بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Sero-epidemiological Study of Camel Brucellosis (*Camelus Dromedarius*) and Associated Risk factors in the Butana Plain Area and Al-Gadarief State – Sudan

دراسة مصلية وبائية لمرض البروسيلا في الإبل (وحيدة السنام) وعوامل الخطر المرتبطة بالمرض في منطقتي سهل البطانة وولاية القضارف ـ السودان

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الآيـــــــــــــة

قال تعالي:

بسم الله الرحمن الرحيم

(أَفَلَا يَنْظُرُونَ إِلَى الْإِبِلِ كَيْفَ خُلِقَتْ)

صدق الله العظيم

سورة الغاشية الآية (17).

DEDICATION

This work is dedicated to the souls of my parents.

To my brothers and sisters.

To my teachers and friends.

With warm wishes and best regards…

BADRELDEIN…

Acknowledgement

Above all, praise is to my almighty Allah for giving me good health, wisdom, ability, and strength to carry out this work and for all other graces.

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List of abbreviations and acronyms

Abbreviation	Meaning
BPAT	Buffered Plate Agglutination Test
BTWC	Biological and Toxin Weapons Convention
CDC	Centers for Disease Control and Prevention
Chi^2	Chi Square
CI	Confidence Interval
DNA	Di Nucleic Acid
Co_2	Carbon Dioxide
ELISA	Enzyme Link Immunosorbent Assay
FAO	Food and Agriculture Organization
Ig	Immunoglobulin
ISAbs	International Standard Anti-*Brucella abortus* Serum
LA-OTC	Long-acting Oxytetracycline
LPS	Lipopolysaccharides
MGB	Minor Groove Binding protein
µg	Microgram
µl	Micro liter
OIE	Office International des Epizooties
OR	Odd Ratio
PCR	Polymerase Chain Reaction
R-LPS	Rough Lipopolysaccharides
RAPD-PCR	Random Amplified Polymorphic-PCR
RBPT	Rose Bengal Plate Test
SAT	Standard Agglutination Tube
Sd	Standard Deviation
SSA	Sub-Saharan Africa

List of abbreviations and acronyms - Continued

Abbreviation	Meaning
S-LPS	Smooth Lipopolysaccharides
SCAHAW	Scientific Committee on Animal Health and Animal Welfare
SNPs	Single Nucleotide Polymorphisms
Spp.	Species
SPSS	Statistical Package for the Sciences
USDA	United States Department of Agriculture
WAHID	World Animal Health Information Database
WHO	World Health Organization

Abstracts

Across-sectional sero-epidemiological study was conducted during the period 25thJanuary to 10th –May /2015, with aim of determining the prevalence of camel brucellosis and to investigate the potential risk factors associated with the disease in the Butana plain area and Al-Gadareif State, Sudan.

A total of 320 camels in 12 herds were included in this study and blood samples (320 samples) were collected randomly from selected camels from 12 areas in 6 different locations in the two areas of this study.

In this study, (289/320) 90.3% and (31/320) 9.7% were females and male camels, respectively. Serum samples were tested by Rose Bengal Plate test, and confirmed by Standard Agglutination Test (SAT).

Among all serum samples screened by (RBPT), (23/320) 7.2% were sero-positive, and only (4 /23) 1.2% were confirmed positive by the SAT test. The study showed that only two herds among the 12 herds included in the study were sero-positive. The results of the study showed that the prevalence of camel brucellosis in the two areas of the study was 7.2% (23/320), and the prevalence according to the location of study was; western Butana 18.7% (23/123), northern Butana, southern Butana, eastern Butana, central Butana, and Al-Gadarief State were (0%). The prevalence of the disease was higher in Butana 7.2%, than Al-Gadarief State (0%) with significant statistical difference (p-value $\leq$ 0.25). Sero-prevalence of brucellosis in female camels was 7.6% relatively higher than that of male camels which was 3.2%.

In univariate analysis by using chi-squire to analyze the risk factors involved in the susceptibility of brucellosis had a significant association

with the occurrence of the disease. Thirteen risk factors had significant association with the occurrence of brucellosis, while five risk factors had no significant association with brucellosis in camels (p-value ≤ 0.25).

The multivariate analysis by logistic regression to determine the strength of significance of risk factors associated with the occurrence of brucellosis showed that only two risk factors are strongly associated with brucellosis in camels (p-value ≤ 0.05).

The results obtained in the current study of the present study revealed the status of sero-positivity prevalence of brucellosis in camels (*Camelus Dromedarius*) and the potential contribution of risk factors which contribute in the occurrence of the disease, and indicated the distribution of the disease between camel herds in Al-Gadarief State and Butana plain area. Moreover, the study indicated that brucellosis is one of the major problems affecting the health and production of camel breeds.

مُلخص البَحث

أجريت دراسة مقطعية مَصلية في الفترة من ٢٥ يناير حتي ١٠مايو /٢٠١٥م ، لتحديد مدي إنتشار داء البروسيلا في الإبل (وحيدة السنام)، ولتقصي عوامل الخطر المرتبطة بالإصابة بهذا المرض في منطقة سهل البطانة و ولاية القضارف – السودان.

تمت الدراسة على عدد ٣٢٠ رأس من الإبل في ١٢ من القطعان. حيث تم جمع ٣٢٠ عينة من دم الإبل المختارة عشوائيا من ١٢ موقع مقسمة علي ٦ إتجاهات مختلفة في هذه الدراسة.

في هذه الدراسة كانت أعداد الاناث ٢٨٩ من العدد الكلي (٣٢٠) أي بنسبة ٩٠,٣% و عدد ٣١ من ذكور الإبل من العدد الكلي (٣٢٠) أي بنسبة ٩,٧%. تم اختبار عدد ٣٢٠ مصل من الإبل المنتخبة بواسطة إختبار الروز بنقال وأُكدت النتائج بإختبار "السات".

كانت نتائج فحص عينات المصل بواسطة إختبار الروز بنقال موجبة في ٢٣ عينة من العدد الكلي (٣٢٠ مصل) أي بنسبة ٧,٢% وعند تأكيد الإختبار بتقنية "السات" تبين أن عدد أربعة عينات فقط كانت موجبة من بين العينات الموجبة باختبار الروز بنقال (٢٣ عينة).كما تبين من خلال هذه الدراسة أن إثنين من القطعان كانت موجبة من بين ١٢ من قطعان الإبل التي شملتهم هذه الدراسة.

أظهرت نتائج هذه الدراسة أن نسبة الإصابة في منطقتي الدراسة كانت ٧,٢% (٣٢٠/٢٣) وكانت نسبة الإصابة حسب المواقع على النحو التالي: غرب البطانة ١٨,٧% (١٢٣/٢٣)، شمال البطانة، جنوب البطانة، شرق البطانة، ووسط البطانة، والقضارف كلها كانت نسبتها ٠%، نسبة الإصابة كانت أعلي في منطقة البطانة ٧,٢%، مما عليه في ولاية القضارف ٠% حسب الفرق الإحصائي المعنوي ($p\text{-value} < 0.25$)ونسية الإصابة بالبروسيلا في الإناث ٧,٦% حيث كانت أكثر من نسبة الإصابة في الذكور ٣,٢%.

أظهر التحليل وحيد المتغير بإستخدام مربع كاي لتحليل عوامل الخطورة المتوقعة إرتباطها معنوياً بحدوث داء البروسيلا حيث وُجِدَ أن ١٣ عاملاً من عوامل الخطورة مرتبطة إرتباطاً معنوياً بِحدوث داء البروسيلا في الإبل بينما تبين أن خمس فقط من عوامِل الخطورة ليس لها إرتباطاً معنوياً بِحدوث داء البروسيلا في الإبل ($p\text{-value} \leq 0.25$).

أظهر التحليل مُتعدد المُتغير بإستخدام التشتُت اللوجستي لتحديد قوة إرتباط عوامل الخطورة بِحدوث داء البروسيلا في الإبل أن عاملان فقط لهما إرتباطاً معنوياً بحدوث داء البروسيلا في الإبل ($p\text{-value} \leq 0.05$).

النتائج المتحصل عليها من خلال هذه الدراسة بينت حالة إنتشار داء البروسيلا في الإبل (وحيدة السنام) من خلال الفحص المصلي بواسطة الروز بنقال وتأكيد هذه النتائج بتقنية السات، ذلك بالاضافة للتعرف على عوامل الخطور التي تسهم في حدوث الإصابة بداء البروسيلا،كما شارت الدراسة إلي مدي إنتشار المرض بين قطعان الإبل في منطقتي سهل البطانة وولاية القضارف. وأكدت الدراسة أن داء البروسيلا من أهم الأمراض التي تؤثر علي صحة وإنتاج سلالات الإبل.

INTRODUCTION

1.1. Background

Camels (*Camelus Dromedarius*) are vital domestic animal species that are best adapted to harsh environments and fluctuating nutritional conditions of arid and extreme arid zones. These animals are endowed with extraordinary features that enable them to survive and perform in such hard conditions (Tassew, 2014). *Dromedarius* are versatile living assets that ensure food security even during the dry periods and also serve as means of transportation and draught power (Higgins *et al.*, 1992). In spite of its vital importance particularly to the marginalized communities in the dry zones of tropics and subtropics, studies about camel are very few, due to the fact that camel production is in remote, migratory and poor infrastructure conditions. Available studies were based on small animal numbers, one time survey, interviewing, questionnaires, estimation and simulations (Schwartz and Dioli, 1992).

The One-humped camel(*Camelus Dromedaries*) play an important socio-economic role within the pastoral and agricultural systems in dry and semi-dry zones of Asia and Africa(Gwida *et al*., 2011).Camels are not known to be primary hosts of *Brucella*, but they are susceptible to *B. abortus, B. melitensis* and *Brucella ovis* (Seifert, 1996). Consequently, the prevalence depends upon the infection rate in primary hosts being in contact with them (Musa *et al*., 2008). The economic and public health impact of brucellosis remains of concern in developing countries (Gessese *et al*., 2014).

The disease can generally cause significant loss of productivity through late first calving age, long calving interval time, low herd fertility and comparatively low milk production in camels. The disease poses a

barrier to export and import of animals constraining livestock trade and is an impediment to free animal movement (Zinsstag *et al*., 2011). *B. melitensis* is considered to have the highest zoonotic potential, followed by *B.abortus*, and *B. suis.* The disease presents as an acute or persistent febrile illness with a diversity of clinical manifestations in humans (Bechtol *et al*., 2011).

Camels in Sudan were previously reared in arid and semi-arid lands. They moved to higher rainfall areas side by side with other domestic livestock and wildlife. This change resulted in exposure of camels to diseases that were uncommon in their natural habitat, e.g. fasciolosis, dermatomycosis, tick paralysis, trypanosomosis, theileriosis and brucellosis (Musa and Shigidi, 2001). Many tribes in different parts of the Sudan depend entirely on camels for their livelihood. Camel meat is consumed throughout the country and the animals contribute effectively to the economy by their use in agricultural practices and exportation. However, brucellosis has emerged as a major cause of abortion, hence a constraint to their breeding (Musa, 1995; Agab *et al*., 1996), and has had a negative impact on the export of camels. Reports from veterinary laboratories have indicated that the prevalence of brucellosis in camels in some localities in Sudan is increasing (Omer *et al*., 2010).

Brucellosis is a highly contagious, zoonotic and economically important bacterial disease of animals worldwide (OIE, 2000). It causes significant economic losses including abortion, loss in milk production and low fertility rate in animals. Among the genus, *Brucella abortus* and *Brucella melitensis* are the leading cause of brucellosis in livestock (Tilahun *et al*., 2013). One of the major factors contributing to the spread of disease is free movement of nomadic pastoralists who are accustomed

to the traditional extensive system of management (Al-Majali *et al.*, 2008).The disease is manifested by late term abortions, weak calves, stillbirths, infertility and characterized mainly by placentitis, epididymitis and orchitis. *Brucella melitensis*, *B. abortus* and *B. suis* are zoonotic pathogenic species which can also infect humans. *B. canis* may cause infections in immunosuppressed individuals (Young, 2000). Globally, this disease is under-reported because of its vague clinical symptoms, difficult laboratory diagnosis and lack of familiarity of the medical professionals (Corbel, 2006). Within Sub-Saharan Africa (SSA), many of the known infectious diseases occur commonly and are poorly controlled, both in livestock and in human populations (Mangen *et al*., 2002). It has been stated that in SSA, the epidemiology of brucellosis in humans and livestock is not well understood, and available data is limited (Schelling *et al*., 2003). Brucellosis is common in rural areas because farmers live in close contact with their animals and often consume fresh unpasteurized dairy products. However, the vending of dairy products may also bring the disease to urban areas (Amenu *et al*., 2010; Bekele *et al*., 2013).

Since *Brucella* species isolated from camels, consumption of milk and meat has led to a high number of human brucellosis cases accordingly, serious public health concern has aroused. Most farmers from nomadic areas believe that camel milk is a healer for many diseases. They drink raw camel milk, and they do not believe that non pasteurized milk can cause disease (Al-Salihi, 2013). There are many difficulties that arise in diagnosis of camel brucellosis, because this disease shows only few clinical signs in comparison to its clinical appearance in cattle (Mousa *et al*., 1987). In addition, camel herds are usually raised in a remote area synchronizes with missing infrastructure. Isolation of the causative agent is very necessary to diagnose the microorganism that

camels are susceptible to; in order to plan for a proper vaccination program. Educational programs and leaflets to aware the Bedouin about the risk of brucellosis will reduce the human infection percentage (Al-Salihi, 2013).

Camels of both species *(Camelus Dromedarius* and *Camelus Bactrianus)* are frequently infected with *Brucella* organisms, especially when they are in contact with infected large and small ruminants (Radwan *et al*., 1992). In addition, abortions have been reported in pregnant camels and *B. abortus* has been isolated from aborted fetuses, genital discharges, urine and milk (Radwan *et al*., 1995). Moreover, *Brucella melitensis* biovars 1 and 2 have been isolated recently from camel milk in Saudi Arabia (Radwan *et al*., 1992).

The purpose of this study was to determine the effect of breed types, sex and age group on somebody measurements of the five Sudanese breed types camel (Amir *et al*., 2015).Livestock population in Sudan was about 141.9 million including 52.1 millions of sheep, 43.4 millions of goats, 41.8 millions of cattle and 4.6 millions of camels (Aoad, 2011).Livestock in the Sudan produces about 46.9% of total agricultural production, 20-25% of total local production and 23.1% of exports revenues. It is the main non- oil export in recent years. Camels (*Camelus Dromedarius*) are important in the Sudan due to adaptation to harsh environments, high population ranking second to Somalia in world 20 million camel population and socioeconomic impacts. Camels are important in the Butana plain due to high population forming about 25% of camel population in the country. Sudanese camels were classified according to location, tribal ownership, colours and function (Agab *et al*., 2014).

In eastern Sudan, camels (*Camelus Dromedarius*) are raised mainly in Butana region and Red Sea coast. In the former, the camel population was estimated around 750.000 head representing 25% of total Sudan camel herd population (Darosa, 2005)**N.** The main camel keeping tribes in Butana region are the Lahawiyin, Kawahla, Shukriya, Rashaida, Bija and Bawadra (Drosa and Agab, 2008).

1.2. Justification

Brucellosis is considered by the Food and Agriculture Organization of the United Nations, the World Health Organization and Office International des Épizooties as one of the most widespread diseases in the world (Bekele *et al*., 2013). According to the OIE, it is the second most important zoonotic disease in the world, accounting for the annual occurrence of more than 500,000 human cases (Pappas *et al*., 2006a).

Brucellosis can affect almost all domestic species, and cross transmission can occur between cattle, sheep, goats, camels and other species (Ghanem *et al*., 2009), causing significant reproductive losses in sexually mature animals (Radostiti *et al*., 1994).

Camels have become a national export commodity to the Middle East. Brucellosis remains a major constraint and there is need to investigate and generate valuable information related to the camel in this traditional socio-cultural environment. Brucellosis has considerable public health importance as owners consume raw camel milk and camel liver is considered as delicacies. The prevalence is higher in intensive camel production system where large herd size kept at close proximity in a farm (Wanjohi *et al*., 2012).

1.3. Objectives of the study

- **Specific objectives**

As the disease has veterinary, public health and economic importance, it is necessary to assess the current status among camels brucellosis data base in selected districts of certain nomadic areas, therefore, the objectives of this study are:

1) To estimate the prevalence of camel brucellosis in Butana plain area and Al-Gdarief state.
2) To investigate the potential risk factors associated with the sero-positivity of camel brucellosis.
3) To determine the attitudes and awareness of owners towards safety and hygiene of brucellosis in these communities.

- **General objectives**

To keep healthy productive herds in order to increase the production and make an effort to present basic data on the epidemiology of camel brucellosis in the selected areas. This will enable planning of strategic control policies to reduce the undesirable and deleterious effects below the economic injury level.

CHAPTER ONE

LITERATURE REVIEW

2.1. Classification of camels (*Camelus Dromedarius*)

Order: *Artiodactyla* (even-toed ungulates).

Suborder: *Tylopoda* (pad-footed animals), *Suiformes* (pig-like), and *Ruminantia* (ruminants).

Family: *Camelidae*.

Genus: *Camelus* (Old-World Genus).

Species: *Camelus dromedarius* (the dromedary, One-humped or Arabian camel), and *Camelus bactrianus* (the Bactrian or the Two-humped camel).

Genus: *Lama* and *Vicugna* (New World camels).

Species: *L. glama*, *L. guanicoe, L. pacos,* and *L. vicugna*, (Gwida *et al*., 2012).

2.2. Distribution of camels (*Camelus Dromedarius*)

The *Camelid* was probably among the last of major domestic species to be put to regular use by man. The most likely time of domestication is about 4000 years before present or slightly earlier. The presumed area of domestication is the southern Arabian Peninsula, probably the area of Yemen and Oman. From presumed center of domestication, dromedary has subsequently been distributed to almost the rest of the world (Wilson, 1998). Environmental, social and cultural factors have great influence on the distribution and production of camels. Arid and semi-arid zones of tropical and subtropical countries of Africa

and Asia are found to be convenient ecology. The greatest cultural influences in recent distribution of camels was the advent of Islam, when Arabs spread their gospel, consolidating its ranges north and east wards in Asia, and along the Mediterranean littoral. There have been many attempts to introduce camels outside the "normal" ranges, in Brazil, Colombia, USA, Cuba, Spain, Italy and France. Generally, there has been steady increase in camel population since about 1980s. However, decrease in numbers has been observed in some countries for instance, where oil is the principal commodity and the nomadic way of life is no longer the major one (Wilson, 1998).Eastern Africa is known to be the heartland for camel production as 80% and 63% of the Africa and world population of camels, respectively produced in the region (Yosef *et al.*, 2015).

Table1: Camel population in some selected countries.

Country	Number (0,000)	Density (No per km^2)	Proportion to total National ruminants (%)
Djibouti	60	0.00	34
Egypt	170	0.16	5.8
Ethiopia	1030-1040	0.33	3.4
India	1100	0.83	0.4
Kenya	620-780	0.08	5.3
Niger	415	0.32	8.3
Saudi Arabia	165	0.00	14.9
Somalia	5800-63500	8.93	46.6
Sudan	2800-3100	0.99	11.1

(Bati, 2004).

Figure 1: Distribution of camel breeds in Sudan, (Ishag *et al*., 2011).

2.3. Potential importance of camels

Camels are primarily the domestic animals of pastoral communities that ensure food security. They produce milk, meat, hair and hides, and also serve as a draught animal for agriculture and transport for people as well as goods (Schwartz and Dioli, 1992). Milk and meat are the important products that camels produce elsewhere. Tefera and Gebreab (2001), reported that the average daily milk yield of a she camel is 2.5 liters. Long lactation and ability to maintain milk production over long dry spells are important facets of camel productivity. Apart from home consumption, the majority of households sell at least one-third of the milk produced to generate cash income (Getahun and Bruckner, 2000). Daily

milk yield can be as high as 20 liters with improved management conditions (Schwartz and Dioli, 1992).

Until the arrival of motorized transport in the arid and semi-arid zones, camels have been the sole means of transport in the areas where they are adapted. They are also used for wheel transport, water lifting and source of power for oil mill. Camel racing and other leisure activities such as camel safaris and trekking have recently become a tourist attraction and luxurious in some parts of the world (Wilson, 1998). From global perspective, the economic production of camels seems minimal. The most significant merit of camels is that they live and perform well in areas where other livestock species do not thrive and perhaps do not survive due to the economic use of water in almost all metabolic functions and wide range of feed resource utilization (Yagil, 1985). In mixed species, the camel feeds on plants or part of plants that are not eaten by other conventional livestock due to its size which enables them to browse the highest strata, thus reducing competitions and enhancing complementarities (Bati, 2004).

2.4. Brucellosis

2.4.1. Historical prospective of brucellosis

Brucellosis, formerly known as Mediterranean fever, Malta fever or undulant fever, is a bacterial infection characterized by wave-like variations in the body temperature of afflicted victims. It presents with migratory myalgia and arthralgia, diaphoresis and headaches, with late complications of granulomatous hepatitis, endocarditis, blood dyscrasias, and neuro-ophthalmologic sequelae. Its modern name bears tribute to Sir David Bruce, the military physician who discovered the etiologic agent. He isolated the organism which he termed ‘micococcus’ and infected

monkeys, and produced clinical signs similar to those observed in humans, thus fulfilling Koch's postulates. Bruce sent the micrococcus to Pasteur Institute in Paris, who identified the causative organism as *Micrococcus melitensis*, now renamed *Brucella melitensis*

Other *Brucella* species that can infect animals, such as those that cause abortions in cattle, have since been identified. In 1905, Themistocles Zammit, a scientist-member of the Bruce-led Mediterranean Fever Commission, proved that the reservoir for the organism was goat's milk. Goat's milk was eliminated from the British soldier's diet, and in the process, brucellosis was removed from Malta (Tan *et al*., 2011).

2.4.2. Definition of the diseases

Brucellosis is a contagious disease caused by bacteria of the genus *Brucella.* Taxonomically, the genus *Brucella* is divided into ten classified species and subdivided into biovars (Wernery, 2014).Brucellosis is considered by the (FAO), (WHO) and the (OIE) as one of the most widespread zoonosis in the world (Schelling *et al.*, 2003).

According to OIE, it is the second most important zoonotic disease in the world after rabies. The disease affects cattle, swine, sheep, goats, camels and dogs. It may also infect other ruminants and marine mammals. Synonyms of Brucellosis include: undulant fever, Malta fever, Mediterranean fever, enzootic abortion, epizootic abortion, contagious abortion, and Bang's disease. It causes significant reproductive losses in sexually mature animals (Wadood *et al*., 2009).The disease is manifested by late term abortions, weak calves, still births, infertility and characterized mainly by placentitis, epididymitis and orchitis, with excretion of the organisms in uterine discharges and milk (England *et al.*, 2004). It also causes morbidity and considerable loss of productivity. The

disease is important from economic point of view; it is one of the most devastating trans-boundary animal diseases and also a major barrier for trade (Abubakar *et al*., 2012).

2.4.3. Etiology

There are six species that have so far been known in the Genus *Brucella*: *B. abortus*, *B. melitensis*, *B. canis*, *B. suis*, *B. ovis* and *B. neotome* (Bati, 2004). Camels are not known to be primary host for any of *Brucella* organisms but they are susceptible to both *B. abortus* and *B. melitensis* (Wanjohi *et al*., 2012).

Table 2: Summary of *Brucella* isolates from camels (*Camelus Dromedarius*) in different countries.

Country	Authors	Organs or specimens	Species isolated
Egypt	Abou – Eisha (2000)	Milk	*B. melitensis*, biovar3
Iran	Zowghi and Ebadi (1988)	Lymph node	*B. melitensis*, biovar1
Kuwait	Zowghi and Ebadi (1988) Al-Khalaf and El-Khaladi (1989)	Lymph node Fetal stomach Content	*B. melitensis*, biovar3
Libya	Gameel *et al.* (1993)	Milk, aborted fetus, vaginal swab	*B. melitensis*, biovar 1
Saudi Arabia	Radwan *et al.* (1992) Radwan *et al.* (1995) Ramadan *et al.* (1998)	Milk Milk Carpal hygroma	*B. melitensis*, biovar1,2 *B. melitensis*, biovar1,2,3 *B. melitensis*
Senegal	Verger *et al.* (1979)	Milk	*B. abortus* biovar1,3
Sudan	Agab *et al.* (1994)	L. node testes, vaginal swab	*B. abortus* biovar3

Source: adopted from Abbas and Agab, (2002); Wernery and Kaaden, (2002).

2.4.4. Classification of *Brucella*

Considering their high degree of DNA homology (> 90 % for all species), *Brucellae* have been proposed as a mono specific genus in which all types should be regarded as biovars of *B. melitensis* (Verger *et*

al., 1985). This proposal has not met with complete agreement, the old classification of the genus (and relevant nomenclature) into six species, *i.e. B. melitensis, B. abortus, B. suis, B. neotome, B. ovis* and *B. canis* (Corbel and Morgan, 1984), is the classification used world-wide. The first 4 species are normally observed in the smooth form, whereas *B. ovis* and B. *canis* have only been encountered in the rough form. Three biovars are recognized for *B. melitensis* (1-3), seven for *B. abortus* (1-6 and 9), and five for *B. suis* (1-5). Species identification is routinely based on lysis by phages and on some simple biochemical tests (oxidase, urease).For *B. melitensis*, *B. abortus, B. suis*, the identification at the biovar level is currently performed by four main tests, *i.e.* carbon dioxide (CO_2) requirement, production of hydrogen sulphide (H_2S), dye (thionin and basic fuchsin) sensitivity, and agglutination with mono specific A and M anti-sera. Moreover, a recently developed co-agglutination test, using latex beads coated with a pair of monoclonal antibodies directed against the rough lipopolysaccharide (R-LPS) and the 25 kDa outer membrane protein (Omp 25), respectively (Bowden *et al.*, 1997), makes it possible to accurately differentiate *B. ovis* from *B. canis* and the occasional rough isolates of the smooth *Brucella* species *B. melitensis* biovar 3 appears to be the most frequently biovar isolated in Mediterranean countries. The precise recognition of biovar 3, especially its differentiation from biovar 2 appears sometimes equivocal. Due to the use of insufficiently discriminating mono specific sera, a number of strains identified initially as biovar 2 were later confirmed as biovar 3 by expert laboratories. Intermediate strains are occasionally found due to the instability reported for some of the phenotypic characteristics used for the current classification of *Brucella*. This situation sometimes impedes the identification of the species and their biovars. Therefore, the identification of stable DNA-specific markers is considered a high

priority for taxonomic, diagnostic and epidemiological purposes.Several methods, mainly PCR-RFLP and Southern blot analysis of various genes or loci, have been employed to find DNA polymorphism which would enable the molecular identification and typing of the *Brucella* species and their biovars (Vizcaino *et al.*, 1997). Among these methods, detection of polymorphism by PCR-RFLP is considered to have an advantage over Southern blotting, since it is easier to perform and is less time-consuming when applied to large numbers of samples. Of all the DNA sequences investigated by PCR-restriction, the major outer membrane protein (*omp*) genes of *Brucella* are the most interesting as they exhibit sufficient polymorphism to allow differentiation between *Brucella* species and some of their biovars (Cloeckaert *et al.*, 1996). Studies of the RFLP patterns of two closely related genes, *omp2a* and *omp2b*, encoding and potentially expressing the *Brucella* spp. major *omp* of 36 kDa (Ficht *et al.*,1989), showed that the type strains of the six *Brucella* species could be differentiated on this basis. More recently, Cloeckaert *et al.*, (1995) using PCR-RFLP and a greater number of restriction enzymes detected *Brucella* species, biovar, or strain-specific markers for the *omp25* gene, encoding the *Brucella* 25 kDa major *omp*, and for the *omp2a* and *omp2b* genes. The *omp31* gene (Vizcaino *et al.*, 1996), encoding a major outer-membrane protein in *B. melitensis*, is also an interesting gene for the differentiation of *Brucella* members. Using a combination of *omp31* PCR-RFLP patterns and Southern blot hybridization, profiles of *Brucella* species were differentiated with the exception of *B. neotomae* which was indistinguishable from *B. suis* biovars 1, 3, 4 and 5. It was also shown that *B. abortus* lacks a large DNA fragment of about 10 kb contained in *omp31* and it's flanking DNA (Vizcaino *et al.*, 1997).

More highly conserved *Brucella* genes may also be useful for taxonomic and epidemiological purposes, even if they contain less polymorphism than the OMP genes. In this respect, the *dna*K locus which allows the identification of *B. melitensis*, the main *Brucella* pathogen for sheep, is of particular interest. All *B. melitensis* biovars showed a specific PCR-RFLP pattern with *Eco*RV, consistent with the presence of a single site instead of two for the other *Brucella* species (Cloeckaert *et al.*, 1996). Taxonomic knowledge of *Brucella* has progressed a great deal since the techniques of molecular biology have been applied to these bacteria. A number of molecular tools (nucleic acid probes, primers...) are now available which make the elaboration of a more objective and reliable classification of the genus possible. Judging by the emergence of new *Brucella* types from marine mammals, the genus is far from being completely identified. In the near future, efforts should be concentrated on the harmonization of these tools to propose the most suitable method for the molecular identification and typing of *Brucella* (Sci. com. Anim. HAW, 2001).

2.4.5. Morphology and characteristics of microorganism

Brucellae are small, short rod, coccobacilli or short rod (measuring 0.5 x 0.7 to 0.6x 1.5μm) occurring singly, in pairs or short chains. They are non-spore forming, non-motile, partially acid fast and Gram-negative facultative intracellular bacteria. With modified Zeihl Neelsen staining (0.5% acetic acid), *Brucella* appears as red staining coccobacilli (Quinn *et al.*, 2002). Most strains are aerobic (some are micro-aerophilic) but many of them are carboxyphilic (capnophilic) and best grow in CO_2 enriched atmosphere. Growth is unlikely on an ordinary media. They are catalase and oxidase positive, produce H_2S, hydrolyze urea and reduce nitrate with

some exceptions. The organisms neither produce indole, acetyl methyl carbinol nor utilize citrate (Bati, 2004).

Brucella are generally susceptible to heat, direct sun light, acidic conditions and common disinfectant (Radostits *et al.*, 1994). However, in favorable conditions the organisms may survive 4 to 6 days in urine, 6 weeks in dust, and 4 to 10 weeks in water, 40 to 75 days in aborted fetus. They also survive the production process of soft cheese up to 6 months, in butter up to 4 months, in milk up to 6 months and ice cream up to 30 days (Bati, 2004). Variants of smooth colony are more virulent than non-smooth ones. This suggests the role of the O-chain of smooth lipopolysaccharide (LPS) in determining virulence. The A and M dominant surface antigens are also found in varying concentration among different smooth variants (Walker, 1999).

2.4.6. Clinical signs

Brucellosis is characterized by abortion and to a lesser extent by orchitis and infection of the accessory sex glands in males. According to various researchers, the clinical signs of brucellosis in breeding *camelids* are the same as those in bovines and small ruminants, although infection in breeding *camelids* causes fewer abortions than it does in bovines and small ruminants (Wernery, 2014). Infections may cause stillborn calves, retained placenta, fetal death mummification and reduced milk yield. Also, delayed service age and fertility have been reported (Musa and Shigidi, 2001). A retained placenta is rare in *Camelidae*. This may be a result of the difference in the placental attachment (Fowler, 2010).

Brucella spp. are fairly hardy; organisms that have been recovered from fetal and manure samples that remained in a cool environment for >2 months. However, exposure to sunlight kills the organisms within a

few hours, and the organisms are susceptible to many common disinfectants (Glynn and Lynn, 2008).

2.4.7. Brucellosis in some species

1) **Bovine brucellosis**

There are six different species of *Brucella*, whereby *Brucella abortus* is the predominant species infecting cattle (Rekha, 2013).*Brucella* infection in pregnant cows can cause abortion or premature calving. Furthermore, *Brucella* infection can lead to temporary sterility, death from acute metritis and decreased milk production. In Africa, infection of cattle with *Brucella* spp. has been reported to result in the formation of hygromas, but these do not appear to be a consistent feature of infection. Mangen *et al*., (2002) highlighted that infection does not necessarily lead to clinical signs.

Large quantities of the bacteria are excreted with the fetus, the placenta and the uterine fluid, mainly at the time of calving. After abortion or parturition, the organism continues to be excreted mainly via the milk of infected cows. Infected breeding bulls can transmit the infection to cows at the time of service via the semen. Apart from direct contact between animals, other sources of infection within and between herds are contaminated water and feed supplies (DFRA, 2002)**.**

2) **Sheep and goats brucellosis**

Sheep and goats brucellosis (excluding *Brucella ovis* infection which is not pathogenic for humans) is a zoonotic infection with important effects on both public health and animal health and production and is widespread in many areas of the world, particularly in some Mediterranean and Middle Eastern countries.

Brucella melitensis, the main etiologic agent of brucellosis in small ruminants, was the first species in the genus *Brucella* described. It was first isolated by Bruce in 1887 from the spleens of soldiers dying of Mediterranean fever on the island of Malta. The origin of the disease remained a mystery for nearly 20 years until it was discovered that goats were the source of infection for human populations (Sci. com. Anim., 2001).

3) Equine brucellosis

None of the *Brucella* spp. is adapted to the horse. Equine infections are usually by the cattle pathogen *B. abortus*, although infection with *B. suis* has been reported. There are no reports of natural infection of horses with *B. canis* (Mair and Divers, 2010).*B. abortus* infections in domestic animals have been reported worldwide, but have been effectively eradicated from several European countries, Japan and Israel. There is no apparent age, gender or breed predisposition to infection in horses, although most cases have been reported in horses aged >3 years (Nicoletti, 2007).

4) Canine brucellosis

Canine brucellosis, caused by *Brucella canis*, is an important cause of reproductive failure, particularly in kennels. This organism causes abortions, stillbirths, epididymitis, orchitis and sperm abnormalities in dogs. Although dogs that have been spayed or neutered do not have reproductive signs, they occasionally develop other conditions such as ocular disease and disco-spondylitis. *B. canis* can persist in an animal even after antibiotic treatment. In kennels, infected dogs are often euthanized to prevent them from infecting other dogs or people. Canine

brucellosis is sometimes difficult to diagnose with the currently available tests.

Other *Brucella* species occasionally associated with disease in dogs include *Brucella abortus*, *B. melitensis* and *B. suis*. In addition to the organisms found in dogs, humans can be infected with the less virulent M- strain of *B. canis*, which is used as an antigen for serological testing. (Iowa State University, 2012).

5) Wildlife brucellosis

The occurrence of the disease in humans depends largely on the occurrence of brucellosis in an animal reservoir, including wildlife. The epidemiological link between wildlife and many diseases in livestock is now well recognized. The longstanding conflict between livestock owners and animal health authorities, on the one hand, and wildlife conservationists on the other, is largely based on differing attitudes to controlling livestock diseases which are, or can be, associated with wildlife. The creation of new interfaces between livestock and wildlife due to human activity is the most important factor in disease transmission (Godfroid *et al*., 2013).

Despite their respective host preferences, *B. abortus* and *B. suis* have also been isolated from a great variety of wildlife species, such as bison (*Bison bison*), elk/red deer (*Cervuselaphus*), feral swine and wild boar (*Sus scrofa*), the red fox (*Vulpes vulpes*), the European brown hair (*Lepus europaeus*), African buffalo (*Syncerus caffer*), reindeer (*Rangifer tarandustarandus*) and caribou (*Rangifer tarandus groenlandicus*). Forthis reason, wildlife must be considered a potential reservoir for brucellosis in livestock (Godfroid, 2002).*Brucella melitensis* is rarely reported in wildlife. However, sporadic cases have been reported in

Europe in chamois (*Rupicapra rupicapra*) and ibex (*Capra ibex*) in the Alps (Garin-Bastuji *et al*., 1990). *Brucella ovis* and *B. canis* (responsible for ovine epididymitis and canine brucellosis, respectively) have never been reported in wildlife in Europe. (Ridler *et al*., 2000). For *B. neotomae*, only strains isolated from desert rats in Utah in the United States (USA) have been reported. Since the first description of an abortion due to *Brucella* spp. in a captive dolphin in California in 1994, several reports have described the isolation and characterization of *Brucella* spp. from a wide variety of marine mammals, such as seals, porpoises, dolphins and whales, in almost all the waters covering the globe. Although brucellosis seropositivity has been documented in Antarctic seals, there has been no isolation of *Brucella* spp. from this animal so far. The overall characteristics of marine mammal strains are different from those of any of the six 'classical' *Brucella* species and, since 2007, *B. ceti* and *B. pinnipedialis* (preferentially infecting cetaceans and pinnipeds, respectively) have been recognized as new *Brucella* species (Godfroid *et al*., 2013).

Experimental studies and epidemiological evidence suggest that birds are very resistant to *Brucella* spp. infection. On the other hand, it has been shown that *B. melitensis* biovar 3 can be cultured from both skin swabs and the visceral organs of Nile catfish (El-Tras *et al*., 2010).These findings suggest that fish may also be susceptible to *B. ceti* and *B. pinnipedialis* infection, which would have veterinary public health implications, and this warrants further investigation (Godfroid *et al*., 2013).Nowadays, the control of brucellosis in wildlife should be based almost exclusively on good management practices, and it is thus vital to assess them. As dramatically shown in the GYA, elk (considered to be

dead-end hosts when ranging freely) are becoming maintenance hosts for *B. abortus* when winter feeding is practiced (Campen and Rhyan, 2010).

6) Human brucellosis

Human brucellosis was known in the Mediterranean region since ancient times and was named differently as Malta fever, undulant fever and Mediterranean fever. The new genus *Brucella* was established in 1920. Brucellosis is caused by the organisms of genus *Brucella*. It occurs all over the globe. Domestic animals like sheep, goat, cattle, dogs, pigs and camels are primarily infected. Man is affected by handling animals or their products. The association of man and animals is age old. This close association led to the transmission of various zoonosis including brucellosis from animals to man. Today, human and animal brucellosis is prevalent all over the world and developing countries are the major sufferers (Dakshayani *et al*., 2013).

Today, the disease is mainly occupational in humans (abattoir, animal industry, hunters and health workers) but transmission through the consumption of raw milk and milk products remains important in developing countries. Symptoms such as undulant (rising and falling) fever, tiredness, night sweats, headaches and chills may drag on for as long as three months before the illness becomes so severe and debilitating as to require medical attention (Godfroid *et al*., 2013).

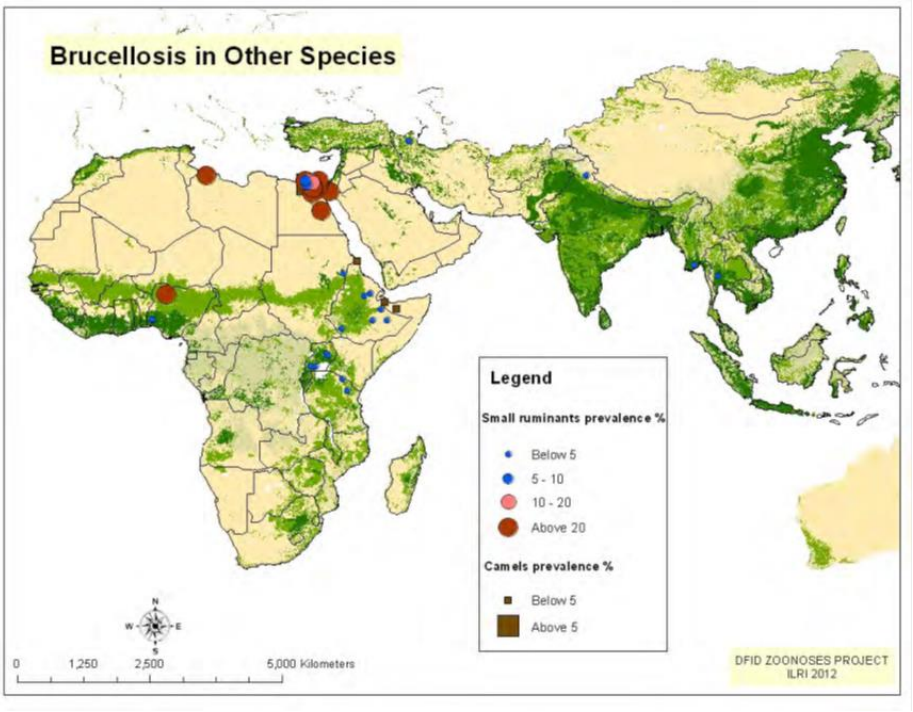

Figure 2: Brucellosis in other species, Report to Department for International Development, UK, (2012).

2.4.8. Pathogenesis and pathological finding

Brucella species are facultative intracellular bacteria able to multiply within human or animal phagocytosis cells, survive intracellular conditions and escape to the host's immune system (Baldwin and Goenka, 2006). Epithelial cells, placental trophoblasts, dendritic cells and macrophages are the target cells (Gorvel, 2008). Transmission of brucellosis to humans is usually the result of direct or indirect contact via ingestion or inhalation, or through conjunctiva or skin abrasions (Young, 2009). Inter human transmission is rare and only, some anecdotic cases are reported as following blood exposure, primary exposure to infected tissues or after sexual contact. Human brucellosis presents in various forms with signs mostly non-specific and similar in patients whatever the route of transmission. The main symptoms are fever or chills, arthralgia, sweating and hepatomegaly and splenomegaly (Santis *et al.*,

2011).Following exposure, the organisms penetrate intact mucosal surface. In the alimentary tract the epithelium covering the ileal Peyer's patches are the preferred sites of entry. After penetration the organisms may be engulfed by phagocytic cells and localized to regional lymph nodes. Then they proliferate, disseminate homogenously and localize in the reticuloendothelial and reproductive tract. Various mechanisms are employed by *Brucella* organisms to survive inside the phagocytic cells: inhibiting phagolysosome fusion, blocking bactericidal action of phagocytes and suppressing the myeloperoxidase H_2O_2 halide system (Bati, 2004).

In pathology these bacteria have a predilection for the pregnant uterus, udder, testicles, accessory male sex glands, lymphnodes, joint capsules and bursae. Lesions may be found in these tissues. They found inflammation of the uterus lining with reddening, oedema and necrotic foci in the uterus epithelium, as well as fibrosis of the endometrium and atrophy of the uterine glands. The authors also observed an increased number of ovariobursal adhesions and hydro-bursae. The adhesions occurred between the bursa ovarica and the ovary and in several cases also between the bursa ovarica and the salpinges, causing a severe in duration of the latter. Hydrobursitis was often observed in brucellosis-positive dromedaries causing an enlargement of the bursa, which was then filled with a clear amber colored fluid. No lesions have been described so far in aborted *camelids* and in brucellosis-positive *camelid* males except orchitis and epididymitis. The testes and epididymis of 360 dromedaries were examined for gross and histopathological lesions. Around 12% of the tested organs originated from seropositive camel bulls. However, from the investigations it was not clear if the

epididymitis, orchitis or testicular degeneration was caused by *Brucella* infection or was a normal pathological feature (Wernery, 2014).

A pregnant llama was experimentally infected by inoculating viable *B. abortus* bacteria into the conjunctival sac. Forty-three days post inoculation; the llama aborted an eight month-old fetus. *Brucella abortus* was isolated from the placenta and all fetal specimens, including the brain, small and large intestines, spleen, kidney, liver, stomach fluid, heart blood and lung. *Brucella* was also isolated from numerous mammary gland lymph nodes in the dams. Histologically there was a moderate, multifocal, lymphocytic and histiocytic, subacute placentitis, with a marked loss of trophoblastic epithelial cells. The chorioallantoic stroma contained abundant necrotic and mineralized debris and the swollen capillaries were expanded by large numbers of *Brucella* organisms (Gilsdorf *et al*., 2001). A few lesions in non-pregnant *B. abortus*-infected dromedaries and in lactating dromedaries that were seropositive for *B. melitensis* and *B. melitensis* was also isolated from milk samples. Cranial and genital lymph nodes from which the pathogen was isolated showed marked sinusoidal edema and follicular hyperplasia of cortical and paracortical areas, with active germinal centers and histocytosis. There were no lesions in the reproductive tract. In Saudi Arabia, pathological and histopathological studies of non-pregnant *dromedaries* naturally infected with *B. melitensis* biovar3 were reported (Wernery, 2014).

Omer *et al*., (2010), described the following changes in different organs:

1) **Lymph node (especially supramammary):** oedema, enlargement, lymphoid hyperplasia, granulomatous reaction in the cortical area of the lymphoid follicle.

2) **Spleen:** enlargement with granular surface in some cases, depletion of some lymphoid follicles, proliferation of fibrous tissue, histiocytosis.
3) **Mammary gland:** granulo-mastitis in some cases, proliferation of interlobular fibrous connective tissue.
4) **Uterus:** moderate amount of mucous and ulceration of endometrial mucosa, endometrial stroma showed edema and diffuse heavy infiltration of mainly macrophages and lymphocytes in the lamina propria, blood vessels dilated and congested.

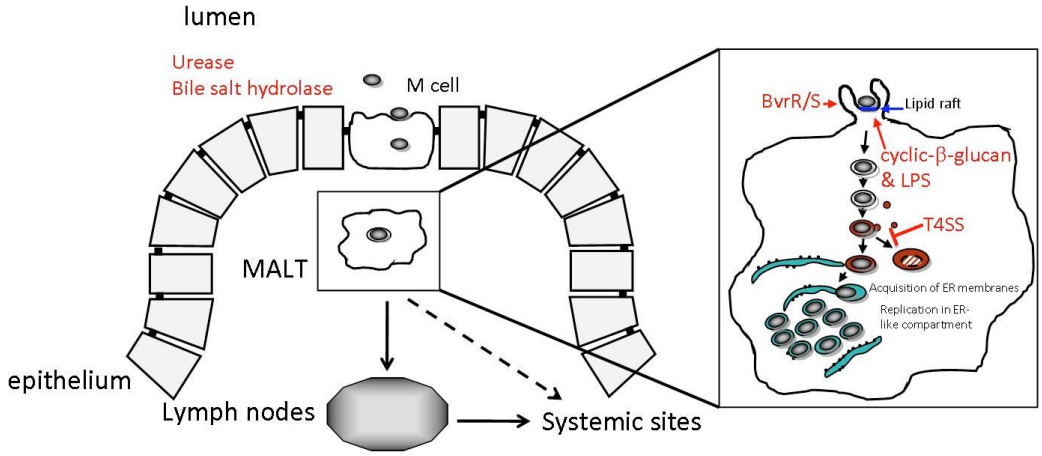

Figure 3: Schematic representation of *Brucella* invasion through the digestive tract. Entry is through M cells and subsequently the bacteria are taken up by macrophages of the mucosa associated lymphoid tissue (MALT). These macrophages transport the bacteria to the lymph nodes and on to systemic sites. Blown up macrophage shows trafficking within the macrophage from entry *via* lipid rafts, through the endosomal pathway to the ER-like compartment in which *Brucella* replicates. In red are *Brucella* virulence factors that are involved in establishing infection (Mariana *et al*., 2010).

2.4.9. Transmission

Both vertical and horizontal transmissions exist in animal brucellosis. Horizontal transmission occurs through ingestion of contaminated feed, skin penetration, via conjunctiva, inhalation and udder

contamination during milking. Congenital infection that happens during parturition is frequently cleared and only few animals remained infected as adult (Radostits *et al.,* 1994). Spread of the disease is due to movement of infected animals to disease free herds. Proximity of infected herd to clean herds happens at water points where a number of camels come together. Epidemiologically important risk factors are large herd size, poor managements, and active abortions, milking more animals by single person and herding with other ruminants. Survival of the organisms in the environment may also play a role in the epidemiology of the disease (Abbas *et al*., 1987; Abuo-Eisha, 2000). 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 (Bati, 2014).

2.4.10. Host factors

Infection may occur in animals of all age groups, but persists commonly in sexually mature animals (Radostits *et al*., 1994). Generally, infection is acquired after three years of age with increase in the subsequent age groups (Abou-Eisha, 2000). Some studies revealed equal distribution of *Brucella* antibodies among males and females (Radwan *et al.,* 1992). In other findings it appeared that females are more susceptible to the disease than males. This was attributed to the fact that females are physiologically more stressed than males (Walker, 1999). Female animals have essential epidemiological importance not only in susceptibility but also in disseminating the disease via uterine discharge and milk. The role of males in the spread of disease under natural conditions not important (Bati, 2014).

The extent to which infection rate varies due to breed difference is not well known. Wernery and Wernery, (1990), reported that breeding camels had lower brucellosis infection rate than racing animals. This was probably because racing camels utilize unpasteurized cow milk.

2.4.11. Diagnosis and diagnostic methods

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). This cross-reactivity exists to:

1) *Yersinia enterocoliticaO*: 9.
2) *Escherichia hermannii.*
3) *E. coli O*: 157.
4) *Francisella tularensis.*
5) *Stenotrophomonas maltophilia.*
6) *Vibrio cholera O*: 1.
7) *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, 1991), making laboratory testing essential. An incorrect diagnosis of brucellosis may occur when based on serology alone (Wernery, 2014).

a) Bacteriological examinations

- **Culturing, isolation and identifications**

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. The recommended medium for isolation of *Brucella* 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). During intensive investigations using selective media it was found that in a camel farm in Saudi Arabia 34% of all *Brucella* seropositive milking dromedaries were *Brucella* shedders. The high number of seropositive animals suggests that it is preferable to use selective media. Tissue specimens from *Brucella*-positive dromedaries were examined by (Omer *et al.*, 2010) using the immune peroxidase test, and obtained very good results. *Brucella* organisms were detected in the cytoplasm of macrophages (visible as brown granules), in the lymphocytes of the lymph nodes and spleen, within the epithelial lining of the endometrium and endothelium of blood vessels, and within mononuclear cells around blood vessels (Radwan *et al.*, 1995).

Farrell's modified medium is used for *Brucella* culture as described by (Radwan *et al*., 1992):

- Fresh milk samples (30 ml taken separately from each quarter) were collected aseptically from all 120 milking camels with *Brucella* antibodies.

- Each milk sample was streaked (with a sterile cotton swab) onto four plates of the selective medium, for determination of *Brucella* shedder camels.
- Similar samples from the same seropositive camels were also culture each week throughout treatment and, after calving, udder secretion samples were also taken, at monthly intervals, from the remaining treated camels (which were not lactating at the time of initiation of treatment).
- The samples were cultured using the same procedure.
- Two *Brucella* shedder camels were kept as controls and used for bacteriological examination.
- One camel was sacrificed immediately prior to initiation of the treatment regimen, and the second was sacrificed four months after the initiation of treatment.
- The samples collected aseptically from the two control camels were: udder secretions and/or udder tissues; supra mammary, pre scapular, iliac, precrural, mediastinal, mesenteric and head lymph nodes; sections of brain, uterus, ovary, liver and spleen; bone marrow from the long bones of the front and hind limbs.
- Each tissue specimen was separately homogenized in a tissue grinder, and aliquots were spread with sterile cotton swabs onto four freshly-prepared plates of culture medium.
- The plates were incubated at 37°C for seven days in the presence and absence of 5% Co_2 atmosphere.
- *Brucella* was successfully isolated.
- The isolated *Brucella* cultures were identified morphologically, microscopically, biochemically and serologically.

- The biotyping of the identified isolates was performed at the Central Veterinary Laboratory, Weybridge, United Kingdom (Radwan *et al*., 1995).

Great care should be employed during handling any material containing *Brucella* organisms. Generally, the 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. The commonly used basal media include: Serum dextrose, serum tryptose agar, glycerol dextrose agar, trypticase, and soya agar (Bati, 2004).

Terzolo *et al.* (1991) suggested that Skirrow agar is a satisfactory medium for both *Brucella* species and *Campylobacter fetus*. Contamination is prevented by use of selective media containing actidione (30 mg/l), bacitracin (25mg/l), polymixin B (5mg/l) and vancomycin (20mg/l) (OIE, 2000). Milk samples, vaginal swabs, semen and aborted fetus are useful for recovering the organisms at antemortem. Samples collected at necropsy include multiple lymph nodes, spleen, udder, pieces of uterus and testicular tissue. Tissue specimens are directly cultured on solid media, whereas milk cultures are performed by centrifuging milk at 5900 to 7700 x g for 15 minutes (Walker, 1999). Cultures are then incubated at 37°C in 5-10% CO_2tension for three days and more. Characteristic colonies have small convex, smooth translucent appearance (Agab *et al.*, 1994). Demonstration of the bacteria is by staining with Gram-negative stain or modified-Zeihl Neelsen stain. Animal inoculation (an old method) can also reveal characteristic lesions in the liver, spleen and epididymis of a guinea pigs (Walker, 1999). Further characterization is based on serotyping, phage typing, dye sensitivity, and biochemical tests. Fluorescent antibody test and

polymerase chain reaction methods have been described for the identification of *Brucella* species (Quinn *et al.*, 2002).

b) Serological examinations

The use of serological tests is the core of the control or eradication of brucellosis. Many such tests are available but, they must be used in accordance with strict standardization rules and meet the requirements laid down by the OIE. The activity of immunoglobulin's during infection in the different serological tests allows the distinction between acute and chronic infection. Hence, the presence of both IgM and IgG indicates an acute brucellosis, whereas chronic brucellosis is characterized by the presence of IgG alone (Wernery, 2014).

- **Rose Bengal plate test (RBPT)**

Isolation of *Brucella* organisms from patients is not always possible. Therefore, serological tests play a major role in the routine diagnosis of the disease (Alton *et al.*, 1975). Serum agglutination tests (slide or tube agglutination), card test and Rose Bengal plate test (RBPT) have been the principal serological methods used. RBPT has been found more efficient than other serum agglutination tests although antigens produced by different laboratories and working procedures may affect the sensitivity. Accordingly, RBPT is considered as a satisfactory screening test (Bati, 2004).

- **Complement fixation test (CFT)**

Complement fixation test (CFT) on the other hand, is considered to be the most accurate test. Some researchers reported its superiority to the other tests (Asfaw *et al.*, 1998). CFT detects predominately IgG antibodies as most of IgM is destroyed during serum deactivation and so used as a confirmatory test (Bati, 2004). The test distinguishes reaction

caused by other factors like vaccines and other bacterial infections. *Escherchia coli O:157, Yersinia entrocolitica O:9, Vibrio chcolerae, Psuedomonas mallophilia and Salmonella* serotypes which share common chain of LPS antigen with smooth *Brucella* strains and do cross react. *Francella tularensis* also cross reacts for unknown reason. Rough *Brucella* strains also cross-react with *Actinobacillus equuli, Pasteurella multocida* and *Pseudomonas aerugenosa* (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 c-ELISA and CFT minimize the risk of cross-serological reactions between *Brucella* and these groups of bacteria (OIE, 2000). Several attempts have been made to use milk ring test for camel brucellosis. Camel milk however, lacks the agglutinating substances required to cluster fat globules **(**Bastawrows, 2000).

- **Enzyme linked-immunosorbent assay (ELISA)**

Recently, ELISA has been used not only for detecting *Brucella* antibodies in sera but also in camel milk (Azwai *et al*., 2001). Besides its higher sensitivity than other conventional tests, ELISA is found to detect sera as positive about 2 to 4 weeks earlier. It can also be used both for screening and confirmatory tests. Other tests such as 2- mercapto ethanol test, rivanol and Coomb`s (antiglobulin) tests have been used for specific purposes. The use of several tests for reliable detection of brucellosis suggests shortcomings in each of these tests. Hence, consideration should be given to all factors that have impact on the relevance of test methods and test results to a specific diagnostic interpretation and application (Bati, 2004).

c) Molecular examinations

- **Polymerase chain reaction (PCR species-specific)**

PCR DNA-based methods such as gene probes and PCR utilize primers derived from different polymorphic regions in the genomes of *Brucella* species. Different PCR methods for the detection of *Brucella* spp. that utilize primers derived from different polymorphic regions in the genomes of *Brucella* species such as:

1) A gene encoding a 31-kDa *B. abortus* antigen which is conserved in all *Brucella* species (primers B4/B5).
2) A sequence 16S rRNA of *B.abortus* (primers F4/R2).
3) A gene encoding an outer membrane protein of 26-k-Da (omp2) (primers JPF/JPR and primers P1/P2).
4) Outer membrane proteins (omp 2b, omp2a and omp31).
5) Proteins of the omp25/omp31 family of *Brucella* spp. the entire bp26 gene of *B. melitensis* 16M, encoding the BP26 protein (omp 28) (primers 26A/26B) were described.

However these techniques allow the differentiation of a limited number of species. The comparison of PCR sensitivity for *Brucella* DNA detection shows different values for distinct assays, i.e. the limit of sensitivity was 8 FG for B4/B5, 5 pg. for F4/R2 and 20 pg for JPF/JPR. Decrease of PCR sensitivity was observed in the presence of human genomic DNA for primers F4/R2 and B4/B5, from 8 fg to 800 fg and from 5 pg. to 50 pg. respectively, while JPF/JPR were not affected. Another comparison evaluating the sensitivity of the PCR primer pairs B4/B5, JPF/JPR, P1/ P2 and 26A/26B, applied in about 5000 samples (Buffy coat, whole blood, and serum) was described. The results of the study showed a detection limit for B4/B5 and JPF/JPR primers pairs of 10 to 100 fg and 25 to 250 fg, respectively while the sensitivity for P1/P2 and 26A/26B primers pairs was of 12.5 to 125 fg and 20 to 200 fg

respectively. All four assays had also an excellent diagnostic sensitivity ranging from 95.5 to 100% in acute infection, depending on the PCR assay and the type of specimen. As blood is known containing inhibitory substances for PCR, the PCR detection limit was investigated testing four primers pairs including B4-B5, ISP1-ISP2, F4-R2, JPF-JPR and modifying the previously reported methods. Results indicated that the detection limit varied between 25 to 800 CFU/ml. depending on the extraction and amplification method, B4-B5 was the most sensitive primers pair (25 and 100 CFU/ml suspended in one ml water and blood, respectively) followed by ISP1-ISP2 and F4-R2, while the JPR-JPF pair was unable to detect *Brucella* DNA. These data were apparently in conflict with the results indicating F4/ R2 as the most sensitive primers, but the differences could be due to the different DNA sources. A PCR assay using seven individual reactions for the rapid detection of the *Brucella* genus, and the differentiation among six recognized *Brucella* species. This assay that can be used in both real-time and conventional PCR used the multiple insertion elements, IS711, which is stable in both number and position in the *Brucella* chromosomes as a target. The PCRs for species differentiation were based on unique genetic loci of *B. melitensis*, *B.abortus*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomae* (Santis *et al*., 2011).

- **Multiplex PCR typing**

Several multiplex PCRs which identify the genus *Brucella* at the species level and partly at the biovar level using different primer combinations have been reported. The first multiplex PCR, called AMOS PCR assay (AMOS is an acronym from ‘‘*abortus – melitensis – ovis - suis’’*), comprised five oligonucleotide primers for the identification of selected biovars of four species of *Brucella*. The assay exploited the polymorphism arising from species-specific localization of the genetic

element IS711 in the *Brucella* chromosome. Identity was determined by the size of the product amplified from primers hybridizing at various distances from the element. This method could identify three biovars (1, 2, and 4) of *B. abortus*, all three biovars of *B. melitensis*, all *B. ovis* biovars and biovar 1 of *B. suis*. An abbreviated multiplex AMOS PCR assay based on three additional primers was developed to differentiate *B. abortus* vaccine strains S19 and RB51 from field strains (Ewalt and Bricker, 2000). In 2005 the finding of a deletion next to one of the IS711 copies in *B. abortus* biovars5, 6, 9 and in some field strains of biovars 3 of *B. abortus* has allowed to design and add a specific primer to the eight primer mixtures of AMOS PCR, allowing enhancing the discrimination power of this assay (Ocampo-Sosa *et al*., 2005). A RAPD-PCR (random amplified polymorphic DNA) was used in order to develop a multiplex PCR that uses the AMOS primers, additional specific loci of the insertion element IS711, and other unique insertions and deletions. This novel PCR assay differentiates between all presently recognized *Brucella* species, including the recently described species *B. ceti* (formerly named '*Brucella maris*' or '*Brucella cetaceae*'), *B. pinnipedialis* (formerly named '*Brucella* maris' or '*Brucella pinnipediae*'), and *B. microti*, including some more recently described strains of the latter species, and also allows accurate differentiation of certain biovars of *B. abortus* and *B. suis.* A new generation of multiplex PCR assays has been developed on the basis of the knowledge arisen from the recent availability of genome data. Garcıa-Yoldi *et al*., (2006), described a multiplex PCR assay for the identification of all six classical species, *Brucella* isolates from marine mammals, the vaccine strains *B. abortus* RB51 and S19 and *B. melitensis* Rev 1. The eight species-specific primer pairs amplified fragments of different sizes that showed a unique profile for each species following agarose gel electrophoresis. However, this multiplex PCR was unable to

differentiate *B. microti* from *B. suis*and *B. ceti* from *B. pinnipedialis*. A similar multiplex approach based on species-specific differences was recently described as being able to distinguish the six classical species but with some problems with *B. canis* and *B. suis* differentiation (Hinic *et al*., 2008).In addition some single target PCRs have proven particularly useful e.g. the presence of an insertion sequence, IS711, downstream of the bp26 gene, a feature specific to the marine mammal *Brucella* strains (Cloeckaert *et al*., 2000). An advancement of the differentiation of all currently described *Brucella* species was published by Mayer-Scholl *et al*., (2010), the primer pair identifying the multiplex PCR and the assay was set up on the DNA of *Brucella* reference strains and field isolates. The assay allowed the identification of all currently known *Brucella*, distinguishing also between the marine species *B. ceti* and *B.pinnipedialis* and identifying the recently described species *B. microti* and *B. inopinata* (Santis *et al*., 2011).

- **Real-time PCR**

Real-time PCR is more rapid and more sensitive than conventional PCR. It does not require post amplification handling of PCR products, thereby reducing the risk of laboratory contamination and false-positive results. Real-time PCR assays have been recently described in order to test *Brucella* cells (Redkar *et al*., 2001).Urine, blood, and paraffin-embedded tissues. Three separate real-time PCRs were developed to specifically identify seven biovars of *B. abortus*, three biovars of *B. melitensis* and one biovar of *B. suis* using fluorescence resonance energy transfer (Santis *et al*., 2011).

The upstream primers used in these real-time PCRs derived from the insertion element, IS711 whereas the reverse primer and FRET probes are selected from unique species or biovar-specific chromosomal loci. Sensitivity of *B. abortus*-specific assay was as low as 0.25 pg. DNA

corresponding to 16-25 genome copies and similar detection levels were also observed for *B. melitensis* and *B. suis*-specific assays. Light Cycler real-time PCR with SYBR Green I targeting bcsp31, a gene found in all *Brucella* species and biovars, was described (Queipo-Ortuno *et al*., 2005). The assay was performed on DNA extracted by urine samples and showed a sensitivity of 10 fg corresponding to one genome copy. Another real-time PCR assay for the rapid laboratory diagnosis of human brucellosis on whole blood and paraffin-embedded tissues was developed using three assays with hybridization probe detection. These assays targeted conserved and specific regions of the *Brucella* genome: The ribosomal 16S–23S ITS region, omp25 and omp31 and the ITS-PCR clinical specificity was 100% and showed a limit of detection as low as 3 genome copies per reaction while omp25 and omp31 assays targeting only a single copy gene. Various molecular techniques differentiating *Brucella* at the species level and/or at the biovar level have been described (Ferrao-Beck *et al*., 2006).

These methods are usually less labor-intensive, faster than biochemical typing, but the techniques adopted were not set up with the aim of obtaining clear-cut species and biovar assignment in a very short time for routine laboratory testing. A real-time 5’ nuclease PCR assay specific for amplification of a 322 bp fragment of the perasamine synthetize (per) gene, a highly conserved region present in the naturally rough *Brucella* species *B. ovis* and *B. canis* and spontaneously rough strains of *B.abortus* and *B. melitensis* (Santis *et al*., 2011).

- **Single nucleotide polymorphisms (SNPs) typing**

Single nucleotide polymorphisms (SNPs) represent powerful markers that allow accurately describing the phylogenetic framework of a species, particularly in a genetically conserved group as *Brucella*. The approach is based on a series of discrimination assays interrogating SNPs

that shown to be specific to a particular *Brucella* species. Scott *et al*., (2007), described the use of SNPs in order to develop a multiplex SNP detection assay, based on primer extension technology that can rapidly and unambiguously identify an isolate as a member of one of the six classical *Brucella* species or as a member of the recently identified marine mammal group. An alternative approach based on Minor Groove Binding protein (MGB) probes applied on a real-time PCR platform was described (Gopaul *et al*., 2008; and Foster *et al*., 2008). The assay distinguishes all members of the classical species, but the differentiation between *B. suis*and *B. canis* was difficult as no *B. suis* specific SNP has been identified. However, as a specific *B. canis* SNP has been identified, it was possible to discriminate *B. suis*from *B. canis* specific SNP and the *B. canis* specific SNP. A new SNP signatures for the rapid identification and biovar characterization of *B. suis* (Santis *et al*., 2011). Allelic profiles unique for each *B. suis* biovar were defined and the most relevant signatures were determined. Biovars assigned with both present and classical methods were globally consistent except for some biovar 3 field strains which matched the allelic profile of biovar 1. An advancement of this method has been represented by a novel SNP based typing platform that, incorporated targets that define the three *Brucella* vaccine strains and allowed the differentiation of the live *Brucella* vaccine strains from field isolates (Gopaul *et al*., 2010).

No test as yet devised is 100% accurate, so generally serological diagnosis consists of testing sera by several methods and techniques. However, a screening test of high sensitivity is usually conducted, followed by a confirmatory test of high specificity for accurate diagnosis of brucellosis (Nielsen and Wu, 2010).

d) Other diagnostic test

- **Skin test**

Brucellosis skin tests have been tried by some researchers, particularly on *Bactrian* camels in the former USSR, using different allergens. The skin test is highly specific but its sensitivity is low, making it a good herd test. The antigen does not sensitize the animal's immune system and therefore will not induce interference in the diagnosis of the disease (Wernery, 2014).

2.4.12. Epidemiology of brucellosis

Brucellosis occurs worldwide in both humans and animals. Distribution of human brucellosis has changed over the last fifty years because of different factors as sanitary, socioeconomic, and political conditions, together with the increase of international travel and population migration (Memish and Balkhy, 2004). Several areas traditionally considered endemic e.g. France and Israel, have achieved the control of the disease while new foci of human brucellosis have emerged, particularly in central Asia and Middle East e.g., - Syria (Pappas, *et al*., 2006). Now adays the infection is most common in the Mediterranean basin, the Middle East, India, Central Asia, Mexico, and Central and South America (Hoover and Friedlander, 1997).While in Northern Europe, Australia, the USA and Canada control programs allowed eradicating the infection (Whatmore, 2009). In Europe the distribution of brucellosis varies widely. Brucellosis-free status has been granted by the European Union (EU) to Sweden, Denmark, Finland, Germany, UK (excluding Northern Ireland), Austria, Netherlands, Belgium, and Luxembourg. Norway and Switzerland are also considered brucellosis-free countries (European Commission, 2003).While the Mediterranean

basin is known to be an endemic region of human brucellosis (Institut de Veille Sanitaire, France, 2005). Indeed, in Italy human brucellosis has travelled to the south, because of socioeconomic factors (Ministry of Health, Italy, 2005), as Portugal and Greece, endemic areas, are characteristically the poorest regions of the EU (Ministry of Health, Portugal, 2005). In the Northern United States, brucellosis cases are due mainly to the importation of the disease through international travel or infected food preparations coming from endemic areas. In the rest of the United States (USA) *B. melitensis* is the main cause of *Brucella* infections, especially in the Hispanic population, localized in areas neighboring Mexico. Indeed, since Mexico is the principal reservoir of infection, the immigration into the USA prevents the eradication of the disease. In Africa the brucellosis is endemic, especially in North Africa, where sanitary data are available, while in most African countries the fragmentary collection of clinical data doesn't allow to have a reliable status of the prevalence of the disease (Wernery, 2014).

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).

The infection occurs via the mucous membranes, including oral nasopharyngeal, conjunctival and genital mucosa, and also through cutaneous abrasions. Animals become infected through feed, water, colostrums, contaminated milk and, especially, by licking or sniffing at placentas and aborted fetuses. The spread of brucellosis during sexual activity plays a subordinate role. The primary shedding routes of *Brucella* organisms remain uterine fluids (lochia) and placenta expelled from

infected animals. In cattle it is known that abortion is associated with the shedding of 1012 to 1013 *Brucella* bacteria. Survival of the organisms in the environment is enhanced by cool temperatures and humidity; however, it was proven that two dromedaries in a *Brucella*-negative dromedary herd were infected with *B. melitensis* through contaminated dust particles from aborted camel fetuses 500 m apart, indicating that the organisms can also survive in a hot desert environment. Many placental mammals, including herbivores, participate in placentophagy, with *camelids* as a noted exception, which may contribute to the spread of *Brucella* bacteria through wind. In bovines, shedding of up to 103 *B. abortus* bacteria/ml through milk following abortion may last for a period of up to three months, which is considered an important fact from an epidemiological point of view. The situation in *camelids* is unknown. Excretion of the pathogen through milk is intermittent (Wernery *et al*., 2007a). However, in chronically infected (serologically positive) dromedaries from the UAE which gave birth to healthy offspring, no *Brucella* organisms were isolated from expelled placentas, and no shedding occurred through milk. Also, the blood of dromedary calves was negative in culture and polymerase chain reaction (PCR). Interestingly, camel calves of serologically positive dams were all serologically negative, using RBT and cELISA techniques, at the age of six months. The calves therefore do not appear to be at risk for an acute brucellosis infection even after the disappearance of maternal antibodies. However, for confirmation of these findings, further investigations need to be performed (Von Hieber, 2010).

It is recommended to separate calves from their dams at the age of seven to eight months, when their maternal antibodies have disappeared; otherwise, they may contract infection from infected dams at the next parturition. The *Brucella*-negativity of female camel calves from

chronically infected dams is controversially discussed between Dubai based veterinarians and some researchers who believe that confirmation of the *Brucella*-negativity can only be confirmed when camel calves remain serologically negative after parturition. In males, it is an even more complicated unsolved issue. In general, abortions occur mainly during the first pregnancy and infected *camelids* are clinically well. The pathogen is found intracellular in mononuclear phagocytes, in which it also multiplies. In pregnant camels, the bacteria localize in the placenta and are most abundant in abortion material (up to 1013 bacteria) including the fetal stomach, vaginal discharge and colostrum. *Brucella melitensis* and/or *B. abortus* organisms have been isolated from camel milk, aborted fetuses, the placenta, fetal stomach fluid, lymph nodes, vaginal swabs, testes and hygromas. It was also shown by von Hieber (2010), that, during a period of two years, 5% of the dams had fluctuating titers from positive to negative to positive and 20% of the serologically positive dams turned negative with RBT and cELISA (latent infection?). This indicates that the pathogens can conceal themselves, most probably in lymph nodes, and do not produce detectable antibodies in those intracellular hiding places. However, evidence of spontaneous recovery from brucellosis had also been described (Ostrividov, 1954; Gatt Rutter and Mack, 1963), with no further explanation. Further research by Wernery *et al.* (2007b), who investigated the question of where *Brucella* organisms were concealed in serologically positive lactating dromedaries which gave birth to healthy calves, revealed that the organisms were found in internal lymph nodes. They were mainly isolated in lungs, lymph nodes, indicating infection by inhalation route. These investigations in *camelids* clearly show that there are important epidemiological differences in dromedaries which abort (acute brucellosis) and chronically infected animals which do not abort. A

chronic infection is certainly the most common occurrence, and in bovines it is known that 75% to 90 % of cows abort only once (Acha and Szyfres, 2003).

Theoretically, the three *Brucella* species known to cause brucellosis in camels (*B. abortus, B. melitensis, and B. ovis*) can cause infection anywhere (Higgins, 1986). However, it is surmised that *B. melitensis* is widespread in Africa and the Middle East and *B. abortus* is widespread in the former USSR. Solonitsyn, (1949) reported mixed infections with various *Brucella* species in *Bactrian* camels in Russia. Although camels appear to be very susceptible to *Brucella* infection, isolation of *Brucella* organisms from camel samples is rare. But attempts to isolate *Brucella* from milk have been successful. *Brucella abortus* biovars 1 and 3 were isolated from camels in Senegal from herds with an increased incidence of abortion, but the samples were culture-negative (Verger *et al*., 1979). However, these workers authors succeeded in isolating *B. abortus* from the gastric fluids of five aborted fetuses. Pal'gov (1950) was able to isolate *B. abortus* from *Bactrian* camels in Russia. In the herds examined, 2% of all animals aborted in the first half of pregnancy, and 15 % of the herds were seropositive to brucellosis using the complement fixation test (CFT). Zowghi and Ebadi, (1988), cultured 3,500 lymph nodes from 300 slaughtered dromedaries from Iran for *Brucella* organisms. *Brucella melitensis* biovars 1 and 3 were isolated from these lymph nodes in 1% (3/300) of the camels. The authors had the opinion that *B. melitensis* infections in the dromedaries originated from neighboring sheep and goat herds. Radwan *et al.* (1995) examined a large camel herd with 2,536 dromedaries in Saudi Arabia from which a 12% abortion rate had been reported. *Brucella abortus* biovar3 was recovered from an inguinal lymph node, three vaginal swabs and one supramammary lymph node obtained from free-ranging camels in Eastern

Sudan which had histories of abortion, presence of hygromas or testicular lesions (Agab *et al*., 1996). It is worth mentioning that both isolates of *B. abortus* biovar3 from Senegal and Sudan are the only oxidase-negative biovars reported in the literature. Ramadan *et al.,* (1998), have recovered *B. melitensis* from a hygroma of an Indian camel. *Brucella melitensis* was isolated twice from two-quarters of milk samples from three seropositive camels in the UAE (Wernery *et al*., (2007).

Three llamas died at London zoo after they came in contact with camels which were newly imported from Moscow. The authors claimed that the high serological titer (type of test not given) for *B. melitensis* was indicative of an acute infection (cited by Wernery, 2014).

2.4.13. Epidemic seasons

In general, brucellosis can be found in any season of the year. The epidemic peak occurs from February to July and is closely related to the months associated with delivery and abortion in animals. In humans, prevalence of the disease is high (39.5%) in summer (Gul and Khan, 2007). Notifications of human brucellosis, which are mandatory in Italy, reach a peak between April and June. However, considering the standard incubation period of 2-4 weeks, and the fact that lamb slaughter is traditionally at a peak during the Easter period, it might be expected that occupational exposure would result in a peak of human cases between March and May (De-Massis *et al.,* 2005).

2.4.14. Environmental and climatic factors

Atmospheric conditions and season of the year may have influence on the management and contact of the infected and susceptible host. In dry areas, water resources are sparsely distributed (Helland, 1982). As a result, the congregation of a large number of mixed ruminants at water

points facilitates disease spread. The coincidence of parturition in wet season enhances the viability of the organisms in the environment, thus increasing the chance of infecting susceptible animals (Corbel, 1990). Baumann and Zessin, (1992) reported higher brucellosis reactor rate in wet seasons than dry seasons. The incidence of brucellosis in camel population appears to be related to breeding and husbandry practices. Herd sizes, density of animal population, and poor management are directly related to prevalence (Wernery and Kaaden, 2002).

2.4.15. Geographical distribution of brucellosis

Brucellosis was first recognized as a disease affecting humans on the Island of Malta in the early 20th century. Though its distribution is worldwide; yet brucellosis is more common in countries with poor standard of animal and public health hygiene (Capasso, 2002). The routes of infection are multiple i.e., food-borne, occupational or recreational, linked to travel and even to bioterrorism. New *Brucella* strains or species may emerge and existing *Brucella* species adapt to changing social, cultural, travel and agricultural environment (Godfroid *et al.*, 2005).

The incidence of reactors in newly established cattle farms may be more than 30%, however, the highest rate (72.9%) of infection till now has been reported in the Palestinian Authority (Shuaibi, 1999). It is interesting to note that the second highest prevalence (71.42%) of brucellosis has been reported in mules from Egypt (Anonymous, 2007). Invariably, all domestic animals suffer from this disease. Brucellosis in buffaloes has been reported from Egypt (10.0%) and Pakistan (5.05%). Since cattle are found throughout the world, prevalence of brucellosis (0.85 to 23.3%) in cattle has been reported from a wide range of countries. In camels, brucellosis has been reported from Arabian and

African countries (0.0- 17.20%), where the disease also occurs in buffaloes, equines and swine. Variable prevalence of this disease has been reported in sheep and goats. Bio-varieties of *Brucella* vary with respect to the geographic region. *B .melitensis* biovar1 from Libya, Oman and Israel and *B. melitensis* biovar2 from Turkey and Saudi Arabia have been isolated. *B. melitensis* biovar3 is the most commonly isolated species from animals in Egypt, Jordan, Israel, Tunisia and Turkey (Refai, 2002). *B. abortus* biovar1 in Egypt, biovar2 in Iran, biovar3 in Iran and Turkey and biovar6 in Sudan have been reported (Halling and Boyle, 2002).

The countries with the highest incidence of human brucellosis include Saudi Arabia, Iran, Palestinian Authority, Syria, Jordan and Oman. Bahrain is reported to have no incidence (Refai, 2002). The percent prevalence of bovine brucellosis has been reported to decrease in Ireland and Italy during the year 1999-2000 but there had been a trend towards a significant increase in Azores (Jacques and Kasbohrer, 2002).Brucellosis is the most common zoonosis in the world, accounting more than 500,000 cases in animals and humans alike, annually (Pappas *et al.,* 2006). Advances in control and eradication practices have led to complete eradication from many developed countries like USA, Israel, Canada, Japan and New Zealand, however it remains an uncontrolled problem in highly endemic areas such as Africa, Middle East, Asia and Latin America (Refai, 2000).Geographically brucellosis has been reported in Asia, Africa, South and Central America, the Mediterranean Basin, Sahara (McDermott and Arimi, 2002) and the Caribbean and these are the regions where cattle raising are mostly preferred. Infected or exposed animals have also been found along the Atlantic and Pacific coasts of North America; the coasts of Peru, Australia, New Zealand and

Hawaii (OIE, 2009). Incidence of brucellosis is reported to be the highest in bovines and prevalence range of 0.85-23.3% has been reported from a wide range of countries. In camels, brucellosis has been reported from Arabian and African countries (0.0-17.20%), (Refai, 2000).

Brucellosis is widespread in African countries, although with varying prevalence (Abubakar *et al*., 2012). The worldwide distribution of brucellosis has been reviewed by Memish and Balkhy (2004). They observed that in Central American countries, bovines are the most affected hosts with herd infection rates ranging from 10-25%. In Mexico, brucellosis is one of the most serious bacterial diseases in livestock and humans alike, even after the development of control strategies at the national level. Brucellosis has been a well-known disease in Latin American countries with prevalence rates of 10-25%. The Netherlands and England were considered to be free of bovine brucellosis by the turn of the century (Godfroid and Kasbohrer, 2002). Brucellosis-positive herds were still reported in France, Ireland and Italy, but the incidence has been declining (Godfroid *et al.*, 2002).In the countries of central and south-eastern Europe, namely Greece, Macedonia, Yugoslavia and Bulgaria, sheep and goats remain a major reservoir of the disease, while cows are less important hosts. While bovine, caprine, ovine and porcine brucellosis exist in most sub-Saharan African countries, the true prevalence is either poorly reported or completely unknown. High incidence of brucellosis has also been reported from the Sub-continent countries particularly India and Pakistan (Abubakar *et al*., 2012).

Table 3: Prevalence of brucellosis in livestock in different countries

Country	Species	Prev. (%)	*Brucella* spp
Algeria	Sheep	2.18	_
	Goat	12.00	_
Egypt	Buffalo	10.00	*Br. Abortus*
	Cattle	23.30	*Br. Melitensis bio.3.*
	Donkey	7.30	_
	Horse	5.88	_
	Mule	71.42	_
Eretria	Cattle	8.20	_
	Sheep	1.40	_
	Goat	3.80	_
	Camel	3.10	_
	Horse	0.00	_
India	Equine	12.89	_
	Bovine	6.37	_
	Sheep	3.42	_
	Goat	5.53	_
Iran	Cattle	0.85	*Br. Abortus*
	Goat	10.18	_
	Camel	8.00	_
Iraq	Sheep	15.00	*Br. Melitensis*
	Cattle	3.00	*Br. Abortus*
	Camel	17.20	_
Libya	Camel	4.10	*Br. Melitensis bio. 1.*
Nigeria	Cattle	5.82	_
	Goat	0.86	_
Oman	Camel	8.00	*Br. Abortus*
	Cattle	3.30	_
	Sheep	1.60	_
	Goat	6.40	*Br. Melitensis*
Pakistan	Horse	5.78	_
	Dog	9.33	_
	Poultry	4.00	_
	Buffalo	5.05	_
	Cattle	5.46	_
	Camel	2.00	_
Saudi Arabia	Camel	8.00	*Br. Meltitensis bio 2.*
	Cattle	18.70	_
	Sheep	6.50	_
	Goat	9.70	_
Sri Lanka	Cattle	4.7	*Br. Abortus*

	Buffalo	4.2	_
Sudan	Camel	6.95	*Br. Abortus*
	Camel	0.00	_
	Cattle	5.00	_
	Sheep	1.00	_
	Goat	4.00	_
UAE	Camel	2.00	*Br. Abortus*
	Cattle	1.30	_
	Sheep	2.00	_
	Goat	3.40	_

(Gul and khan, 2007).

2.4.16. Zoonotic importance of brucellosis

In humans, brucellosis can be caused by *B. abortus*, *B. melitensis*, *B. suis* biovars 1-4 and, rarely, *B. canis*. From public health point of view, brucellosis is considered to be an occupational disease that mainly affects farm labor, slaughter-house workers, butchers, veterinarians (Yagupsky and Baron, 2005). Transmission typically occurs through contact with infected animals, materials with skin abrasions, inhalation of aerosols or ingestion of contaminated or unpasteurized dairy and food products (Christopher *et al.*, 2010).

Worldwide prevalence of brucellosis in human population has been studied and reviewed. The Mediterranean Basin, south and Central America, Eastern Europe, Asia, Africa, the Caribbean and the Middle East are considered as high-risk countries. In the Eastern Mediterranean Region, the incidence of disease ranges from 1 per 100,000 to 20 per 100,000 populations. Brucellosis is endemic in Saudi Arabia, where the national sero-prevalence is 15% (Abubakar *et al*., 2012).

Mukhtar and Kokab (2008), showed that brucellosis is also a public health problem in Pakistan by conducting a sero-prevalence study of brucellosis in abattoir workers of Lahore. Symptoms in human brucellosis

can be highly variable, ranging from non–specific, flu-like symptoms (acute form) to undulant fever which may progress to a more chronic form and can also produce serious complications affecting the musculoskeletal, cardiovascular, and central nervous systems, other problems like arthritis, orchitis and epididymitis. It also gives rise to a chronic granulomatous infection, causing clinical morbidity that requires combined prolonged antibiotic treatment (Grillo *et al.*, 2006).Human incidence of brucellosis can only be controlled by decreasing the incidence of disease in animals, especially livestock species. It is a serious public health challenge having socio-economic problems and an unaccounted financial burden which needs joint efforts, promotion of inter-sectoral action, regional and international cooperation, as well as technical and financial support (Abubakar *et al*., 2012).

Table 4: Zoonotic Potential and Host Preference of *Brucella* Species.

Species	Zoonotic Potential	Host Preference
Brucella melitensis	High	Sheep, goat
Brucella abortus	Moderate	Cattle
Brucella suis	Moderate	Pig
Brucella canis	Mild	Dog
Brucella ovis	Absent	Sheep
Brucella neotomae	Absent	Desert wood rat (*Neotoma lepida)*
Brucella ceti	Mild	Cetaceans
Brucella pinnipedialis	Mild	Seals
Brucella microti	Absent	Common voles (*Microtus arvalis*)

(Mariana *et al*., 2010).

2.4.17. Economic importance of camels

Camels are primarily the domestic animals of pastoral communities that ensure food security. They produce milk, meat, hair and hides, and also serve as a draught animal for agriculture and transport of people as

well as goods (Bati, 2004). Milk and meat are the important products that camels produce elsewhere. A study in eastern Ethiopia indicated 3 to 6 liters of daily milk yield over 13 to 15 months of lactation length (Getahun and Bruckner, 2000), while Tefera and Gebreab, (2001) reported the average daily milk yield of 2.5 liters. Long lactation and ability to maintain milk production over long dry spells are important facets of camel productivity. Apart from home consumption, the majority of the households sell at least one-third of the milk produced to generate cash income. Daily milk yield can be as high as 20 liters with improved management conditions (Bati, 2004).

2.4.18. Economic importance of brucellosis

Brucellosis is characterized by abortion, non-viable offspring birth in female, and orchitis and epididymitis in male animals. Abortion is the major feature that is manifested in camels. The disease is also associated with infertility and prolonged calving intervals, and has considerable impact on camel production. Chronic inflammation of epididymis, of the joints, tendon sheath and synovial bursae especially at the carpus may also occur in camels (Bati, 2004). The disease can generally cause significant loss of productivity through late first calving age, long calving interval time, low herd fertility and comparatively low milk production, as in cattle may also happen in camels (Radostits *et al.*, 1994).

The disease can also have an impact on export and import of animals constraining livestock trade. Sub-clinical brucellosis can pose problems in racing camels by reducing the performance and productivity of these animals in the Arabian Peninsula where camel racing is highly popular (Bati, 2004)**.**

2.4.19. Impact of brucellosis on human Public health

Brucellosis in human represents a major public health hazard, which affects social and economic development in various countries. Groups at high risk for brucellosis are animal health workers, butchers, farmers, and those who habitually consume raw milk and come in contact with animals (Bati, 2004). In man, transmission occurs as a result of ingestion of milk, contact via skin abrasion, mucous membranes and inhalation (Seifert, 1996). Masoumi *et al.* (1992) recorded higher prevalence rate among butchers and people who habitually consume raw milk. Camel keepers consume camel milk as well as liver without heat treatment. This is even considered as delicacy. There is also a close contact between herds men and the animal during watering, grooming, riding, nursing sick ones and delivery assistance (Bati, 2004).The isolation of the two major pathogenic *Brucella* species: *B. melitensis* and *B. abortus*, from milk and other samples of camel origin (Hamdy and Amin, 2002) clearly indicate the potential public health hazards of camel brucellosis (Straten *et al*., 1997). The disease in man may be misdiagnosed due to the prevailing malaria infections in dry areas (El-Ansary *et al.*, 2001).

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 (Wernery, 2014). Infection prevalence in the animal reservoirs determines the incidence of human cases (Von Hieber, 2010). *Brucella* spp. is also potential agents of bioterrorism is classified in group B (second-highest priority agent) of the Centers for Disease Control and Prevention (CDC) in the USA. *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 and, especially, placentas, and by consuming raw milk and other unheated dairy products. Airborne infections occur in animal pens, stables, laboratories and abattoirs (Wernery, 2014). Some cases have also occurred from accidental self-inoculation with live vaccines (Saleem *et al*., 2010;OIE, 2012). Moreover, it was also shown by Bradenstein *et al.*, (2002), that Rev 1 vaccine strain can cause human infections. In their study humans became infected after consuming milk from vaccinated adult pregnant animals which excreted the vaccine strain in milk for a long period of time. The high and increasing herd and animal prevalence of camel brucellosis in many countries is of grave concern (Sprague *et al*., 2012); therefore, veterinary authorities, consumers, camel owners and camel keepers, as well as responsible persons in the Ministry of Health and Agriculture of each country, should make every effort to address this issue. During investigations conducted by Radwan *et al*., (1995), it was found that brucellosis was diagnosed in 30% of the camel handlers and milkers and the same *B. melitensis* biovars were cultured from aborted sheep and goats sharing the same premises. In humans, the incubation period lasts from five to 60 days, but can also be longer. Clinical signs are not specific and can be acute or chronic (Wernery, 2014). *Brucella* infections in pregnant women in early pregnancy may lead to high rates of fetal loss (up to 40%) and infection in men can lead to orchitis and epididymitis. *Brucella melitensis* DNA persists in human blood for many years after infection despite appropriate treatment and apparent recovery (Vrioni *et al.*, 2008). Humans are at risk through consumption of unheated milk or through handling *Brucella*-positive animals (Wernery,

2014). Shimol *et al.* (2012) described a brucellosis outbreak that affected 15 people who consumed unpasteurized camel milk. Affected people suffered mainly from arthralgia and fever and 50% had positive blood culture for *B. melitensis*, whereas 60% had serum agglutination titers of 1:60 or higher. During a *B. melitensis* outbreak which occurred in a herd of alpacas in Peru, over 25% of the alpaca handlers were seropositive to brucellosis and some developed clinical signs (Wernery, 2014). Extreme care must be exercised when working with *Brucella* organisms in laboratories. It is estimated that up to 2% of all diagnosed brucellosis cases are laboratory-acquired infections, mainly through inhalation when handling diagnostic specimens (Ergonul *et al*., 2004).

2.4.20. Bioterrorism of brucellosis

Brucella species, particularly *B. melitensis* and *B. suis*, have traditionally been considered biological weapons, although brucellosis is characterized by a long incubation period, often asymptomatic infections and low mortality. Indeed, the airborne transmission through mucous membranes such as the conjunctiva, oropharynx, respiratory tract or the transmission through skin abrasions make *Brucella* highly contagious, as demonstrated by efficient human airborne transmission during abortions of infected animals or aerosolisation in laboratory manipulations (Pappas *et al*., 2006). Furthermore, factors as the low number of bacteria constituting an infectious aerosol dose (10-100 organism), the nonspecific clinical symptoms of brucellosis, the worldwide circulation of the infection, the onset of chronic debilitating disease, make *Brucella* spp. a category B bioterroristic agent, according the Center for Disease Control and Prevention definition. Although *Brucella* is sensitive to inactivation standard methods as heating and disinfectants, it often survives for up to two years in the environment (Santis *et al*., 2011).

The most virulent of several strains of the *Brucella* bacteria, code name US, was the most advanced and the only standardized agent in (1950). By the summer of 1951 the Chemical Corps Biological Department scheduled the production of *B. suis* and *B. melitensis*. In 1954, *B. suis* became the first agent weaponized by the USA and tested on animals. By 1955, the USA filled cluster bombs with this agent for the US Air Force at the Pine Bluff Arsenal in Arkansas (Endicott and Hagerman, 1998). In 1967 the development of *Brucella* as a bioweapon was stopped, and Richard Nixon on 1969 in the Statement on Chemical and Biological Defense Policies and Programs unilaterally renounced to use chemical weapons and banned the development of all biological weapons. A preliminary treaty prohibiting the development, storage and acquisition of biological weapons was completed in 1972 and ratified as Biological and Toxin Weapons Convention (BTWC) in 1975 from 144 countries, however several nations of the Middle East did not sign the treaty and the Soviet Union, in spite of the Convention, expanded its biological weapons program (Roffey *et al*., 2002).

The correlation of suspicious cases to a possible attack involving a biological agent represents the mainly difficult in the forensic investigations. An effective public health response to a possible biological terrorism crime or terrorism threat include 1) sensitive, specific, and rapid laboratory diagnosis of patients and characterization of biological agents; 2) early detection through improved surveillance; 3) effective communication; and 4) coordinated local, state, and federal response in the investigation of unusual events or unexplained illnesses (CDC, 2000). The early detection is essential to ensure a prompt response to a biological terrorist event, but also the discrimination between natural outbreaks and/or intentional release of micro-organism agents is of

crucial importance in the context of the bioterrorism. Therefore it is very important to have a strain typing epidemiological tool for source trace back in outbreaks. Characterization of *Brucella* at species and biovar level using differential microbiological approaches for phenotyping often may result in complicating interpretation where a more accurate identification is necessary. Furthermore, these typing methods are time consuming and potentially hazardous for laboratory operators, as *Brucella* spp. need BSL3 facilities. Thus, genetic characterization using molecular DNA technology has been developed and several molecular techniques for sub typing have been proposed (Santis *et al*., 2011).Brucellosis is not only a major zoonotic problem but is also linked with bioterrorism and belongs to category B (Gul and Khan, 2007). The severity of this disease, lack of vaccines suitable for use in man and frequent failure of clinical laboratories to correctly identify isolates led to the investigation of *Brucella* as an agent for bioterrorism. Before 1954, when Britain was focusing on anthrax, brucellosis was the first microorganism chosen by the United States to develop as a weapon. This microorganism could be effectively disseminated in four pound bombs. Indeed, the American military weaponized *Br. suis*in 1954, however, changing global politics resulted in abandonment of these efforts following the biological and toxic weapons convention in 1972. *Brucellae* are not difficult to grow and disperse, and transmission to humans may result in prolonged illness and long-term sequelae (Yagupsky and Baron, 2005). Aerosol or food contamination could be the sources of dispersion. This microorganism has the advantage of being debilitating without being fatal. The infective dose for these organisms is very low, if acquired via the inhalation route. It has been estimated that 10-100 organisms are sufficient to constitute an infectious aerosol dose for humans. The economic impact of a brucellosis bioterrorist attack would cost $ 477.7

million per 100,000 persons exposed. Although *Brucella* has long been considered a potential microorganism for bioterrorism, no application in a bioterrorist attack has been reported so far (Gul and Khan, 2007).

2.4.21. Control and management practices

Brucella has been eradicated in many regions of the world, but in others it is widespread and an economically important disease. Many cases of human brucellosis are found in regions where the disease has not been eliminated in livestock. Different strategic options can be adopted to first decrease the prevalence of brucellosis to an acceptable level (brucellosis control) and secondly to remove the foci of infection (brucellosis eradication).The choice of control strategy depends on a number of considerations, such as infection prevalence in different animal species, human clinical incidence and the capacity of Veterinary Services. However, a pre-requisite for any control program is the implementation of an efficient animal disease surveillance network. Eradication in small ruminants has never been achieved (Smits, 2013), and may be also very difficult to achieve in OWCs due to the complexity and expense of treating animals across widespread areas. In cattle and small ruminants, when prevalence is low (between 3% and 5%), vaccination comes first followed by slaughter (Wernery, 2014). Abbas and Agab, (2002), suggested whole-herd vaccination in low-prevalence countries, and test-and-slaughter followed by vaccination in high prevalence countries. In camel-racing countries, the culling method cannot be applied because racing dromedaries are often extremely valuable animals and play a very important role in Bedouin culture. Therefore, it is preferable to castrate all *Brucella*-positive bulls, not to breed positive females, and to vaccinate. No compromise should be made when it comes to camel dairy farms. They must be free of brucellosis (Wernery, 2014).The control and

prevention of brucellosis in farm animals depend on animal species involved, *Brucella* species, management practices and availability and efficacy of vaccines. The options to control the disease include immunization, testing and removal, and improving management practices and movement control (Wernery and Kaaden, 2002).

Control of camel brucellosis should suit 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 countries where intensification is practiced. Movement control of herds, Improvement of management practices is one way of attempting to control brucellosis. This would aim to improve hygiene and reduce the chances of contact between infected and non-infected animals. Although it would not be easy under many circumstances, where resources are lacking and the movement of livestock is difficult to restrict, the following points can be attempted in reducing infection rates:

1) Public awareness is of vital importance in successful control and prevention of brucellosis.
2) Isolation of infected animals and female at parturition.
3) Proper disposal of aborted fetus, placental tissue and uterine discharge.
4) Disinfection of contaminated areas (Bati, 2004).

2.4.22. Brucellosis eradication programs

Currently, about half a million human brucellosis cases are annually reported worldwide but the estimated number of unreported cases due to the unspecific clinical symptoms of the disease is supposed

to be 10 times higher. In endemic countries prevalence rates often exceed 10 cases per 100,000 populations (Pappas *et al*., 2006). Brucellosis is transmitted to humans from direct contact with livestock (occupational disease for abattoir personnel, farmers and veterinarians for example) or more often by ingestion of unpasteurized milk or milk products (Godfroid *et al*., 2005). In heifers that aborted, *B. abortus* is found in the uterus, in milk, in the mammary glands and associated lymph nodes. Of significant epidemiological importance, *B. abortus* was also found in weak and healthy calves born from experimentally infected heifers (Xavier *et al*., 2009). The consumption of cattle, sheep and goat meat does not seem to play a role, although meat from animals that appear to be sick at the time of slaughter should not be consumed (Glynn and Lynn, 2008). However, bacteria can be transmitted to humans by unsafe butchering and consumption of under-cooked meat. A recent report from Botswana suggests that household bush meat processing practices represent a significant *Brucella* spp. exposure risk to family members and the community (Alexander *et al*., 2012). Person to person transmission of brucellosis through breast feeding or by sexual intercourse, although reported (Ruben *et al*., 1991), is epidemiologically anecdotal and therefore brucellosis in humans almost always originates from an animal reservoir and results from different risk factors and behavioral traits (Zinsstag *et al*., 2007).

In the developed world, for more than four decades, control and eradication programs of brucellosis in livestock have been implemented by national veterinary services. Classically after a first phase in which the infection is controlled by compulsory vaccination, then vaccination is gradually restricted and eventually prohibited whereas a “test and slaughter” policy is implemented in order to eradicate the infection. More

than a decade is usually needed to complete the brucellosis eradication program by a “test-and-slaughter” policy and key for success is a sufficient financial compensation scheme for farmers for their culled livestock. In the European Union (EU), such national programs are co-financed by the EU and the Member States (MSs).This policy has been successfully implemented for bovine as well as ovine and caprine brucellosis in Northern MSs (with the notable exception of bovine brucellosis in the United Kingdom), whereas eradication programs, particularly ovine and caprine brucellosis eradication programs are not yet completed in some Southern European MSs (http://ec.europa.eu/food/animal/diseases/eradication/eradication bovine, sheep, goats, brucellosis, en.pdf). Countries are reporting on the national animal health situation to the OIE via the World Animal Health Information Database (WAHID) Interface. This interface provides access to all data held within OIE and can be accessed following the link: http://web.oie.int/wahis/public.php?page=home. Unfortunately, the information related to brucellosis that is provided by some developing countries is scarce or absent. In such resource poor countries, the implementation of an efficient eradication policy is impossible and thus innovative approaches taking into account the scarcity of financial resources as well as the perceptions and attitudes of communities have to be defined where human brucellosis is documented to be a public health problem (Marcotty *et al*., 2009). One example of such an innovative approach has been studied in Mongolia where the economic benefit, cost-effectiveness, and distribution of benefit of improving human health through the control of brucellosis by mass vaccination of livestock has been estimated (Zinsstag *et al*., 2005). In Tajikistan biannual conjunctival vaccination of small ruminants with Rev 1 reduced the sero prevalence by 80 per cent in 5 years and the prevalence of households with evidence of

infection in their animals dropped from 25.1 to 7.5 per cent (Ward *et al*., 2012). However, the extent to which this success is to be attributed to the implementation of a conceptually sound "One Health" framework remains to be analyzed. Interestingly, in Nigeria, the most populated country in Africa, from a medical perspective, brucellosis has been classified as a sporadic zoonosis and it is therefore unlikely that specific veterinary public health measures will be prioritized, even more so given that links between medical and veterinary officers in Nigeria can be said to be non-existent or at best very weak (Coker *et al*., 2000). Lastly, in the scientific literature related to human brucellosis in Sub-Saharan Africa, prevalence rates are based on serological results, with less than a handful reports over the last four decades on the isolation of any *Brucella* strain in patients. The absence of isolation of any *Brucella* spp. inducing seropositivity and disease in patients makes it difficult to trace back the origin of the infection when brucellosis seropositivity is detected in different species of the animal reservoir (Godfroid *et al*., 2011).

2.4.23. Treatment and the main therapeutic agents

Treatment was implemented without regard to age, stage of lactation, number of previous pregnancies, and date of most recent pregnancy or number of previous abortions due to *Brucella.* The date of previous abortions in relation to the date of initiation of treatment was not recorded on the farm. *Brucella* organisms are Gram-negative coccobacilli which are sensitive to many broad-spectrum antibiotics, but the use of antibiotics is forbidden in many countries because of the uncertainty related to the infective status of the treated animals and because of the spread of antibiotic resistance. Treatment is unlikely to be cost-efficient or therapeutically effective because of the intracellular sequestration of the organisms, mainly in the lymph nodes. However, cure rates between

65% and 100% have been reported in infected goats by daily intraperitoneal injection of 500 mg and 1,000 mg tetracycline's (Radwan *et al.*, 1992).

They have also treated 202 seropositive *dromedaries* with a combination of oxytetracycline (25 mg/kg body weight) every two days for 30 days and streptomycin (25 mg/kg body weight) every two days for 16 days. In addition to this parenteral treatment, milking camels received 10 ml of oxytetracycline as intra-mammary infusions in each teat every two days for eight days. This regimen of treatment was effective in eliminating the shedding of *Brucella* organisms through milk. All treated *dromedaries* also became serologically negative within 16 months of treatment. But the single untreated control camel remained positive over the same period of time. Using antibiotics may be a way to save valuable animals (e.g. racing camels) from being culled, but it is doubtful if antibiotic treatment on a herd level basis can be successful. It is not clear from this investigation whether or not the shedding would have stopped anyway, without any antibody treatment, because the study did not include any untreated controls. However, the author's unpublished treatment protocol clearly demonstrated that dromedary brucellosis is not treatable with antibiotics, although it is claimed otherwise. These results also clearly demonstrate the sensitivities of four different tests in chronically infected *dromedaries*.

- **The main therapeutic agents are**

1) Long-acting Oxytetracycline (LA-OTC) injectable solution (from France) containing 200 mg/ml OTC base.
2) Streptomycin sulphate (ST) (from Egypt) supplied in vials, each containing 1 g or 4 g, which were reconstituted in sterile distilled water (3 ml or 12 ml, respectively) just prior to use.

3) OTC intramammary infusion (IMI) (from the Netherlands) in 10 ml syringes, each containing 200 mg tetracycline hydrochloride, 250 mg neomycin base, 2,000 international units bacitracin, 10 mg prednisolone and excipient to 8 g. (Wernery, 2014).

2.4.24. Vaccination and Vaccines of brucellosis

Because of the serious medical and economic consequences of brucellosis, serious efforts have been undertaken to prevent the infection through the use of vaccines. In OWCs, both inactivated and attenuated *Brucella* vaccines have been used successfully. *Dromedaries* were vaccinated with *B. abortus* strain S19 and with *B. melitensis* Rev 1 (Wernery, 2014). Young (three months) dromedaries received a full dose of the vaccine and adults (10 years) a reduced dosage. Both groups developed *Brucella* antibodies with titers 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) vaccinated five dromedaries with a reduced dose (5 × 108 cfu in 2 ml) of *B. abortus* strain S19. All five camels seroconverted after one week and their antibodies declined six to seven weeks later. The dromedaries tested negative 14 weeks later. So far, no challenge infections have been performed after vaccination. In cattle, the optimum age for vaccination is between four and eight months of age. Serum agglutination test returns negative results by the time the bovines are of breeding age, except in 6% of cases (Radostits *et al*., 2007). It is obvious that post-vaccination titers increase with the increasing age and therefore cattle vaccination is recommended only in young stock. Vaccination of bulls with S19 is of no value because it often resulted in the development of orchitis and the presence of strain S19 in the semen (Saegermann *et al*., 2010). Very little is known about the

optimal vaccination age in camels and their serological response. Before vaccination is started in dromedaries, thorough investigations are of paramount importance in order to find out if animals are naturally infected by *B. abortus* or *B. melitensis*, this can only be determined by culture or PCR. *Brucellosis melitensis* Rev 1 is an attenuated vaccine and must be very carefully used otherwise infections of considerable virulence may occur in both vaccinated and in-contact humans. Vaccination of pregnant goats and sheep may result in abortion and excretion of live *B. melitensis* vaccine bacteria in milk and vaginal discharge. The situation in *dromedaries* is unknown (Wernery, 2014).

2.4.25. Immunization and immune response

The live attenuated *B. abortus* S19 and *B. melitensis* Rev-1 proved to be effective vaccines against the disease in camels and other ruminants. Both vaccines have disadvantages of causing abortion, being pathogenic to human beings and interference with serological tests. 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 (Bati, 2004).

- **Humeral Immunity**

Naturally infected and vaccinated animals can be serological reactors. After infection, the level of immunoglobulin isotypes: IgM, IgG and IgA will significantly increase in serum (Radostits *et al.*, 1994). IgM antibodies, which appear initially after infection and low levels of IgG, will cause complement-mediated lysis of *Brucella.* Secretary IgA is tending to be abundant in milk whereas IgG is high in serum (Walker, 1999). The O-chain of smooth lipopolysaccharide complex of the cell envelope together with the outer protein epitopes have contributory role

as protective immunogens. On the other hands, the immunogenicity of the non-smooth variant is relatively low (Corbel, 1990; WHO, 1997). The O chain specific antibodies play a major role in protective immunity, but don't eliminate the organisms as they are protected being intracellular. This indicates lack of correlation between protection and high antibody level (Bati, 2004).

- **Cellular Immunity**

Characteristic chronic granulomatous lesions develop in infected tissue where macrophage, neutrophils and lymphocytes respond to *Brucella* antigens. As the organisms are facultative intracellular organisms, phagocytes play a key role in initiating T-cells by processing and presenting antigens. Sensitized T-cells release cytokines that activate macrophages which in turn combat *Brucella* by reactive oxygen intermediate. Both CD4 and CD8 subsets are involved in cell-mediated protection. Cytokines also play a role in controlling *Brucella* infections (WHO, 1997). Neutrophils effectively utilize the myeloperoxidase-hydrogen peroxide halide system in killing *Brucella*. However, the organisms inhibit degranulation and the respiratory oxidative burst, and able to survive in the cells (Riley and Robertson, 1984).

Macrophages readily ingest *Brucella* when opsonized with either complement or specific antibodies. The survival of the organisms in macrophages may result from a failure of phagosome-lysosome fusion and resistance to oxidative killing by producing superoxide dismutase and catalase (Quinn *et al.,* 2002). Tatum *et al.* (1992) suggested that anti-oxidant Cu-Zn superoxide dismutase plays a role in the survival of *Brucella* species in phagocytic cells.

CHAPTER TWO

MATERIALS AND METHODS

3.1. Study areas

A. Description of the Butana plain area

Butana plain is a semi-arid clay region, covers most of the present Kassala and Al-Gdarief States in Eastern Sudan. It lies between Latitude 13 40' and 17 50' North and Longitude 32 40' and 36 00' East. It is bound by the Main River Nile on its northwestern border, the Blue Nile on its southwestern edge, the Atbara River in the northeast and by the railway connecting Kassala and Sennar in the south.

The area is composed of mountainous ranges intersecting the plain to the western and southern borders. It is crossed by many seasonal rivers namely, Atbara, Seitite, Ba-Salam, El-Gash and Rahad Rivers. Small temporary seasonal valleys do run through these plains during the rainy season. The rocky basement complex forms the geological underlining of Butana plains with sandy and stony soils in the north, light non-cracking clay in the Central, Eastern and Western regions and dark cracking clay in the South. As a result of this and with the exception of small water catchments in the mountains mentioned before, very limited water resources are available. Seasonal shallow surface water wells are present as well as few very deep bore wells. However, the amount of water and the persistence of reserves during the summer dry season depend on the quantity of rainfall during the wet season. In the Butana, a tropical continental climate prevails ranging from a sub-equatorial condition with rain in the south to desert climate in the north. Most of the rains are in the form of showers or thunderstorms. The rainfall in Butana region is highly variable from one year to the other. It ranges between 600 mm/year in the

southeast to less than 100 mm/year in the northwest. As always in the semi-arid regions, rainfall is the most important climatic factor in Butana because people and their livestock depend on this factor which supports the growth of the vegetation for their animals. The annual mean temperature ranges from 32 ºC during the day to 16 ºC at night in January (winter) and from 46 ºC during the day to 27 ºC at night in May-June (summer). Two vegetation zones are existing in the area, namely semi-desert *Acacia* shrub and short grasslands of the North Central Sudan and secondly, the low woodland savannah of central Sudan. The vegetation of Butana is constantly changing as a result of annual rainfall, accidental fire outbreaks and expansion of agriculture and grazing (Saint-Martin *et al.*, 1992).

The Butana area is inhabited by Tran's human camel owning tribes in its northern part while its southern part is populated by agro-pastoralists who practice mainly mechanized rain-fed agricultural activities for production of sorghum and sesame grains besides considerable livestock raising activities (Darosa and Agab, 2008).

B. Description of the Al-Gadarief State

This study was conducted in Gadarief State, which is located in the eastern part of the Sudan between 33 – 37° E Longitudes and 12 – 16° N Latitudes with an area of approximately 78,000 km^2. It is bounded in the north by Kassala and Khartoum States, in the west by Gezira, in the south by Blue Nile State and shared boundary with Ethiopia from the east.

Two hundred and eighty head of five types of Sudanese camel were randomly selected from Gadarif state [Bishari (n = 40), Arabi (n = 50), Daili (n = 70),Anafi (n = 60) and Kenani (n = 60)] according to, sex (males = 108, females = 72) and according to six age group ≤ 3years (n =

40), 3-5years (n = 40), 6-7years (n = 62), 8-9 (n = 61), 10-12 (n = 47) and ≥ 12 (n = 30) (Amir *et al*., 2015).

3.2. Study population

The last estimation of camels' population in the Sudan was about 4.7million head (MARF, 2011). Camels in Sudan are concentrated in two main regions; the Eastern region, where camels are found in the Butana plain and the Red Sea hills, and Western regions (Darfur and Kordofan). Phenotypic characterization to assess the existing biodiversity and differences among the Sudanese camel breed subtypes is necessary prerequisite to facilitate the conservation and utilization program in an effective and meaning full way. In spite of that Sudanese camel breed subtypes are not well classified or defined, with very limited information available (Ishag *et al*., 2010). Camels in the Sudan are classified as pack (heavy) and riding (light) types according to the function they perform and probably as a result of selection applied for these traits by the various camel-owning tribes. The Sudanese heavy type constitutes the majority of the camels kept by nomads in Sudan. In this group two types can be identified on the basis of conformation and tribal ownership: The Arab and Rashaidi camels. On the other hand, the riding camels are restricted to the north-east of the country between the Nile and Red Sea. Two main types are recognized, namely Anafi and RedSea Hills (Bishari) camels. (Amir *et al*., 2015).

Figure 4: Geographic map of the study areas.

3.3. Study design

Data was collected as part of a study on the sero-epidemiology of brucellosis infection in camels herding in Butana plain area and Al-Gadarief state. Across-sectional study was carried out during (January – May 2015) to estimate the prevalence of camel brucellosis and to investigate the associate risk factors. Multistage random sampling was designed based on state, governorate, localities, herds and animals, selection between locality, herd and individual animals based on simple random sampling.

A cross-sectional study was performed which involve the selection of sample of individuals from a large population and then the determination for each individual of the simultaneous presence or absence of disease and hypothesized risk factors association were investigated (Thrusfield, 2007).

The study areas were divided into six locations as fllow: From Butana plain area: Tumbol 115, Al saiala 8, (western butana). Saheilan 11, Kagmer 11, (southern Butana). Al khanger 21, Om sarha 18, (northern butana). Al hasheeb 15, Kardash 15, (eastern Butana). Alsobagh 41, Al takon 34, (central Butana). From Al-Gadarief State: Al showak 10 and Al rawashda 21, and selected two administration unit randomly by simple random sampling from each location then one epidemiological unit (farm or camel camps) had been chosen randomly from each administration unit, and then samples were taken randomly from each individual camel.

3.4. Sample size determination

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

$$\mathbf{N} = \frac{(\mathbf{1.96})^{\mathbf{2}}\mathbf{P_{exp}}(\mathbf{1}-\mathbf{P_{exp}})}{\mathbf{d^2}}$$ Where:

N = regarded sample size.

$\mathbf{P_{exp}}$ = expected prevalence.

$\mathbf{d^2}$ = allowable error (Thrusfield, 2005).

The expected prevalence in the previous study was (5.8%), according to Mohamed, (2013), in a study conducted in Khartoum State-Sudan. So the sample size was calculated as follows:

$$\mathbf{N} = \frac{(1.96)^2(5.8\%)(94.2\%)}{(0.05)^2}$$

$$\mathbf{N} = \frac{(1-96)^2(0.058)(0.942)}{(0.0025)} = 87.4 \text{ animal.}$$

This calculated sample size was thought not enough to represent all study population in these selected localities; therefore, this sample size was inflated by multiplying by three to increase accuracy of the study according to Thrusfield theory (Thrusfield, 2007). The new calculated sample size is (262), and then completed to (320).

The sample size was distributed proportionally among each locality according to the information obtained from the Ministry of Agriculture Animal Resources and Irrigation in order to represent all camel population in the study areas.

3.5. Sampling techniques

Multistage random sampling had been carried in this study because of its practical advantages and flexibility. All of the constituent members could be sampled or further stages of sampling could be undertaken, corresponding to the progressively high level of sub-sampling, then camels on each stage are usually sampled by simple random sampling technique (Thrusfield, 2007).

The following steps were followed in taking samples:

Blood samples (10 ml) were collected from the jugular vein using sterile disposable syringes. The blood was transferred into clean and sterile plain tubes. The tubes were labeled (farm and camel name or number). Serum was separated within 12 hours of collection by centrefuge and transported to the laboratory in an ice box where they stored at -20 ºC until laboratory testing was performed by the RBPT and SAT methods.

All serum samples from Butana plain area were transported to the Bacteriology Laboratory - Soba Veterinary Research Institute and tested by Rose Bengal Plate Test (RBPT), and the serum samples from Al-Gadarief stae were transported to the Laboratory of Al-Gadarief Veterinary Research Center and tested by Rose Bengal Plate Test (RBPT) then all sero-positive samples were further tested by the Standard Agglutination Tube test for confirmation.

3.6. Diagnostic techniques

a. Rose Bengal Plate Test (RBPT)

All serum samples were initially screened by the rose Bengal plate test using RBPT antigen (Soba Veterinary Research Institute).The serum samples were initially kept in the refrigerator at 4 ºC. Before testing, the sera and antigen were left at room temperature for half an hour before testing to maintain room temperature.

The test procedure was according to the methods and techniques of Alton *et al*, (1975); and was as follows:

30 µl of RBPT antigen was added to each circle on the plate and 30 µl of test serum was placed alongside the antigen. The antigen and test serum were mixed thoroughly using a wooden applicator. The plate was shaken for 4 minutes and the degree of agglutinating reactions were read and recorded as; + + + + (coarse clumping and clearing), + + + (clumping and some clearing), + + (visible fine agglutination), + (weak fine agglutinations using magnifying glass) in case of positive reactions, and 0 (no agglutinations) in case of negative reactions.

b. Serum agglutination test (SAT)

The serum agglutination test adopted in Europe was the tube agglutination test recommended by Alton *et al.,* (1988). The test procedure was as follows:

The test was conducted in glass tubes suitable for working with 1 ml volumes. In view of the occasional occurrence of prozone phenomena, at least five tubes were used for each serum under test. Using an automatic pipette, 0.8 ml of phenol-saline (0.85% sodium chloride and 0.5% phenol) was placed in the first tube and 0.5 ml in each succeeding tube; 0.2 ml of the serum under test was transferred to the first tube and mixed thoroughly with the phenol-saline; 0.5 ml of the mixture was carried over the second tube and mixed, after mixing 0.5 ml was transferred to the third tube, and so on. This process was continued until the last tube and after mixing, 0.5 ml of the serum dilution was discarded. To each tube 0.5 ml of antigen at the recommended dilution was added and the contents of the tubes were thoroughly mixed, thus giving final serum dilutions of 1:10, 1:20, etc. The tubes were then incubated at 37 ºC for 20 ± 1 hour before recording the results.

The degree of agglutination was assessed on the amount of clearing that had taken place in the tubes as compared to standard tubes. The tubes were examined without being shaken against a black background with a source of light directed from above and behind the tubes. Complete agglutination and sedimentation with water-clear supernatant was recorded as; + + + +, nearly complete agglutination and 75% clearance recorded as; + + +, marked agglutination and 50% clearing recorded as; + +, some sedimentation and 25%clearing recorded as; +, and no clearing recorded as; 0.

The accuracy and reliability of the reading were improved by standard tubes simulating the degree of agglutination for comparison purposes. Standards were prepared at the same time the tests were conducted and incubated with the test samples.

The results of agglutination tests were expressed in International Units and interpreted according to the recommendations of the 5th FAO/WHO Expert Committee of Brucellosis. By definition the International Standard of Anti-*Brucella abortus* serum (ISAbs) contained 1000 IU per ampoule, therefore, using an antigen that gave a titer of 1:500 with the ISAbs, a serum under test gave a titer of 1:40 contained $1000 \times 40 / 500 = 80$ IU/ml.

3.7. Questionnaire used for the Survey

Information about each sampled camel was obtained and camel owners were interviewed. This enabled highlighting the risk factors associated with brucellosis in support of the serological results.

A semi-structured questionnaire format was formulated for camel owners with the objective of elucidating the multi-factorial background of camel brucellosis. The questionnaire was completed by asking the owners the following questions: Location, age, gender, breed, body condition, parity, history of abortion, history of retained placenta, infertility, herd size, source of new camels, grazing system, watering, contact with other animals, production type, parturient room, awareness of brucellosis and awareness of fetus and proper fetal membrane disposal.

3.8. Statistical analysis

Data on tested serum and questionnaire were stored in Microsoft excel spread sheet (Microsoft Office Crop- 195-2007) as data base.

Statistical analysis was performed using "statistical package for the sciences" (SPSS), version 16.0 software for windows (SPSS-Inc., Chicago, IL, USA).

The sero-prevalence of animal level was calculated on the basis of RBPT positivity, dividing the number of *Brucella* reactors by total number of tested animals. Similarly, herd level prevalence was calculated as the number of herd with at least one positive animal divided by the total number of herds tested.

Data collected from the questionnaire survey was analyzed using descriptive statistics methods. Frequency distribution showed the frequency of occurrence of the observation in the present data set. Since the present data was categorical the frequency distribution of the variables compared the frequency of occurrence of observations in every category.

Cross tabulation was used in 2×2 tables and multi way tables to measure the degree of observation between these tables and related statistics. Association between the outcome variable (status of brucellosis) and its potential risk factors were first screened in a univariate analysis using the chi-square (χ^2); potential risk factors with p-value ≤ 0.25 were considered significant at this level.

Significant risk factors in univariate analysis were subjected to further multivariate analysis using logistic regression.EXB was used to indicate the strength of association with risk factors involved in the occurrence of the disease. All risk factors with p-value ≤ 0.05 were considered significant for brucellosis.

The linear relationship between RBPT agglutination intensities and SAT titers were also calculated.

CHAPTER THREE

RESULTS

4.1. Overall frequency

The overall prevalence of brucellosis in camels (*Camelus Dromedarius*) in the areas of the study was 7.2 % and 1.2 % when sera tested by RBPT and SAT were respectively. A total of 320 camels belonging to 12 herds were screened by RBPT among which 23camels were identified as seropositive by the RBPT reactions, and further confirmation by SAT identified 4 sero-positive reactions out of 320 with titter as 1:10 (13 IU), 1:20 (36 IU), 1:10 (18 IU), and 1:80 (246 IU) (Table 5 and 6).

Table 5: Estimate frequency distribution of positive and negative serum samples of camel brucellosis tested by RBPT.

Results		Frequency	Relative frequency (%)	Cumulative frequency
Valid	-ve	297	92.8	92.8
	+ve	23	7.2	100.0
	Total	320	100.0	

Table 6: Estimate frequency distribution of positive and negative serum samples of camel brucellosis tested by SAT.

Results		Frequency	Relative frequency (%)	Cumulative frequency
Valid	-ve	316	98.8	98.8
	+ve	4	1.2	100.0
	Total	320	100.0	

4.2. Frequency distribution of camels examined by RBPT and SAT:

A total of 320 camels were sampled from six areas (northern, western, southern, eastern, central Butana and Al-Gadarief State), in 12

selected locations (Tumbol, Al saiala, Saheilan, Kagmer, Al khanger, Om sarha, Al hasheeb, Kardash, Al sobagh, Al takon, Al showak, and Al rawashda).

Regarding locations 39, 22, 123, 30, 75, and 31 camels were from northern, southern western, eastern, middle Butana plain and Al-Gadarief state were respectively, selected and were as follows: As for age (year) 54 young (≤ 5) and 266 adult (≥ 6) camels. For gender 289 were females and 31were males. As for breed Arabi, Anafi, Bushari, and Cross 192, 108, 6, 14 camels, respectively. Regarding bodily condition, 250 camels were in good bodily condition and 70 camels were in poor bodily condition. For parity 257 she camel had 1 to 4 calving, 11 she camel had more than 4 clving, and 52 she camel not calved. As for history of abortion two she camels aborted and 318 did not abort, and for history of retained placenta only one she camel had retained placenta and 319 had no history of retained placenta. Regarding infertility only one she camel had infertility and 319 had no history of infertility. Regarding herd size 18, 112, and 190 small (≤ 10), medium (11-30), and large (> 30), were respectively, selected. As for source of new camels 67, 57, 152, and 44 Darfur, Kurdofan, Kassala, and Butana, respectively, were selected. Regarding the grazing system eight camels were fed indoor and 312 outdoor were tested. As for watering system 123, 31, and 166 tap water, surface water, and ponds, respectively. As for in contact with other animals 34 camels were in contact with other animals and 286 were not in contact with other animals. Regarding production type 60, 138, 4 and 118 were milk, meat, racing and mixed production were respectively, examined. For parturient room 34 had parturient rooms and 286 had no parturient rooms. As for awareness of brucellosis 299 owners had awareness of the disease and 21 were not aware of the disease. Regarding awareness of fetus and fetal

membrane disposal 34 owners were aware of proper disposal of fetal membranes and 286 were not aware of proper disposal of fetal membranes. All these camels were sampled and their sera were tested (Table 7).

Table 7: Frequency distribution of 320 camels examined for brucellosis by RBPT and SAT.

Risk factors	Frequency	Relative frequency	Cumulative frequency
Location			
N. Butana	39	12.2	12.2
S. Butana	22	6.9	19.1
W. Butana	123	38.4	57.5
E. Butana	30	9.4	66.9
M. Butana	75	23.4	90.3
Al-Gadarief	31	9.7	100.0
Age (year)			
Young (≤ 5)	54	16.9	16.9
Adult (≥ 6)	266	83.1	100.0
Gender			
Male	289	90.3	90.3
Female	31	9.7	100.0
Breed			
Arabi	192	60.0	60.0
Anafi	108	33.8	93.8
Bushari	6	1.9	95.6
Cross	14	4.4	100.0
Body condition			
Good	250	78.1	78.1
Poor	70	21.9	100.0
Parity			
(1-4) calves	257	80.3	80.3
(>4) calves	11	3.4	83.8
No caves	52	16.2	100.0
History of abortion			
Yes	2	0.6	0.6
No	318	99.4	100.0
History of retained placenta			
Yes	1	0.3	0.3
No	319	99.7	100.0
Infertility			
Yes	1	0.3	0.3
No	319	99.7	100.0
Herd size			

Table7 - Continued

Small (≤ 10)	18	5.6	5.6
Medium (11-30)	112	35.0	40.6
Large (> 30)	190	59.4	100.0
Source of new camels			
Darfur	67	20.9	20.9
Kurdofan	57	17.8	38.8
Kassala	152	47.8	68.2
Butana	44	13.8	100.0
Grazing system			
Indoor	8	2.5	2.5
Outdoor	312	97.5	100.0
Watering system			
Tap water	123	38.4	38.4
Surface water	31	9.7	48.1
Bounds	166	51.9	100.0
Contact with other animals			
Yes	34	10.6	10.6
No	286	89.4	100.0
Production type			
Milk	60	18.8	18.8
Meat	138	43.1	61.9
Racing	4	1.2	63.1
Mix	118	36.9	100.0
Parturient room			
Yes	34	10.6	10.6
No	286	89.4	100.0
Awareness of brucellosis			
Yes	299	93.4	93.4
No	21	6.6	100.0
Awareness of fetus & fetal membrane disposal			
Yes	34	10.6	10.6
No	286	89.4	100.0

4.3. Effect of risk factors of camel brucellosis tested by RBPT/SAT:

The distribution of seropositive camels according to the potential risk factors in this study were western Butana 23 (18.7%) by RBPT and 4 (3.3%) by SAT, but camels from northern Butana, southern Butana, eastern Butana, central Butana, and Al-Gadarief State were 0 (0%) and were found negative by the two test.

Regarding age (year) young (≤ 5) 6 (11.11%) were positive in RBPT and 2 (3.7 %) in SAT, adult (≥ 6) 17 (6.4%) in RBPT and 2 (0.8%) in SAT were found positive. As for gender male 1 (3.23%) in RBPT and 1 (3.2%) in SAT, female 22 (7.6 %) in RBPT and 3 (1.04%) in SAT were found positive. As for breed Arabi 13 (6.8%) in RBPT and 2 (1.04%) in SAT, Anafi 10 (9.3 %) in RBPT and 2 (1.9 %) in SAT were found positive. As for body condition good 22 (8.8%) in RBPT and 4 (1.6%) in SAT, poor 1 (1.4%) in RBPT were found positive. Regarding parity (1- 4 clves) 20 she camel (7.8%) in RBPT, and 2 she camel (0.8%) were found positive, also 3 she camel not calving (5.8%) in RBPT and 2 she camel not calving (3.9%) were found positive. As for history of abortion those without history were 23 (7.2%) positive in RBPT and 4 (1.3%) in SAT, also in the history of retained placenta without history 23 (7.2%) in RBPT and 4 (1.3%) in SAT were found positive. As for infertility, 23 (7.2%) have infertility in RBPT and 4 (1.3%) in SAT were found positive. Regarding herd size small (≤ 10) 3 (16.7%) were positive to RBPT, in the large 20 (10.6%) were positive in RBPT and 4 (2.11%) in SAT. As for source of new camels, those from Darfur 5 (7.5%) were positive in RBPT and 2 (2.99%) in SAT, Kurdofan 14 (24.6%) in RBPT, Kassala 2 (1.3%) in RBPT, and Butana 2 (4.6%) in RBPT were found positive. In the grazing system, those kept indoor 3 (37.5%) were positive in RBPT, outdoor 20 (6.4%) in RBPT and 4 (1.3%) in SAT. As for watering system, tap water 23 (18.7%) in RBPT and 4 (3.3%) in SAT were found positive. Regarding contact with other animals, camels without contact were 23 (8.04%) positive in RBPT and 4 (2.4%) in SAT. As for production type, camel used for meat production were 23 (16.7%) positive in RBPT and 4 (2.9%). As for parturient room, without room 23 (0.04%) in RBPT and 4 (1.4%) in SAT were found positive. Regarding awareness of brucellosis, have awareness 23 (7.7%) in RBPT and 4

(1.34%) were found positive. As for awareness of fetus and fetal membrane disposal, not done 23 (0.04%) in RBPT and 4 (1.4%) in SAT were found positive (Table 8).

Table 8: Effect of risk factors of camel brucellosis tested by RBPT/SAT

Risk factors	No. tested in (RBPT/SAT)	No. positive in RBPT (%)	No. positive in SAT (%)
Location			
N. Butana	39	0 (0)	0 (0)
S. Butana	22	0 (0)	0 (0)
W. Butana	123	23 (18.7)	4 (3.3)
E. Butana	30	0 (0)	0 (0)
M. Butana	75	0 (0)	0 (0)
Al-Gadarief	31	0 (0)	0 (0)
Age (year)			
Young (≤ 5)	54	6 (11.11)	2 (3.7)
Adult (≥ 6)	266	17 (6.4)	2 (0.8)
Gender			
Male	31	1 (3.23)	1 (3.2)
Female	289	22 (7.6)	3 (3.04)
Breed			
Arabi	192	13 (6.8)	2 (3.04)
Anafi	108	10 (9.3)	2 (3.9)
Bushari	6	0 (0)	0 (0)
Cross	14	0 (0)	0 (0)
Body condition			
Good	250	22 (8.8)	4 (1.6)
Poor	70	1 (1.4)	0 (0)
Parity			
(1-4) calves	257	20 (7.8)	2 (0.8)
(>4) calves	11	0 (0)	0 (0)
No calves	52	3 (5.8)	2 (3.9)
History of abortion			
Yes	2	0 (0)	0 (0)
No	318	23 (7.2)	4 (1.3)
History of retained placenta			
Yes	1	0 (0)	0 (0)
No	319	23 (7.2)	4 (1.3)
Infertility			
Yes	1	0 (0)	0 (0)
No	319	23 (7.2)	4 (1.3)
Herd size			
Small (≤ 10)	18	3 (16.7)	0 (0)
Medium (11-30)	112	0 (0)	0 (0)
Large (> 30)	190	20 (10.6)	4 (2.11)

Table8 - Continued			
Source of new camels			
Darfur	67	5 (7.5)	2 (2.99)
Kurdofan	57	14 (24.6)	2 (3.5)
Kassala	152	2 91.3)	0 (0)
Butana	44	2 (4.6)	0 (0)
Grazing system			
Indoor	8	3 (27.5)	0 (0)
Outdoor	312	20 (6.4)	4 (1.3)
Watering system			
Tap water	123	23 (18.7)	4 (3.4)
Surface water	31	0 (0)	0 (0)
Bounds	166	0 (0)	0 (0)
Contact with other animals			
Yes	34	0 (0)	0 (0)
No	286	23 (8.04)	4 (2.4)
Production type			
Milk	60	0 (0)	0 (0)
Meat	138	23 (16.7)	4 (2.9)
Racing	4	0 (0)	0 (0)
Mix	118	0 (0)	0 (0)
Parturient room			
Yes	34	0 (0)	0 (0)
No	286	23 (0.04)	4 (1.4)
Awareness of brucellosis			
Yes	299	23 (7.7)	0 (0)
No	21	0 (0)	4 (1.34)
Awareness of fetus & fetal membrane disposal			
Yes	34	0 (0)	0 (0)
No	286	23 (0.04)	4 (1.4)

4.4. Univariate analysis:

In the univariate analysis, the chi-squire test showed that there were 13 out of 18 risk factors statistically significantly associated with brucellosis prevalence (p-value ≤ 0.25), these were; location, age (year), herd size, source of new camels, and watering system (in the two test), body condition, grazing system, contact with other animals, production type, parturient room, awareness of brucellosis, and awareness of fetus and fetal membrane disposal (in RBPT alone), and parity (in SAT alone).

Using chi-square, showed the significant difference with brucellosis prevalence in location between the northern Butana, southern Butana, western Butana, eastern Butana, central Butana, and Al-Gadarief (χ^2 = 39.60, df = 5, p-value = 0.000) were highly significant association in RBPT with the rate of infection showed 23 (18.7%) in western Butana, and (χ^2 = 6.49, df = 5, p-value = 0.136) in SAT with the rate of infection showed 4 (3.3%) in western Butana. Age (year) showed the significant difference between adult and young (χ^2 = 1.499, df = 1, p-value = 0.222) in RBPT with the rate of infection showed 6 (11.11%) in young and 266 (6.4%) in adult and (χ^2 = 3.17, df = 1, p-value = 0.076) in SAT the infection rate showed 2 (3.7%) in young and 2 (0.8%) in adult. In body condition showed the significant difference between good and poor body condition (χ^2 = 4.455, df = 1, p-value = 0.035) in RBPT alone with rate of infection showed 22 (8.8%) in good and 1 (1.4%) in poor body condition. As for parity the significant difference showed between (1 to 4), (> 4) and no calve (χ^2 = 3.44, df = 2, p-value = 0.084) in SAT alone with the rate of infection showed 2 (0.8%) in (1-4) calve and 2 (3.9%) in more than 4 calve. Regarding herd size showed significant difference between small, medium and large (χ^2 = 14.27, df = 2, p-value 0.095) in RBPT with infection rate showed 3 (16.7%) in small and 20 (10.6%) in large herd size and (χ^2 = 2.77, df = 2, p-value = 0.122) in SAT with infection rate showed 4 (2.11%) in large herd size. As in source of new camels showed the significant difference between Darfur, Kurdofan, Kassala, and Butana (χ^2 = 34.12, df = 3, p-value = 0.011) in RBPT with infection rate showed 5 (7.5%), 14 (24.6%), 2 (1.3%) and 2 (4.6%) Darfour, Kurdofan, Kassala and Butana respectively, and (χ^2 = 6.47, df = 3, p-value = 0.031) with infection rate showed 2 (2.99%) in Darfur and 2 (3.51%) in kurdofan. In the grazing system the significant difference showed between indoor, outdoor (χ^2 = 11.30, df = 1, p-value = 0.001) in RBPT alone which highly

significant with infection rate showed 3 (37.5%) in indoor and 20 (6.4%) in outdoor. Regarding watering system the significant difference showed between tap water, surface water, and pounds (χ^2 = 39.69, df = 2, p-value = 0.000) in RBPT which was highly significant with infection rate showed 23 (18.7%) in tap water, and (χ^2 = 6.49, df = 2, p-value = 0.015) in SAT with rate of infection 4 (3.3%) in tap water. In the contact with other animals the significant difference showed between camels with other animals and not (χ^2 = 2.95, df = 1, p-value = 0.07) in RBPT alone with the infection rate was 23 (.04%) in camels found without other animals. As for production type showed the significant difference between milk, meat, racing and mix (χ^2 = 32.69, df = 3, p-value = 0.016) in RBPT alone with an infection rate showed 23 (16.7%) in meat camels. In the parturient room, showed significant difference between camel have parturient room and not (χ^2 = 2.95, df = 1. P-value = 0.087) in RBPT alone with infection rate found 23 (0.04%) in camels have not parturient room. Regarding awareness of brucellosis the significant difference showed between with aware and not aware (χ^2 = 1.74, df = 1, p-value = 0.188) in RBPT alone with the infection rate showed 23 (7.7%) in the camel with aware. As for awareness of fetus and fetal membrane disposal the significant difference between aware and without aware (χ^2 = 2.95, df = 1, p-value = 0.087) in RBPT alone with the rate of infection 23 (0.04%).

In this study there was 6 out of 18 (tested by RBPT) and 12 out of 18 (tested by SAT) risk factors found not to have a significant association with brucellosis infection in camels (p-value $\geq$ 0.25), there were; gender, breed, history of abortion, history of retained and infertility (in RBPT and SAT), parity (in RBPT alone) and body condition, grazing system, contact with other animals, production type, parturient room, awareness

of brucellosis and awareness of fetus and fetal membrane disposal (in SAT alone) (table 9 and 10).

Table 9: Summary of univariate analysis using chi-square test for potential risk factors associated with brucellosis in 320 camel serum samples tested by RBPT.

Risk factors	No. tested	No. +ve (%)	d.f	X^2	p-value
Location			5	39.69	0.000
N. Butana	39	0 (0)			
S. Butana	22	0 (0)			
W. Butana	123	23 (18.7)			
E. Butana	30	0 (0)			
M. Butana	75	0 (0)			
Al-Gadarief	31	0 (0)			
Age (year)			1	1.499	0.222
Young (≤ 5)	54	6 (11.11)			
Adult (≥ 6)	266	17 (6.4)			
Gender			1	0.808	0.370
Male	31	1 (3.23)			
Female	289	22 (7.6)			
Breed			3	2.294	0.632
Arabi	192	13 (6.8)			
Anafi	108	10 (9.3)			
Bushari	6	0 (0)			
Cross	14	0 (0)			
Body condition			1	4.455	0.035
Good	250	22 (8.8)			
Poor	70	1 (1.4)			
Parity			2	1.145	0.511
(1-4) calves	257	20 (7.8)			
(>4) calves	11	0 (0)			
No calves	52	3 (5.8)			
History of abortion			1	0.156	0.693
Yes	2	0 (0)			
No	318	23 (7.2)			
History of retained placenta			1	0.078	0.781
Yes	1	0 (0)			
No	319	23 (7.2)			
Infertility			1	0.078	0.781
Yes	1	0 (0)			
No	319	23 (7.2)			
Herd size			2	14.27	0.095
Small (≤ 10)	18	3 (16.7)			
Medium (11-30)	112	0 (0)			
Large (> 30)	190	20 (10.6)			
Source of new camels			3	34.12	0.011

Table9 - Continued

Darfur	67	5 (7.5)			
Kurdofan	57	14 (24.6)			
Kassala	152	2 (1.3)			
Butana	44	2 (4.6)			
Grazing system			1	11.30	0.001
Indoor	8	3 (37.5)			
Outdoor	312	20 (6.4)			
Watering system			2	39.69	0.000
Tap water	123	23 (18.7)			
Surface water	31	0 (0)			
Bounds	166	0 (0)			
Contact with other animals			1	2.95	0.087
Yes	34	0 (0)			
No	286	23 (8.04)			
Production type			3	32.69	0.016
Milk	60	0 (0)			
Meat	138	23 (16.7)			
Racing	4	0 (0)			
Mix	118	0 (0)			
Parturient room			1	2.95	0.087
Yes	34	0 (0)			
No	286	23 (0.04)			
Awareness of brucellosis			1	1.74	0.188
Yes	299	23 (7.7)			
No	21	0 (0)			
Awareness of fetus & fetal membrane disposal			1	2.95	0.087
Yes	34	0 (0)			
No		23 (0.04)			

Table 10: Summary of univariate analysis using chi-square test for potential risk factors associated with brucellosis in 320 camel serum samples tested by SAT.

Risk factors	No. tested	No. +ve (%)	d.f	X^2	p-value
Location			5	6.49	0.136
N. Butana	39	0 (0)			
S. Butana	22	0 (0)			
W. Butana	123	4 (3.3)			
E. Butana	30	0 (0)			
M. Butana	75	0 (0)			
Al-Gadarief	31	0 (0)			
Age (year)			1	3.17	0.076
Young (≤ 5)	54	2 (3.7)			

Table10-Continued

Adult (≥ 6)	266	2 (0.8)			
Gender			1	1.09	0.298
Male	31	1 93.2)			
Female	289	3 (1.04)			
Breed			3	0.64	0.986
Arabi	192	2 (1.04)			
Anafi	108	2 (1.9)			
Bushari	6	0 (0)			
Cross	14	0 (0)			
Body condition			1	1.13	0.288
Good	250	4 (1.6)			
Poor	70	0 (0)			
Parity			2	3.44	0.084
(1-4) calves	257	2 (0.8)			
(>4) calves	11	0 (0)			
No calves	52	2 (3.9)			
History of abortion			1	0.03	0.873
Yes	2	0 (0)			
No	318	4 (1.3)			
History of retained placenta			1	0.01	0.910
Yes	1	0 (0)			
No	319	4 (1.3)			
Infertility			1	0.01	0.910
Yes	1	0 (0)			
No	319	4 (1.3)			
Herd size			2	2.77	0.122
Small (≤ 10)	18	0 (0)			
Medium (11-30)	112	0 (0)			
Large (> 30)	190	4 (2.11)			
Source of new camels			3	6.47	0.031
Darfur	67	2 (2.99)			
Kurdofan	57	2 (3.51)			
Kassala	152	0 (0)			
Butana	44	0 (0)			
Grazing system			1	0.10	0.748
Indoor	8	0 (0)			
Outdoor	312	4 (1.3)			
Watering system			2	6.49	0.015
Tap water	123	4 (3.3)			
Surface water	31	0 (0)			
Bounds	166	0 (0)			
Contact with other animals			1	0.49	0.488
Yes	34	0 (0)			
No	286	4 (2.4)			
Production type			3	5.34	0.332
Milk	60	0 (0)			
Meat	138	4 (2.19)			

Table10-Continued

Racing	4	0 (0)			
Mix	118	0 (0)			
Parturient room			1	0.49	0.488
Yes	34	0 (0)			
No	286	4 (1.4)			
Awareness of brucellosis			1	0.29	0.594
Yes	299	4 (1.34)			
No	21	0 (0)			
Awareness of fetus & fetal membrane disposal			1	0.48	0.488
Yes	34	0 (0)			
No		4 (1.4)			

4.5. Multivariate analysis

The multivariate analysis using logistic regression showed that there were 2 out of 13 potential risk factors had significant association with brucellosis infection in camels (p-value ≤ 0.05). These were; source of new camels (p-value 0.004) and grazing system (p-value 0.030).

In multivariate 11 out of 13 risk factors found no significant with brucellosis infection in camels these were; location (p-value 1.000), age (p-value 0.460), body condition (p-value 0.066), parity (p-value 1.000), herd size (p-value 0.402), watering system (p-value 1.000), contact with other animals (p-value 0.998), production type (p-value 0.700), parturient room (p-value 0.998), awareness of brucellosis (p-value 0.998) and awareness of fetus and fetal membrane disposal (p-value 0.988) (Table 11).

Table 11: Summary of multivariate analysis using logistic regression for potential risk factors associated with brucellosis in 320 camel serum samples tested by RBPT and SAT.

Risk factors	No. tested	No. +ve in RBPT (%)	No. +ve in SAT (%)	Exp(B)	C.I. 95.0% for Exp (B)		p-value
					Lower	Upper	
Location							1.000
N. Butana	39	0 (0)	0 (0)	Ref			
S. Butana	22	0 (0)	0 (0)	3.72	0.00	-	
E. Butana	30	0 (0)		1.00	0.00	-	
M. Butana	75	0 (0)		1.00	0.00	-	
Al-Gadarief	31	0 (0)		1.00	0.00	-	
W. Butana	123	23(18.7)		1.00	0.00	-	
Age (year)							0.460
Adult (≤ 5)	266	17(6.4)	2 (8)	Ref			
Young (≥ 6)	54	6(11.1)	2 (8)	0.68	0.245	1.892	
Body condition							0.066
Poor	70	1(1.4)	0 (0)	Ref			
Good	250	22(8.8)	4 (1)	0.15	0.019	1.134	
Parity							0.719
(>4) calves	11	0(0)	0 (0)	Ref			
No calves	52	3(5.8)	2 (8)	0.99	0.249	3.972	
(1-4) calves	257	20(7.8)	2 (8)	1.03	0.000	-	
Herd size							1.000
Medium	112	0 (0)	0 (0)	Ref			
Large	190	20(10.6)	4 (1)	0.35	0.077	1.607	
Small	18	3(16.7)	0 (0)	0.41	0.000	-	
Source of new camels							0.004
Kassala	152	2(1.3)	0 (0)	Ref			
Butana	44	2(4.6)	0 (0)	0.66	0.085	5.228	
Darfur	67	5(7.5)	2 (8)	4.66	0.932	23.37	
Kurdofan	57	14(24.6)	2 (8)	0.78	0.207	8.316	
Grazing system							0.030
Outdoor	312	20(6.4)	4 (1)	Ref			
Indoor	8	3(37.5)	0 (0)	0.19	0.041	0.849	
Watering system							1.000
Surface water	31	0 (0)	0 (0)	Ref			
Bounds	166	0 (0)	0 (0)	0.00	0.000	-	
Tap water	123	23(18.7)	4 (1)	0.00	0.000	-	
Contact with other animals							0.998
Yes	34	0 (0)	0 (0)	Ref			
No	286	23(8.04)	4 (1)	1.706	0.000	-	

Table 11- Cnotinued

Production type							0.700
Milk	60	0 (0)	0 (0)	Ref			
Mix	118	0 (0)	0 (0)	1.98	0.000	-	
Racing	4	0 (0)	0 (0)	2.151	0.000	-	
Meat	138	23(16.7)	4 (1)	3.262	0.000	-	
Parturient room							0.998
Yes	34	0 (0)	0 (0)	Ref			
No	286	23(0.04)	4 (1)	0.00	0.000	-	
Awareness of brucellosis							0.998
No	21	0 (0)	4 (1)	Ref			
Yes	299	23(7.7)	0 (0)	0.00	0.000	-	
Awareness of fetus & fetal membrane disposal							0.988
Yes	34	0 (0)	0 (0)	Ref			
No	286	23(0.04)	4 (1)	0.00	0.000	-	

4.6. Comparison between RBPT and SAT:

Rose Bengal plate test gave positive reaction in 23 seropositive out of 320 serum samples and SAT agree with RBPT in 3 out of 23 serum samples which gave seropositive with SAT and only one sample tested positive with SAT out of 297 that tested negative with RBPT (Table 12).

Table 12: Cross tabulation using "kappa test" to compare between RBPT and SAT in examination of 320 serum samples of camel for brucellosis infection.

Count		**RBPT**		**Total**
		-ve	+ve	
SAT	-ve	296	20	316
	+ve	1	3	4
Total		297	23	320

CHAPTER FOUR

5.1. DISCUSSION

In the current study and based on the results obtained from RBPT and SAT, the prevalence of *Brucellosis* of the examined camels was 7.2% and 1.2%, respectively. This result was in accordance with that recorded in Ethiopia (Teshome *et al.,* 2003). However, higher prevalence was recorded in Sudan (Musa and Shigidi 2001; Omer *et al*., 2010; Mohamed and Elsanosi, 2013), Saudi Arabia (Abbas and Agab 2002), Jordan (Al-Majali *et al*., 2008). The differences in the prevalence of camel *brucellosis* from different countries could be attributed to varying husbandry and management practices (Bati, 2004).

The univariate analysis, the evidence showed beyond doubt the occurrence of the disease in camels in western Butana was 18.7% and 3.3% tested by RBPT and SAT respectively, Prevalence of *Brucella* antibodies in all age groups of camels showed that brucellosis infection in the animals was 11.11% and 3.7% in RBPT and SAT for young, but was 6.4% and 0.8% in two tested adults who started early in life probably through sucking and persisted into adulthood means that the young camels are more suscetable to the disese the reasons may be due to separation of young from adult and kept them in farms of pedding ner the place were she camel parturient also found plcenatal dischge and fetal membrane paaed from infected one. Radwan *et al.,* (1992) also reported similarity of seroprevalence among various age groups of adult female dromedaries. Similar patterns were found in cattle, Oloffs *et al*., (1998) reported that 30% of the positive animals in Uganda were younger than three years of age and within them was a 2-year-old bull, which was not introduced for service.As for body condition and production type

wbrucellosis prevalence was (16.7% good body condition, 10.6% poor body condition) thos my be due to poor body conditions ovoid contact with others, and 16.7% in meat camels respectively, and parity had also no effect on positivity in breeding camels, in this study the prevalence rate were 7.8% for camel that had one (1 to 4) calves and 5.8% in camels that have no calves, this might be either due to equal susceptibility of breeding females or possibly as a result of negative impact of brucellosis on fertility, infected animals may have lower number of parity acording to (Wernery and Kaaden, 2002). In watering system the rate of infection is high in camels tht used to take tap water 18.7%. A large number of livestock herds are congregated at water points facilitating the spread of disease. Traditional wells, ponds and few rivers are major permanent water sources in the area (Helland, 1982). Unlike traditional wells (water lifted by people and added to troughs), animals have direct access to pond water contaminated by discharges. Correspondingly, a higher infection rate was recorded in herds often using traditional wells and ponds together. However, the mobile nature of camel herds may not restrict them to a specific category of the water resources, making conclusion difficult (Bati, 2004). In multivariate nalalysis the ssociation of grazing system and source of new camels have significant effect on the disease prevelance.

However, herders in Borena and elsewhere invariably keep small ruminants and cattle alongside with camels. There is high chance of brucellosis transmission from these ruminants to dromedaries as they live in free range in promiscuity in the bush and at water points (Andreani *et al.,* 1982). Acoording to the Radwan *et al.,* (1992), contact between dromedaries and small ruminants is incriminated for the transmission of brucellosis to camels. Abou-Eisha, (2000) also observed higher

seroprevalence in camels that were in contact with sheep and goat. Moreover, higher frequencies of *B. melitensis* isolation from camels perhaps magnify the role of small ruminants in the transmission of brucellosis to camels (Bati, 2004). The prevelance rate of parturient room, and fetus & fetal membrane disposal awareness was 0.04%, 7.7% and 0.04% respectively. The disease can also be a health hazard to human beings particularly to pastoral households who in many ways are exposed to the disease (Abbas and Agab 2002). Camel owners of the study area consume raw milk, and do delivery assistance, clean newborns, assist suckling and carry the young from field to home without any protection. The knowledge about brucellosis is nil among herdsmen. These can put the public health of the area at risk. The disease in man may be misdiagnosed due to the prevailing malaria infections in dry areas (Abou-Eisha, 2000; El-Ansary *et al.,* 2001). Brucellosis in camels seemed to display less clinical signs and antibody levels than in cattle, probably because of a relative resistance of the former to brucellosis. The disease should be controlled by vaccination of camels and primary hosts (Bati, 2004).

Multivariate analysis showed that source of new camels and grazing system was significantly associated with seroprevalence of camel brucellosis (p-value 0.004), (p-value 0.030) respectivily in logistic regression (p-value $\leq$ 0.05). Similar association was recorded by Bati (2004) ($P<0.05$), Al- Majali *et al*., (2008) ($P<0.05$) and Ghanem *et al*., (2009) ($P<0.001$). The seroprevalence findings of the present study is similar to the previous reports from different countries (Abu-Damir *et al*., 1984; Abbas *et al.,* 1987; Baumann and Zessin, 1992; Abou-Eisha, 2000; Omar *et al*., 2010; Azwai *etal*., 2001; Teshome *et al.,* 2003). However, it is lower than some studies in Ethiopia Kenya (Waghela *et al.,* 1978),

Nigeria (Ajogi and Adamu, 1998), Sudan (Ginawi, 1997; Majid *et al.*, 1999), Somalia (Andreani *et al*., 1982), Kuwait (Al-Khalaf and El-Khaladi, 1989) and Saudi Arabia (Radwan *et al*., 1992).

However, reporting lower prevalence rates by some authors could also be due to low diagnostic sensitivity of the tests used (Baumann and Zessin, 1992) or as a consequence of serial multiple tests (Abbas and Agab, 2002). Cross-reacting bacteria such as *Escherchia coli, Yersinia entrocolitica* and *Salmonella serotypes* (Garin-Bastuji *et al.,* 1999) have the potential of affecting the serological findings when tests of low specificity are used. Accordingly, RBPT is considered as satisfactory screening test (OIE, 2000; Quinn *et al.,* 2002). The highest specificity of CFT could be used as confirmatory test in serial testing (OIE, 2000). Therefore, the use of serial testing procedure initially screened all samples by RBPT, and then applying CFT or SAT on positive reactors as employed in the current test improves the efficiency of detecting brucellosis as previously reported (Teshome *et al.,* 2003). Improvement of the diagnostic specificity of the test is particularly useful in control programs when testing and slaughter policy is adopted in camels. There is yet no standards set for the diagnostic test protocol and diagnostic titer for brucellosis. OIE (2000) recommends the test procedure outlined for the diagnosis of bovine brucellosis to be applied for camels.

In the present study titer of 1/10 and above dilutions were considered positive for SAT. As a result sero-positivity was confirmed in 23 out of 320 RBPT positive reactors. The highest titer recorded was lower than what has been reported by Teshome *et al.* (2003) in which 30% had a dilution rate of 1/640.

The results of the present investigation indicated that, *Brucella* existed within camel herds in Butana plain area and Al-Gadarief, the

source of new camel and grazing system are a major risk factors associated with camel brucellosis. Therefore, frequent screening of the camel source and grazing are recommended to assess the status of the disease and to identify the *Brucella* species involved. Moreover, epidemiological studies are needed to explore the current status of the disease in other ruminants to enable the public and veterinary authorities to construct concrete program for prevention of the disease within sources and grazing system of camel herds (Mohamed *et al*., 2013).

5.2. CONCLUSIONS

The current study has shown the overall prevalence of brucellosis antibodies as 7.2% of all tested *dromedarius* in Butana plain area and Al-Gadarief State. Despite the fact that the overall sero-prevalence of brucellosis recorded in this study was low, this implies that animals and family members of those infected herds are above all at risk.

In univariate analysis location, age (year), herd size, source of new camels, and watering system (in the two test), body condition, grazing system, contact with other animals, production type, parturient room, awareness of brucellosis, and awareness of fetus and fetal membrane disposal (in RBPT alone), parity (in SAT alone) categories were significantly associated with sero-prevalence of camel brucellosis (p-value ≤ 0.25).

The multivariate analysis of presumed risk factors indicated that source of new camels and grazing system are a major risk factor associated with camel brucellosis (p-value ≤ 0.05).

Due to lack of awareness about brucellosis together with existing habit of raw milk consumption and close contact with animals these can serve as means of infection in human beings, the practices assessed in this study also indicated that owners need to be trained and educated about the harm of mal practices and regular compliance with hygiene.

5.3. RECOMMENDATIONS

Based on the results of this study the following are recommendations are suggested:

Although the prevalence of brucellosis in camel production is probably related to husbandry practices, there is lack of information regarding the pathogenesis and epidemiology of brucellosis in camels, however, education and training of herdsmen about animal diseases, modern management practices and sanitary measures could play a major role in lowering the prevalence of the diseases.

1) Development of control measures that could minimize the infection with brucellosis by:
   - Using the prophylactic treatment for the disease.
   - Routine vaccination for cattle, sheep and goats should be considered in areas where camels are kept together with these animals.
   - Proper and strict follow-up of treatment should be done by professionals and supervision of the field personnel by experts should be practiced.
   - Culling of old and chronically affected camels, and screening of camel blood and milk should be considered in attempts to reduce prevalence of brucellosis.
2) Isolation and identification of species and biotypes of *Brucella* involved in camel brucellosis warrant further and intensive studies.
3) Extension service and training programs aiming at the creation of awareness about the importance and prevention of brucellosis among dairy camels, screening of camels and milk for brucellosis,

dry therapy, and hygiene at milking and camel hygiene should be considered in attempts to reduce prevalence of brucellosis.

4) Different epidemiological factors associated with brucellosis prevalence that interplays in brucellosis occurrence need confirmation through further studies.
5) The low prevalence rate of camel brucellosis observed in the other studies of some countries may suggest the implementation of a test-and- slaughter policy. However, this remains difficult in our country for the time being due to the free movement of herds in the pastoral areas and unaffordable compensation to the owners. There fore improving management practices can assist in reducing the spread of infection in camel herds.
6) Most of the brucellosis-positive camels are clinically healthy and owners do not allow their positive animals to be culled. There fore, the author proposes that the best way to halt the spread of the disease is to castrate serologically positive bulls.
7) Future, studies are extremely necessary to investigate the risk factors and the public health issues related to camel brucellosis in other areas in Sudan.
8) Governmental and non-governmental organizations are requested to support camel researches, veterinary services to establish adequate veterinary infrastructures concerning intensive care and attention towards the welfare of camel during parturition and production.

CHAPTER FIVE

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APPENDICES

6.2. Appendices 1

Frequency

RBPT					
		Frequency	Percent	Valid percent	Cumulative percent
Valid	+ve	297	92.8	92.8	92.8
	-ve	23	7.2	7.2	100.0
	Total	320	100.0	100.0	

SAT					
		Frequency	Percent	Valid percent	Cumulative percent
Valid	+ve	316	98.8	98.8	98.
	-ve	4	1.2	1.2	100.0
	Total	320	100.0	100.0	

Location					
		Frequency	Percent	Valid percent	Cumulative percent
Valid	Northern butana	39	12.2	12.2	12.2
	Western butana	123	38.4	38.4	50.6
	Southern butana	22	6.9	6.9	57.5
	Eastern butana	30	9.4	9.4	66.9
	Meddle butana	75	23.4	23.4	90.3
	Al-Gadarief	31	9.7	9.7	100.0
	Total	320	100.0	100.0	

Age (year)					
		Frequency	Percent	Valid percent	Cumulative percent
Valid	Young (≤ 5)	54	16.9	16.9	16.9
	Adult (≥ 6)	266	83.1	83.1	100.0
	Total	320	100.0	100.0	

Gender					
		Frequency	Percent	Valid percent	Cumulative percent
Valid	Female	289	90.3	90.3	90.3
	Male	31	9.7	9.7	100.0
	Total	320	100.0	100.0	

Breed					
		Frequency	Percent	Valid percent	Cumulative percent
Valid	Arabi	192	60.0	60.0	60.0
	Anafi	108	33.8	33.8	93.8
	Bushari	6	1.9	1.9	95.6
	Cross	14	4.4	4.4	100.0
	Total	320	100.0	100.0	

Body condition					
		Frequency	Percent	Valid percent	Cumulative percent
Valid	Good	250	78.1	78.1	78.1
	Poor	70	21.9	21.9	100.0
	Total	320	100.0	100.0	

Parity					
		Frequency	Percent	Valid percent	Cumulative percent
Valid	(1-4) calves	257	80.3	80.3	80.3
	(>4) calves	11	3.4	3.4	83.8
	No calves	52	16.2	16.2	100.0
	Total	320	100.0	100.0	

History of abortion					
		Frequency	Percent	Valid percent	Cumulative percent
Valid	Yes	2	0.6	0.6	0.6
	No	318	99.4	99.4	100.0
	Total	320	100.0	100.0	

History of retained placenta					
		Frequency	Percent	Valid percent	Cumulative percent
Valid	Yes	1	0.3	0.3	0.3
	No	319	99.7	99.7	100.0
	Total	320	100.0	100.0	

Infertility					
		Frequency	Percent	Valid percent	Cumulative percent
Valid	Yes	1	0.3	0.3	0.3
	No	319	99.7	99.7	100.0
	Total	320	100.0	100.0	

Herd size					
		Frequency	Percent	Valid percent	Cumulative percent
Valid	Small (≤ 10)	18	5.6	5.6	5.6
	Medium (11-30)	112	35.0	35.0	40.6
	Large (> 30)	190	59.4	59.4	100.0
	Total	320	100.0	100.0	

Source of new camels					
		Frequency	Percent	Valid percent	Cumulative percent
Valid	Darfur	67	20.9	20.9	20.9
	Kurdofan	57	17.8	17.8	38.8
	Kassala	152	47.5	47.5	86.2
	Butana	44	13.8	13.8	100.0
	Total	320	100.0	100.0	

Grazing system					
		Frequency	Percent	Valid percent	Cumulative percent
Valid	Indoor	8	2.5	2.5	2.5
	Outdoor	312	97.5	97.5	100.0
	Total	320	100.0	100.0	

Watering system					
		Frequency	Percent	Valid percent	Cumulative percent
Valid	Tap water	123	38.4	38.4	38.4
	Surface water	31	9.7	9.7	48.1
	Pounds	166	51.9	51.9	100.0
	Total	320	100.0	100.0	

Contact with other animals					
		Frequency	Percent	Valid percent	Cumulative percent
Valid	Yes	34	10.6	10.6	10.6
	No	286	89.4	89.4	100.0
	Total	320	100.0	100.0	

Production type					
		Frequency	Percent	Valid percent	Cumulative percent
Valid	Milk	60	18.8	18.8	18.8
	Meat	138	43.1	43.1	61.9
	Racing	4	1.2	1.2	63.1
	Mix	118	36.9	36.9	100.0
	Total	320	100.0	100.0	

Parturient room					
		Frequency	Percent	Valid percent	Cumulative percent
Valid	Yes	34	10.6	10.4	10.4
	No	286	89.4	89.4	100.0
	Total	320	100.0	100.0	

Awareness of brucellosis					
		Frequency	Percent	Valid percent	Cumulative percent
Valid	Yes	299	93.4	93.4	93.4
	No	21	6.6	6.6	100.0
	Total	320	100.0	100.0	

Awareness of fetus & fetal membrane disposal					
		Frequency	Percent	Valid percent	Cumulative percent
Valid	Yes	34	10.6	10.6	10.6
	No	286	89.4	89.4	100.0
	Total	320	100.0	100.0	

6.3. Appendices 2

a. Cross RBPT

Count				**Location**				
		Northern butana	Western butana	Southern butana	Eastern butana	Meddle butana	Al-gdarief	**Total**
RBPT	-ve	39	100	22	30	75	31	297
	+ve	0	23	0	0	0	0	23
Total		39	123	22	30	75	31	320

Count		**Age (year)**		**Total**
		Yung (≤ 5)	Adult (≥ 6)	
RBPT	-ve	48	249	297
	+ve	6	17	23
Total		54	266	320

Count		**Gender**		**Total**
		Female	Male	
RBPT	-ve	267	30	297
	+ve	22	1	23
Total		289	31	320

Count		**Breed**				**Total**
		Arabi	Anafi	Bushari	Cross	
RBPT	-ve	179	98	6	14	297
	+ve	13	10	0	0	23
Total		192	108	6	14	320

Count		Body condition		Total
		Good	Poor	
RBPT	-ve	228	69	297
	+ve	22	1	23
Total		250	70	320

Count		Parity			Total
		(1-4) calve	(>4) calve	No calve	
RBPT	-ve	237	11	49	297
	+ve	20	0	3	23
Total		257	11	52	320

Count		History of abortion		Total
		Yes	No	
RBPT	-ve	2	295	297
	+ve	0	23	23
Total		2	318	320

Count		History of retained placenta		Total
		Yes	No	
RBPT	-ve	1	296	297
	+ve	0	23	23
Total		1	319	320

Count		Infertility		Total
		Yes	No	
RBPT	-ve	1	296	297
	+ve	0	23	23
Total		1	319	320

Count		Herd size			Total
		Small	Medium	Large	
RBPT	-ve	15	112	170	297
	+ve	3	0	20	23
Total		18	112	190	320

Count		Source of new camels				Total
		Darfur	Kurdofan	Kassala	Butana	
RBPT	-ve	62	43	150	42	297
	+ve	5	14	2	2	23
Total		67	57	152	44	320

Count		Grazing system		Total
		Indoor	Outdoor	
RBPT	-ve	5	292	297
	+ve	3	20	23
Total		8	312	320

Count		Watering system			Total
		Tap water	Surface water	Pounds	
RBPT	-ve	100	31	166	297
	+ve	23	0	0	23
Total		123	31	166	320

Count		Contact with other animals		Total
		Yes	No	
RBPT	-ve	34	263	297
	+ve	0	23	23
Total		34	286	320

Count		Production type				Total
		Milk	Meat	Racing	Mix	
RBPT	-ve	60	115	4	118	297
	+ve	0	23	0	0	23
Total		60	138	4	118	320

Count		Parturient room		Total
		Yes	No	
RBPT	-ve	34	263	297
	+ve	0	23	23
Total		34	286	320

Count		Awareness of brucellosis		Total
		Yes	No	
RBPT	-ve	276	21	297
	+ve	23	0	23
Total		299	21	320

Count		Awareness of fetus & fetal membrane disposal		Total
		Yes	No	
RBPT	-ve	34	263	297
	+ve	0	23	23
Total		34	286	320

b. Cross SAT:

Count				Location				Total
		Northern butana	Western butana	Southern butana	Eastern butana	Meddle butana	Al-gdarief	
SAT	-ve	39	119	22	30	75	31	316
	+ve	0	4	0	0	0	0	4
Total		39	123	22	30	75	31	320

Count		Age (year)		Total
		Yung (≤ 5)	Adult (≥ 6)	
SAT	-ve	52	264	316
	+ve	2	2	4
Total		54	266	320

Count		Gender		Total
		Female	Male	
SAT	-ve	286	30	316
	+ve	3	1	4
Total		289	31	320

Count		Breed				Total
		Arabi	Anafi	Bushari	Cross	
SAT	-ve	190	106	6	14	316
	+ve	2	2	0	0	4
Total		192	108	6	14	320

Count		Body condition		Total
		Good	Poor	
SAT	-ve	246	70	316
	+ve	4	0	4
Total		250	70	320

Count		Parity			Total
		(1-4) calves	(>4) calves	No calves	
SAT	-ve	255	11	50	316
	+ve	2	0	2	4
Total		257	11	52	320

Count		History of abortion		Total
		Yes	No	
SAT	-ve	2	314	316
	+ve	0	4	4
Total		2	318	320

Count		History of retained placenta		Total
		Yes	No	
SAT	-ve	1	315	316
	+ve	0	4	4
Total		1	319	320

Count		Infertility		Total
		Yes	No	
SAT	-ve	1	315	316
	+ve	0	4	4
Total		1	319	320

Count		Herd size			Total
		Small	Medium	Large	
SAT	-ve	18	112	186	316
	+ve	0	0	4	4
Total		18	112	190	320

Count		Source of new camels				Total
		Darfur	Kurdofan	Kassala	Butana	
SAT	-ve	65	55	152	44	316
	+ve	2	2	0	0	4
Total		67	57	152	44	320

Count		Grazing system		Total
		Indoor	Outdoor	
SAT	-ve	8	308	316
	+ve	0	4	4
Total		8	312	320

Count		Watering system			Total
		Tap water	Surface water	Pounds	
SAT	-ve	119	31	166	316
	+ve	4	0	0	4
Total		123	31	166	320

Count		Contact with other animals		Total
		Yes	No	
SAT	-ve	34	282	316
	+ve	0	4	4
Total		34	286	320

Count		Production type				Total
		Milk	Meat	Racing	Mix	
SAT	-ve	60	134	4	118	316
	+ve	0	4	0	0	4
Total		60	138	4	118	320

Count		Parturient room		
		Yes	No	**Total**
SAT	-ve	34	282	316
	+ve	0	4	4
Total		34	286	320

Count		Awareness of brucellosis		
		Yes	No	**Total**
SAT	-ve	295	21	316
	+ve	4	0	4
Total		299	21	320

Count		Awareness of fetus & fetal membrane disposal		
		Yes	No	**Total**
SAT	-ve	34	282	316
	+ve	0	4	4
Total		34	286	320

6.4. Appendices 3

(a) Univariate RBPT

Location

Chi-square Test			
	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	39.690[a]	5	0.000
Likelihood Ratio	46.85	5	0.000
Linear-by-Linear Association	13.607	1	0.000
N of Valid Cases	320		

a. 4 cells (33.3%) have expected count less than 5. The minimum expected count is 1.58.

Age (year)

Chi-square Test					
	value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson chi-square	1.499[a]	1	0.221		
Continuity correction[b]	0.875	1	0.350		
Likelihood Ratio	1.343	1	0.247		
Fisher's Exact Test				0.245	0.172
Linear-by-Linear Association	1.494	1			
N of Valid Cases[b]	320				

a. 1 cell (25.0%) have expected count less than 5. The minimum expected count is 3.88.

b. Computed only for a 2x2 Table.

Gender

Chi-square Test					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson chi-square	0.808[a]	1	0.369		
Continuity correction[b]	0.284	1	0.594		
Likelihood Ratio	0.982	1	0.322		
Fisher's Exact Test				0.711	0.322
Linear-by-Linear Association	0.805	1	0.370		
N of Valid Cases[b]	320				

a. 1 cell (25.0%) have expected count less than 5. The minimum expected count is 2.23.

b. Computed only for a 2x2 Table.

Breed

Chi-square Test			
	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	2.294[a]	3	0.514
Likelihood Ratio	3.675	3	0.299
Linear-by-Linear Association	0.229	1	0.632
N of Valid Cases	320		

a. 1cell (25.0%) have expected count less than 5. The minimum expected count is 0.43.

Body condition

Chi-square Test					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson chi-square	4.455[a]	1	0.035		
Continuity correction[b]	3.418	1	0.064		
Likelihood Ratio	5.990	1	0.014		
Fisher's Exact Test				0.035	0.022
Linear-by-Linear Association	4.441	1	0.035		
N of Valid Cases[b]	320				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 5.03.

b. Computed only for a 2x2 Table.

Parity

Chi-square Test			
	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	1.145[a]	2	0.564
Likelihood Ratio	1.941	2	0.379
Linear-by-Linear Association	0.432	1	0.511
N of Valid Cases	320		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 0.79.

History of abortion

Chi-square Test					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson chi-square	0.156[a]	1	0.693		
Continuity correction[b]	0.000	1	1.000		
Likelihood Ratio	0.299	1	0.584		
Fisher's Exact Test				1.000	0.861
Linear-by-Linear Association	0.155	1	0.693		
N of Valid Cases[b]	320				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 0.14.

b. Computed only for a 2x2 Table.

History of retained placenta

Chi-square Test					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson chi-square	0.078[a]	1	0.780		
Continuity correction[b]	0.000	1	1.000		
Likelihood Ratio	0.149	1	0.699		
Fisher's Exact Test				1.000	0.928
Linear-by-Linear Association	0.077	1	0.781		
N of Valid Cases[b]	320				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 0.07.

b. Computed only for a 2x2 Table.

Infertility

Chi-square Test					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson chi-square	0.078[a]	1	0.780		
Continuity correction[b]	0.000	1	1.000		
Likelihood Ratio	0.149	1	0.699		
Fisher's Exact Test				1.000	0.928
Linear-by-Linear Association	0.077	1	0.781		
N of Valid Cases[b]	320				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 0.07.

b. Computed only for a 2x2 Table.

Herd size

Chi-square Test			
	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	14.273[a]	2	0.001
Likelihood Ratio	21.327	2	0.000
Linear-by-Linear Association	2.781	1	0.095
N of Valid Cases	320		

a. 1 cell (16.7%) have expected count less than 5. The minimum expected count is 1.29.

Source of new camels

Chi-square Test			
	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	34.116[a]	3	0.000
Likelihood Ratio	28.727	3	0.000
Linear-by-Linear Association	6.481	1	0.011
N of Valid Cases	320		

a. 3 cells (37.5%) have expected count less than 5. The minimum expected count is 3.16.

Grazing system

Chi-square Test					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson chi-square	11.302[a]	1	0.001		
Continuity correction[b]	7.122	1	0.008		
Likelihood Ratio	6.250	1	0.012		
Fisher's Exact Test				0.014	0.014
Linear-by-Linear Association	11.266	1	0.001		
N of Valid Cases[b]	320				

a. 1 cell (25.0%) have expected count less than 5. The minimum expected count is 0.58.

b. Computed only for a 2x2 Table.

Watering system

Chi-square Test			
	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	39.690[a]	2	0.000
Likelihood Ratio	46.885	2	0.000
Linear-by-Linear Association	35.917	1	0.000
N of Valid Cases	320		

a. 1cell (16.7%) have expected count less than 5. The minimum expected count is 2.23.

Contact with other animals

Chi-square Test					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson chi-square	2.946[a]	1	0.086		
Continuity correction[b]	1.864	1	0.172		
Likelihood Ratio	5.374	1	0.020		
Fisher's Exact Test				0.151	0.068
Linear-by-Linear Association	2.937	1	0.087		
N of Valid Cases[b]	320				

a. 1 cell (25.0%) have expected count less than 5. The minimum expected count is 2.44.

b. Computed only for a 2x2 Table.

Production type

Chi-square Test			
	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	32.682[a]	3	0.000
Likelihood Ratio	41.061	3	0.000
Linear-by-Linear Association	5.753	1	0.016
N of Valid Cases	320		

a. 3 cells (37.5%) have expected count less than 5. The minimum expected count is 0.29.

Parturient room

Chi-square Test					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson chi-square	2.946[a]	1	0.086		
Continuity correction[b]	1.864	1	0.172		
Likelihood Ratio	5.374	1	0.020		
Fisher's Exact Test				0.151	0.068
Linear-by-Linear Association	2.937	1	0.087		
N of Valid Cases[b]	320				

a. 1 cell (25.0%) have expected count less than 5. The minimum expected count is 2.44.

b. Computed only for a 2x2 Table.

Awareness of brucellosis

Chi-square Test					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson chi-square	1.740[a]	1	0.187		
Continuity correction[b]	0.778	1	0.378		
Likelihood Ratio	3.245	1	0.072		
Fisher's Exact Test				0.382	0.198
Linear-by-Linear Association	1.735	1	0.188		
N of Valid Cases[b]	320				

a. 1 cell (25.0%) have expected count less than 5. The minimum expected count is 1.51.

b. Computed only for a 2x2 Table.

Awareness of fetus and fetal membrane disposal

Chi-square Test					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson chi-square	2.946[a]	1	0.086		
Continuity correction[b]	1.864	1	0.172		
Likelihood Ratio	5.374	1	0.020		
Fisher's Exact Test				0.151	0.068
Linear-by-Linear Association	2.937	1	0.087		
N of Valid Cases[b]	320				

a. 1 cell (25.0%) have expected count less than 5. The minimum expected count is 2.44.

b. Computed only for a 2x2 Table.

(b) Univariate SAT

Location

Chi-square Test			
	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	6.488[a]	5	0.262
Likelihood Ratio	7.730	5	0.172
Linear-by-Linear Association	2.224	1	0.136
N of Valid Cases	320		

a. 6 cells (50.0%) have expected count less than 5. The minimum expected count is 0.28.

Age (year)

Chi-square Test					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson chi-square	3.169[a]	1	0.075		
Continuity correction[b]	1.228	1	0.268		
Likelihood Ratio	2.351	1	0.125		
Fisher's Exact Test				0.134	0.134
Linear-by-Linear Association	3.159	1	0.076		
N of Valid Cases[b]	320				

c. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 0.68.

d. Computed only for a 2x2 Table.

Gender

Chi-square Test					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson chi-square	1.086[a]	1	0.297		
Continuity correction[b]	0.037	1	0.848		
Likelihood Ratio	0.795	1	0.373		
Fisher's Exact Test				0.336	0.336
Linear-by-Linear Association	1.082	1	0.298		
N of Valid Cases[b]	320				

c. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 0.39.

d. Computed only for a 2x2 Table.

Breed

Chi-square Test			
	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	0.638[a]	3	0.888
Likelihood Ratio	0.851	3	0.837
Linear-by-Linear Association	0.000	1	0.986
N of Valid Cases	320		

a. 4 cells (25.0%) have expected count less than 5. The minimum expected count is 0.08.

Body condition

Chi-square Test					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson chi-square	1.134[a]	1	0.287		
Continuity correction[b]	0.208	1	0.648		
Likelihood Ratio	1.989	1	0.158		
Fisher's Exact Test				0.580	0.371
Linear-by-Linear Association	1.131	1	0.288		
N of Valid Cases[b]	320				

c. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 0.88.

d. Computed only for a 2x2 Table.

Parity

Chi-square Test			
	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	3.442[a]	2	0.179
Likelihood Ratio	2.643	2	0.267
Linear-by-Linear Association	2.985	1	0.084
N of Valid Cases	320		

a. 3 cells (50.0%) have expected count less than 5. The minimum expected count is 0.14.

History of abortion

Chi-square Test					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson chi-square	0.025[a]	1	0.873		
Continuity correction[b]	0.000	1	1.000		
Likelihood Ratio	0.050	1	0.822		
Fisher's Exact Test				1.000	0.975
Linear-by-Linear Association	0.025	1	0.873		
N of Valid Cases[b]	320				

a. 3 cells (75.0%) have expected count less than 5. The minimum expected count is 0.03.

b. Computed only for a 2x2 Table.

History of retained placenta

Chi-square Test					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson chi-square	0.013[a]	1	0.910		
Continuity correction[b]	0.000	1	1.000		
Likelihood Ratio	0.025	1	0.874		
Fisher's Exact Test				1.000	0.987
Linear-by-Linear Association	0.013	1	0.910		
N of Valid Cases[b]	320				

c. 3 cells (75.0%) have expected count less than 5. The minimum expected count is 0.01.

d. Computed only for a 2x2 Table.

Infertility

Chi-square Test					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson chi-square	0.013[a]	1	0.910		
Continuity correction[b]	0.000	1	1.000		
Likelihood Ratio	0.025	1	0.874		
Fisher's Exact Test				1.000	0.987
Linear-by-Linear Association	0.013	1	0.910		
N of Valid Cases[b]	320				

a. 2 cells (75.0%) have expected count less than 5. The minimum expected count is 0.01.

b. Computed only for a 2x2 Table.

Herd size

Chi-square Test			
	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	2.771[a]	2	0.250
Likelihood Ratio	4.205	2	0.122
Linear-by-Linear Association	2.392	1	0.122
N of Valid Cases	320		

a. 3 cells (50.0%) have expected count less than 5. The minimum expected count is 0.23.

Source of new camels

Chi-square Test			
	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	6.471[a]	3	0.091
Likelihood Ratio	7.692	3	0.053
Linear-by-Linear Association	4.641	1	0.031
N of Valid Cases	320		

a. 4 cells (50.0%) have expected count less than 5. The minimum expected count is 0.55.

Grazing system

Chi-square Test					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson chi-square	0.104[a]	1	0.747		
Continuity correction[b]	0.000	1	1.000		
Likelihood Ratio	0.204	1	0.652		
Fisher's Exact Test				1.000	1.903
Linear-by-Linear Association	0.104	1	0.748		
N of Valid Cases[b]	320				

c. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 0.10.

d. Computed only for a 2x2 Table.

Watering system

Chi-square Test			
	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	6.488[a]	2	0.039
Likelihood Ratio	7.730	2	0.021
Linear-by-Linear Association	5.871	1	0.015
N of Valid Cases	320		

a. 3 cells (50.0%) have expected count less than 5. The minimum expected count is 0.39.

Contact with other animals

Chi-square Test					
	Value	**df**	**Asymp. Sig. (2-sided)**	**Exact Sig. (2-sided)**	**Exact Sig. (1-sided)**
Pearson chi-square	0.482[a]	1	0.488		
Continuity correction[b]	0.000	1	1.000		
Likelihood Ratio	0.905	1	0.342		
Fisher's Exact Test				1.000	0.637
Linear-by-Linear Association	0.480	1	0.488		
N of Valid Cases[b]	320				

c. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 2.43.

d. Computed only for a 2x2 Table.

Production type

Chi-square Test			
	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	5.342[a]	3	0.148
Likelihood Ratio	6.795	3	0.079
Linear-by-Linear Association	0.940	1	0.332
N of Valid Cases	320		

a. 5 cells (62.5%) have expected count less than 5. The minimum expected count is 0.05.

Parturient room

Chi-square Test					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson chi-square	0.482[a]	1	0.488		
Continuity correction[b]	0.000	1	1.000		
Likelihood Ratio	0.905	1	0.342		
Fisher's Exact Test				1.000	0.637
Linear-by-Linear Association	0.480	1	0.488		
N of Valid Cases[b]	320				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 2.43.

b. Computed only for a 2x2 Table.

Awareness of brucellosis

Chi-square Test					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson chi-square	0.284[a]	1	0.594		
Continuity correction[b]	0.000	1	1.000		
Likelihood Ratio	0.547	1	0.460		
Fisher's Exact Test				1.000	0.761
Linear-by-Linear Association	0.24	1	0.594		
N of Valid Cases[b]	320				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 0.26.

b. Computed only for a 2x2 Table.

Awareness of fetus and fetal membrane disposal

Chi-square Test					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson chi-square	0.482[a]	1	0.488		
Continuity correction[b]	0.000	1	1.000		
Likelihood Ratio	0.905	1	0.342		
Fisher's Exact Test				1.000	0.637
Linear-by-Linear Association	0.480	1	0.488		
N of Valid Cases[b]	320				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 0.43.

b. Computed only for a 2x2 Table.

6.5. Appendices 4

Multivariate

		B	S.E	Wald	df	Sig.	Exp(B)	95.0%CI for Exp(B)	
								Lower	Upper
Step1[a]	**Location**			.000	5	1.000			
	Location(1)	19.733	6.46E3	.000	1	.998	3.716E	.000	.
	Location(2)	.000	1.072E	.000	1	1.000	1.000	.000	.
	Location(3)	.000	9.761E	.000	1	1.000	1.000	.000	.
	Location(4)	.000	7.935E	.000	1	1.000	1.000	.000	.
	Location(5)	.000	9.671E	.000	1	1.000	1.000	.000	.
	Age(1)	-.385-	.522	.545	1	.460	.680	.245	1.892
	Body condition(1)	-1.92-	1.042	3.383	1	.066	.147	.019	1.134
	Parity			.000	2	1.000			

	Continued								
	Parity(1)	.031	1.259E	.000	1	1.000	1.031	.000	.
	Parity(2)	-.005-	.707	.000	1	.994	.995	.249	3.972
	Herd size			1.821	2	.402			
	Herd size(1)	-.886-	5.816E	.000	1	1.000	.412	.000	.
	Herd size(2)								
	Source of new camels			13.420	3	.004			
	Source of new camels(1)	-.243-	.884	.075	1	.784	.784	.139	4.439
	Source of new camels(2)	1.540-	.822	3.512	1	.061	4.667	.932	23.37
	Source of new camels(3)	-.405-	1.051	.149	1	.700	.667	.085	5.228
	Grazing system(1)	-1.68-	.772	4.717	1	.030	.187	.041	.849
	Watering system			.000	2	1.000			
	Watering system(1)	-20.2-	7.623E	.000	1	.998	.000	.000	.
	Watering system(2)	-19.7-	4.756E	.000	1	.997	.000	.000	.
	Contact with other animals(1)	18.955	6.816E	.000	1	.998	1.706E	.000	.
	Production type			.000	3	1.000			
	Production type(1)	19.603	3.976E	.000	1	.997	3.262E	.000	.
	Production type(2)	.766	2.065E	.000	1	1.000	2.151	.000	.
	Production type(3)	.683	6.172E	.000	1	1.000	1.980	.000	.
	Parturient room(1)	18.766	6.893E	.000	1	.998	1.413E	.000	.
	Awareness of brucellosis1)	-18.9-	8.766E	.000	1	.998	.000	.000	.
	Awareness of fetus & fetal membrane1)	18.766	6.893E	.000	1	.998	1.413E	.000	.
	Constant	-20.9-	6.615E	.000	1	1.000	.000		

a. location, age, body condition, parity, herd size, source of new camels, grazing system, watering system, contact with other animals, production type,

parturient room, awareness of brucellosis and awareness of fetus & fetal membrane disposal.

6.6. Appendices 5

Compare of tests using "kappa" test

Count		RBPT		Total
		-ve	+ve	
SAT	-ve	296	20	316
	+ve	1	3	4
Total		297	23	320

6.7. Appendix 6:

Sudan University of Science & Technology
College of Graduate Studies
Department of Epidemiology
Investigation of Camel Brucellosis in Butana plain area and Al-Gadarief State.

Questionnaire

Questionnaire for the evaluation of the effect of potential risk factors on the occurrence of brucellosis.

Date……..…

Locality……. Farm…….……..…..…..…

Name of respondent…….. Age...…….. Sex…… Family size.....…..

Herd size……...….Owner experience (years)…….....

General and Individual Risk Factors:

1- Location:

Northern Butana…..... { }. Western Butane…….. { }.

Southern Butana…..... { }. Eastern Butana…….... { }.

Meddle Butana……... { }. Al gadarief State……. { }.

2- Age (Years):

Young (≤ 5)……...….. { }. Adult (≥ 6)……...……. { }.

3- Gender:

Male……... { }. Female…….. { }.

4- Breed:

Arabi.... { }. Anafi.... { }. Bushari…. { }. Cross…. { }.

5- Body Condition:

Good.....…. { }. Poor…….. { }.

6- Parity:

(1- 4)…… { }. (> 4)…….… { }. No…… { }.

7- History of Abortion:

Yes…… { }. No……..… { }.

8- History of Retained Placenta:

Yes…… { }. No……..... { }.

9- Infertility:

Yes……..… { }. No……..... { }.

Management Risk Factors:

1- Herd Size:

Small (≤ 10)… { }. Medium... (11-30)... { }. Large (> 30)... { }.

2- Sources of New Camels:

Darfur……..... { }. Kurdofan……..…… { }.

Kassala…….. { }. Butana……..... { }.

3- Grazing System:

Indoor……..….. { }. Outdoor……... { }.

4- Watering:

Tap Water.... { }. Surface Water.... { }. Bounds.… { }.

5- Contact With Other Animals:

Yes……... { }. No……. { }.

6- Production Type:

Milk.... { }. Meat.... { }. Racing.…. { }. Mixing…. { }.

7- Awareness Of Brucellosis:

Yes…….. { }. No…… { }.

8- Awareness of fetus & Fetal membrane Disposal:

Yes…..... { }. No…..… { }.

9- Parturient Room:

Yes……. { }. No……... { }.