengal Test	23.3%	31.9%	31.4%
	Red sea	Count	2	46	48
		% within state or origin	4.2%	95.8%	100.0%
		% within result of result of Rose Bengal Test	6.7%	9.8%	9.6%
	Khartoum	Count	8	89	97
		% within state or origin	8.2%	91.8%	100.0%
		% within result of result of Rose Bengal Test	26.7%	18.9%	19.4%
Total		Count	30	470	500
		% within state or origin	6.0%	94.0%	100.0%
		% within result of result of Rose Bengal Test	100.0%	100.0%	100.0%

Table 2: Prevalence of *Brucella* in sex of camel by RBPT

sex of camel			Result of Rose Bengal Test		Total
			positive	negative	
	male	Count	29	425	454
		% within sex of camel	6.4%	93.6%	100.0%
		% within result of Rose Bengal Test	96.7%	90.4%	90.8%
	female	Count	1	45	46
		% within sex of camel	2.2%	97.8%	100.0%
		% within result of Rose Bengal Test	3.3%	9.6%	9.2%
Total		Count	30	470	500
		% within sex of camel	6.0%	94.0%	100.0%
		% within result of Rose Bengal Test	100.0%	100.0%	100.0%

Table 3: Prevalence of *Brucella* in age of camel by RBPT

age of camel			Result of Rose Bengal Test		Total
			positive	negative	
	<4	Count	2	117	119
		% within age of camel	1.7%	98.3%	100.0%
		% within result of Rose Bengal Test	6.7%	24.9%	23.8%
	>4	Count	28	353	381
		% within age of camel	7.3%	92.7%	100.0%
		% within Rose Bengal Test	93.3%	75.1%	76.2%
Total		Count	30	470	500
		% within age of camel	6.0%	94.0%	100.0%
		% within result of Rose Bengal Test	100.0%	100.0%	100.0%

Table 4: Prevalence of *Brucella* in breed of camel by RBPT

breed of camel			Result of Rose Bengal Test		Total
			positive	negative	
	Bishari	Count	0	131	131
		% within breed of camel	0.0%	100.0%	100.0%
		% within Rose Bengal Test	0.0%	27.9%	26.2%
	Baladi	Count	30	339	369
		% within breed of camel	8.1%	91.9%	100.0%
		% within result of Rose Bengal Test	100.0%	72.1%	73.8%
Total		Count	30	470	500
		% within breed of camel	6.0%	94.0%	100.0%
		% within result of Rose Bengal Test	100.0%	100.0%	100.0%

Table 5: Prevalence of *Brucella* in purpose of camel by RBPT

purpose of camel			Result of Rose Bengal Test		Total
			positive	negative	
	race	Count	4	107	111
		% within purpose of camel	3.6%	96.4%	100.0%
		% within result of Rose Bengal Test	13.3%	22.8%	22.2%
	meat	Count	26	363	389
		% within purpose of camel	6.7%	93.3%	100.0%
		% within result of Rose Bengal Test	86.7%	77.2%	77.8%
Total		Count	30	470	500
		% within purpose of camel	6.0%	94.0%	100.0%
		% within result of Rose Bengal Test	100.0%	100.0%	100.0%

APPENDIX 3

Univariate analysis for the prevalence and risk factors of brucellosis diagnosed by RBPT in 500 camels in Suakin quarantine using the Chi-square ($\chi 2$)

Table 1: Chi-square of Association of *Brucella* and states

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.928[a]	3	.587
Likelihood Ratio	1.933	3	.586
Linear-by-Linear Association	.156	1	.692
N of Valid Cases	500		

Table 2: Chi-square Association of *Brucella* and Sex

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.315[a]	1	.252
Continuity Correction[b]	.674	1	.412
Likelihood Ratio	1.679	1	.195
Fisher's Exact Test			
Linear-by-Linear Association	1.312	1	.252
N of Valid Cases	500		

Table 3: Chi-square Association of *Brucella* and age

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5.166[a]	1	.023
Continuity Correction[b]	4.210	1	.040
Likelihood Ratio	6.574	1	.010
Fisher's Exact Test			
Linear-by-Linear Association	5.156	1	.023
N of Valid Cases	500		

Table 4: Chi-square Association of *Brucella* and breed

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	11.330[a]	1	.001
Continuity Correction[b]	9.935	1	.002
Likelihood Ratio	18.900	1	.000
Fisher's Exact Test			
Linear-by-Linear Association	11.308	1	.001
N of Valid Cases	500		

Table 5: Chi-square Association of *Brucella* and purpose

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.453[a]	1	.228
Continuity Correction[b]	.958	1	.328
Likelihood Ratio	1.620	1	.203
Fisher's Exact Test			
Linear-by-Linear Association	1.450	1	.229
N of Valid Cases	500		