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EPIDEMIOLOGY OF BRUCELOSIS IN CAMELS IN WEST KURDUFAN STATE SUDAN

وبائية داء البروسيلات في الابل بولاية غرب كردفان السودان

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DEGREE OF PhD OF PREVENTIVE VETERINARY MEDICINE

BY

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DEDICATION

TO

MY FAMILY, MY FRIENDS AND MY COLLEAGUES

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Abstract

A cross-sectional study was carried out fromSeptember 2018 to April 2019.The aim of this projectwas to study Brucellosis in the camels *(Camelus dromedarius*) to estimation the prevalence of brucellosis in thecamels and investigate risk factors associated with the disease in West Kurdufan state.

Atotal of 500 serum and whole blood samples were collected from camel in three selected localities in West Kurdufan State, the total of camels examined in this study 49% (n=245) from Alnehood, 42.4% (n=212)from Ghubaish, and 8.6% (n=43) from Elkhwai Localities. From the total camels tested 28.8% (n=144) were males, while 71.2 %(n=356) were females respectively. All samples were screened by the RBPT, and 3.8%(19/500) were positive by the test. All samples were further tested by the serum agglutination test (SAT) test, and of the 19 RBPT positive samples,17 were found positive by the SAT test. Among the sixty herds included in the study 15were found positive (25%) .The investigated individual risk factors included: localities, sex, age groups, sexes while herd sizes, management types, having aborted animals in the herd, sharing males between herds for breeding purpose, contact with other ruminants ,contact with other camel herds at pasture and water points and awereness of herdman about brucellosis were the management risks.The study revealed that 3.8% of samples tested and 25% herds were seroposetive for brucellosis with increase in seroprevalence of disease in Elkhwai 16.3% ,Alnehood 3.7% and Ghubaish 1.4%.(OD Ratio =13.546 , CI = 3.347-54.822 , P-value =0.000).The result showed that the prevalence is relatively higher in females 3.9% than males3.5%. The seroprevalence of brucellosis according to herd size were2.8%,3.3% and 4% in small, medium and large herd sizes, respectively.This increase in seropositivity is not significant statistically(p.value=0.920).The study revealed that the camel contacts with other camel herds in pasture and water points were higher prevalence (9.2%) than herds not in contact(2.2%) (OD Ratio =0.119, CI = 0.039-0.363, P-value

=0.000), whereas camels reared with ruminants (sheep, goat and cattle) showed prevalence (6.7%) higher than that kept alone (2.2%). (OD Ratio =0.307, CI = 0.119-0.796, P-value =0.015).

This study showed the status of seroprevalence and with exception of (localities, contact with other herds and species) the statistical analysis by using chi square didn't show any effect of other risk factor on prevalence of brucellosis among camels in West Kurdufan State. Out of the 500 whole blood sample tested by polymerase chain reaction (PCR) none of the sample was found positive.

ملخص البحث

أجريت دراسة مقطعية من سبتمبر 2018إلى أبريل 2019بغرض كشف البروسيلا في دم الإبل بواسطة إختبار البلمرة التفاعلي وتحديد معدل انتشارمرض البروسيلا في الإبل وكذلك تحديدعوامل الخطرللإصابة بمرض البروسيلا في الإبل في ولاية غرب كردفان،السودان, قد إشتملت الدراسة على عدد (500) من الإبل 144(28.8%)منها ذكور وعدد 356(71.2%) إناث تم اختيارها من عدد (60)قطيع من ثلاثة محليات بولاية غرب كردفان . تم جمع عدد (500)عينة دم كامل ومصل من الابل عدد(245) عينه من النهود ,(212)عينه من غبيش و(43)عينه من محلية الخوي , تم فحص جميع عينات الدم بواسطةإختبار الروزبنقال وال (SAT) 19عينه كانت إيجابية للإختبار بواسطه الروزبنقال بينما 17 عينه كانت ايجابية بواسطة إختبار ال (SAT) .وأظهرت الدراسة وجود الأجسام المضادة في3.8٪من العينات التي تم اختبارها , و25% من القطعان. مع وجود إرتفاع في نسبه الإصابة في الخوي 16.3% , النهود3.7% وغبيش 1.4 % , (OD Ratio =13.546 , CI = 3.347-54.822 , P-value =0.000). كان هناك ارتفاع طفيف في معدل الانتشارالمصلي من البروسيلا في الإبل الإناث كان 3.9 ٪أعلى نسبيامن تلك الإبل الذكور3.5٪, كذلك أثبتت نتائج هذه الدراسة إرتفاع معدل انتشار البروسيلا في قطعان الإبل المخالطة لقطعان إبل أخري في أماكن الرعي ونقاط شرب الماء (9.2%) أعلى من القطعان الغير مخالطه (1.2%) (OD Ratio =0.119 , CI = 0.039-0.363 , P-value =0.000) . بينما في الإبل التى تربى مع المجترات (ماعز –أغنام -أبقار) كانت الاصابة بمعدل (6.7%) اعلى من التى تربى في قطعان منفصله عن المجترات (2.2%)

. (OD Ratio =0.307, CI = 0.119-0.796, P-value =0.015)

الانتشار المصلي حسب حجم القطيع كان 2.8٪في القطعان الصغيرة(أقل من 20) ,3.3٪ في القطعان المتوسطه(20-40) و4٪في القطعان الكبيرة(أكبر من 40) لكن هذه الزيادة لم يكن لها أي تأثير إحصائي كبير (p.value=0.920) .أظهرت هذه الدراسة مدى انتشار مرض البروسيلا بين الإبل في ولاية غرب كردفان وباستثناء (المحليات والاختلاط مع قطعان الإبل الأخرى وتربيه الإبل مع المجترات الأخرى) لم يظهر التحليل الإحصائي لنتائج الدراسة أي تأثير كبير لعوامل الخطورة الأخرى على انتشار مرض البروسيلا بين الإبل في ولاية غرب كردفان .تم فحص عدد (500) عيتة دم كامل بواسطة تفاعل البلمرة المتسلسل وكانت جميعها سلبية لوجود البروسيلا.

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Introduction

Camels are an important livestock species in the arid and semi arid zones in Asia and Africa. They contribute significantly to the livelihood of the pastoralists and agro pastoralists living in the fragile environments of the deserts and semi deserts of these countries. Most camels surviving today are domesticated.

The camel is a domestic mammal which due to its physiological attributes is suitable for use in climatic extremes. Diseases, poor nutrition, and traditional management systems have restricted their full utilization (Ghorbani *et al*., 2013).

Two domesticated species of old world camels exist, the dromedary or one-humped camel (*Camelus dromedarius)* and The Bactrian or two humped camel (*Camelus bactrianus)*. The dromedary camel is the most important livestock animal in the semiarid areas of Northern and Eastern Africa as well as in the Arabian Peninsula and Iran.

Approximately 94% of the estimated world's camel populations were thought to be one-humped or dromedary camels. The two-humped Bactrian camel comprises 6% and is primarily in Asia. Seventy percent of the world's camels are located in the tropics, the majority of them in Sub-Sahara Africa. Five adjoining countries -Somalia, Ethiopia, Kenya, Sudan, and Dijibouti-contain 84% of African camels and 60% of the world's camels. In 2001, the total camel population was 19 million of which 17 million were dromedaries (*C. dromedarius*) and 2 million were Bactrian camels (*C. bactrianus*)(Farah,2004).

According to FAO statistics (Global Livestock Production and Health Atlas - GLIPHA, 2006) the world population of camels is about 20 million animals, 15 million in Africa and 5 million in Asia. In most countries, the camel population is increasing after a period of decreasing number due to the introduction of modern transport facilities (Farah, 2004). Sudan has the second largest camel population in the world, estimated at nearly 4, 700,000 (M A R F – 2012).

The one humped camel is an important livestock species in Sudan and their numbers were estimated as more than (4,500,000) head of camels, which constitute a major source of income for their owners and consequently the national economy.

Camels possess an economic importance among the Sudanese nomadic tribes.There is at present an increased awareness of the role of camels as the mainsource of milk and meat for pastoralists. The urban population of many countries (particularly in North Africa and the Middle-East) also consumescamel milk and meat. Camel racing is popular in the Arabian Gulf countriesand northern Africa. (Abbas and Agab, 2002).

Camels in the Sudan are classified as pack (heavy) and riding (light) types according to the function they perform. These traits were probably developed as a result of selection applied by the various camel owning tribes. The Arab breed of camel is well suited for meat production and transportation. Camel milk is important at the subsistence level but is rarely marketed. The export of camels for slaughter -mostly to Egypt, but also to the Libyan Arab Jamahiriya and other countries, is an important source of foreign currency, which is not overlooked in a country with few roads and a harsh environment.

Brucellosis is one of the most important bacterial zoonoses with a global distribution (Teshale*et al*., 2006; Young, 1995; Lopes *et al*., 2010; Angara and A. A. Ali 2014). The disease represent an occupational hazrd for veterinarians, farm, abattoir and labortory workers(Madkour, 1992).It is an infectious disease, almost invariably transmitted by direct or indirect contacts with infected animals or their products(Teshale*et al*., 2006). The *brucellae* are small, nonmotile, aerobic, facultative intracellular and Gram-negative coccobacilli.They belong to the genus *Brucella* which includes *Brucella melitensis* and *B. ovis* as well as many other species (Lopes*et al*.,2010). Brucellosis is a disease caused by varies species of the genus *Brucella*and is the most widely spread zoonosis worldwide (Dawood, 2008)and one of the diseases

that negatively affect animal trade.Brucellosis, also known as “undulant fever”, “Mediterranean fever” or “Maltafever” is a zoonosis, It affects people of all age groups and of both sexes. Although there has been great progress in controlling the disease in many countries, there still remain regions where the infection persists in domestic animals and, consequently, transmission to the human population frequently occurs, Though it has beeneradicated in many developed countries in Europe, Australia, Canada, Israel,Japan and New Zealand (Gul and Khan, 2007).It remains an uncontrolled problem in regions of high endemicity such as Africa, Mediterranean, Middle East, parts of Asia and Latin America (Refai,2002).

Brucellosis is transmitted from animals to humans by ingestion of raw milk, milk products, raw liver, and direct contact with animals (Cooper, 1992).

Camels are not known to be primary hosts of *Brucella* organisms, but they are susceptible to both *B. abortus* and *B. melitensis* (Musa *et al*., 2008).

Brucellosis was reported in camels as early as 1931, later, the disease has been reported from all camel-keeping nations (Gwida*et al*., 2012).

The disease in dromedary can be caused by *B. abortus, B. melitensis* and *B.ovis* (Seifert, 1996).Different studies showed that *B. abortus* and *B. melitensis*are the most frequent isolates (Radwan *et al.,* 1992; Gameel*et al*., 1993; Agab *et al.*, 1994; Abou-Eisha, 2000 and Hamdy and Amin, 2002).*Brucellamelitensis* and *B. abortus* are capable of infecting a wide range of hostsincluding man (Walker, 1999).

Aims of the study

The objectives of this study were to determin the prevalence and potential risk factors of *Brucella* spp in the camels *(Camelus dromedarius*) in West Kurdufan State by using serological and molecular methods.

CHAPTER ONE
LITERATURE REVIEW

1.1. History of camels

The camelides belong to order Artiodactyl (even-toed ungulates), sub-order Tylopoda, that represents with the suborders *Suiformes* (pig-like), Thuscamelids as ruminating animals classified in proximity to ruminants but are not part of the suborder *Ruminantia* differences such as foot anatomy, stomach system andthe absence of horns confirm this fact (Schwartz and Dioli, 1992; Fowler, 1998; Werney, 2003).The family *Camelidae* is divided into three genera: The old world camels (genus *Camelus*) and the new world camels (genus *Lama* with the species *L. glama, L.guanicoe*, *L. pacos* and genus *Vicugna* with the species *V. vicugna*) (Wilson and Reeder, 2005).The new-world Camelidae are smaller versions of the camels and live in the heights of the mountains in South America. Two domesticated species of old world camels exist, the dromedary or one-humped camel (*Camelus dromedarius)* and The Bactrian or twohumped camel (*Camelus bactrianus)* that can be found in the cold deserts and dry steppes of Asia. In the desert Gobi there is still a population of wild two-humped camels classified as *Camelus ferus* (PETERS, 1997; FOWLER, 1998).

The name of the dromedary derived from the Greek, “dromeus” which means runner or droma- running (Jassim and Naji, 2002). The one-humped camel was probably domesticated in the region of today’s Yemen and Oman about 3000 to 4000 years ago (Fowler, 1998).

A Dromedary and Bactrian camels were domesticated in Near East for use as a draft and saddle animals, food source as milk, meat and even may be textile source about 2500-3000 years ago.

The history of the dromedary camel in the Sudan is even more obscure. It is believed to have entered theSudan from Egypt. The oldest evidence is a bronze figure of camel with a saddle found at Merwi and estimated to date between 25-15 B.C. Camels in the Sudan is spread in a belt configuration; it extends

between latitudes12°-16°N. This belt is characterized by erratic rainfall, less than 350 mm. and contains two main regions: theEastern state, whereas camels are found in the Butana plains and the Red Sea hills and the Western regions (Darfour and Kordofan). In Sudan the production systems include: traditional nomadic system, transhumantor semi-nomadic system, sedentary or semi sedentary system and intensive system which is limited to racingand dairy camels.

1.2 .World camel distribution

World camel population is estimated to be around 25.89 million across 47 countries. About 85% of the camel population inhabits mainly eastern and northern Africa and the rest inhabits the Indian subcontinent and Middle East countries. (FAO, 2008)

Somalia and Sudan have the largest populations, with some 70 percent of the African camel herd (Al-Juboori and Baker, 2012).

The camel plays vital socioeconomic roles and supports the survival of millions of people inthe semi dry and arid zones of Asia and Africa. It has been found that camels are the fit domestic animal during severe drought periods. The camel not only survived such droughts, but continued production and reproduction (Schwartz, 1992).

1.3. Economical importance of camels

The Camelidae are bred for production of meat, milk, hair, and hide, besides; carrying and transporting heavy loads. Camels were, and still are, valued as riding and work animals.

Camels known as “dessert ship” are well adapted to severe environmental conditions, and it is the only domestic species that able to survive, and reproduce in harsh climatic conditions.

In their natural desert habitat where camels are usually raised particularly during the long dry season, camels are subjected to severe stress conditions which render them susceptible to many diseases and ailments (Agab, 1993).

Camels can graze on low productive pastures on which the production of milk is possible and economically profitable. For this reason, camels may reduce the dependence of pastoralists on other livestock that is usually much more vulnerable to drought than camels (Farah andFischer 2004).

Camels are the toughest animal species for production and survival under harshenvironmental conditions and have been considered an aid to man for thousands of years.

There has been an increasing demand of camel meat in people and societies that do not breed camels, thus leading to a higher number of camel abattoirs and butcheries in several countries that mainly slaughter young animals (Farah and Fischer, 2004 and Finke, 2005).

Milk of Camel is one of the most important and valuable food resources for nomads in arid regions and can contribute to a better income for pastoralists, as in the last year's milk consumption among the urban population increased (Farah and Fischer, 2004).Another important product is camel wool. It is one of the world's most expensive natural animal fibers. In some countries, camels are kept in the backyards of cities to gain wool, besides milk and meat. An adult camel usually produces 2 – 3 kg per shearing (Wernery, 2003). Camel hides are known for their strength and durability. They are used by camel breeders, but also as fashion accessories (Wernery, 2003). Other products used are dung as fertilizer and source of fuel for pastoralists and bones for production of jewellery or bone-meal for fertilizing purposes.

1.4. Camel husbandry

In Sudan three main types of production systems for camel herds are adopted.

These are:

1.4.1. Traditional nomadic system

This system is dominant in the geographical zone between 13°N to 16°N (Northern part of the camel belt) (Al-Khori*et al*., 2000) this is typically practised by the Kababish tribe in Northern Kordofan State (NKS). The camel

herders are continuously on the move in response to availability of grazing and water supplies.

1.4.2. Transhumant or semi-nomadic system

This system is found in eastern and southern regions of the camel belt and is practised by semi-nomadic tribes. In this system a degree of settlement is experienced during the rainy season where rainfed agriculture is practised forstable food production and the crop residues provide feed supplement for camel populations (Bakheit, S.A., 1999). Several tribes in Eastern Sudan practice a transhumant mode of range utilization (Abbas*et al*., 1992).

1.4.3. Sedentary or semi-sedentary system

This system ispractised in the eastern region of Sudan (East of RiverNile and west of the Red Sea hills) . It is also practised inthe agricultural areas in the central and southern parts of the camel belt (Al-Khori *et al*., 2000).

1.4.4. Intensive system

In the past, this type of productionwas practised in all camel area but it was limited for racing camel only (very small number of animals). Recently anintensive system of camel meat and dairy productionexists as a kind of commercial investment .

Herd sizes of camels per household ranged between 40–120 camels. One breeding camel bull was used for 25females. Males are used for transport by four years old, depending on the load, while 15% of owners use camels for pulling ploughs to cultivate farms.

When the grazing and climatic conditions become harsh in Sudan during the dry season, camel owners migrate southwards to the savannah zone near to watering points. During the rainy season camels graze grasses and browse trees and after the rainy season they stay around cultivated areas to feed on crop residues. However, grazing on Acacia mellifera, Acacia Senegal, Ziziphus spp., Balanities aegyptiaca and Ipomoea spp. caused clashes between settled farmers and nomadic camel herders. The watering interval varies from seven to nine

days in summer, while in winter months when the ambient temperature is lower and the rangeland contains succulent plants, owners extend the watering interval to 20–35 days. Salt and atron (a source of calcium) are supplied, dissolved in drinking water and mash. Salt is used during rainy and winter seasons due to the availability of water; it increases body weight at a time when grazing is scarce. Camels are classified according to their age as follows: at one year age mafrood, two years wadlabun, three years higg, four years gadaa, five years rabaa and seven years sadees.

Camels have been proved that they are susceptible, the same as other livestockor even more, to the common disease causing pathogensaffecting other animal species (Abbas and Agab, 2002).

1.5. Brucellosis

1.5.1. Definition of the disease

Brucellosis is one of the most important zoonoses and affects human welfare and livestock health worldwide. It exists especially in the Mediterranean Basin, the Arabian Peninsula, the Indian Subcontinent and parts of Central and South America. The disease is caused by bacteria of the genus *Brucella*which includes different species mainly *Brucella abortus* and *Brucella melitensis* that vary in their affinity and virulence to several hosts (FAO, 2004a; FAO, 2004b).

1.5.2. History of brucellosis

Brucellosis is an ancient and one of the world's most widespread zoonotic diseases affecting both, public health and animal production (Ariza *et al.*, 2007). The paleo-pathological evidence form the partial skeleton of the late Pliocene *Australopithecus africanus* suggests that brucellosis occasionally affected our direct ancestors 2.3–2.5 million years ago (D'anastasio *et al*., 2011).

The pathological, molecular and electron microscopy findings from the human skeletal remains (Capasso, 2002;D'Anastasio *et al*., 2011), remains of buried cheese(Capasso, 2002) also suggested the presence of brucellosis long time ago.

The causative agent of brucellosis, "*Micrococcus melitensis*" i.e.*Brucella melitensis*, was discovered in 1887 by British surgeon captain David Bruce, his wife Mary Elizabeth Steele and the Maltese microbiologist doctor GiuseppeCaruana-Scicluna from the liver of diseased soldiers in the Mediterranean island of Malta (Wyatt, 2009).

In 1884, Dr. Bruce was able to differentiate between brucellosis (Malta fever) and typhoid outbreaks affected Malta. Three years later, he isolated the causative agent of Maltafever and named the bacterium Micrococcus melitensis. In 1897, Dr.Bang studied the disease in Denmark and could isolate *Brucellaabortus* strains from aborted cattle. He noticed that the pathogen can also infect sheep, goat and horses; the disease became known as Bang's disease. Later on, in 1918, Evans could detect the connection between animal and human cases after he isolated an organism from human aborted foetus which was closely related to Bruces's organism. In the year 1938, it was possible to differentiate among the caprine, bovine and swine forms of Undulant fever caused by *B. melitensis, B. abortus and B. suis*, respectively. Since 1884 till now, brucellosis represents a continuous reemerging zoonoses worldwide (Godfroid *et al* .,2005; Vassallo M,1992).

Investigation of burned cheese rests found in the old Roman city (Herculaneum) which was suddenly destroyed in August 79 AD by the volcanic eruption (Vesuvius) revealed the presence of bacterial colonies morphologically resemble *Brucella*, which may be the first sign of brucellosis in the old ages (Godfroid J *et al* .,2005).

B.melitensis isolation reported in 1958 from Al Gazira region (Central Sudan) when cases of febrile illness observed among foreigners visiting the area (Dafaalla *et al*.,1958). Later,Several studies were conducted mostly towards understanding the epidemiology of brucellosis with the majority of these studies applying serology (Angara *et al*.,2014) rather than isolation (Musa *et al* .,1990) with few researches using molecular tools(Osman AEF *et al*., 2015)

1.5.3. Synonyms

The disease in animals is also called as Bang's disease, Enzootic Abortion, Epizootic Abortion, Slinking of Calves, Ram Epididymitis and Contagious Abortion and in humans it is named as Malta fever, Undulant Fever, Mediterranean fever, Rock Fever of Gibraltar, and Gastric Fever (Neharika 2018)

1.5.4.Zoonoses of brucellosis

The significance of the brucellosis is due to its zoonotic and economic impacts McDermott, Grace and Zinsstag, 2013) . It can be transmitted to people in contact with infected animals or consuming their products, the causative agent has a very low infectious dose; only 10 organisms of *B. melitensis* are sufficient to cause an infection in man (L.B., Lopes R. Nicolino and J.P.A. Haddad (2010).

Direct and indirect contact with diseased animals or foodstuffs of animal origin represents the major source of infection to humans. It was thought that the infected human are the dead end of the infection, however, human to human transmission was recorded recently (Tuon, 2017).

Icecream and homemade cheese play an important role in the spread of thedisease among human as they are prepared in a way which does not eliminate viable *Brucella* bacilli.

Human brucellosis was discovered as early as 1895 in the Pasteur Institute d'Algeria (Refai, 2002).

The prevalence of brucellosis in the animal reservoirs determines the incidence of human cases (Pappas and Papadimitriou, 2006; Von Hieber, 2010). In countries where brucellosis is endemic, humans can be infected via contact with infected animals or consumption of their contaminated products, mostly milk and milk products especially cheese made from unpasteurized milk of sheep and goats and rennet from infected lambs and kids.

The Expansion in business and leisure travel to brucellosis-endemic countries led to transmit the disease into non-endemic areas (Corbel, 2006).

Human get infection of brucellosis via inhalation, animal contacts, and consumption of unpasteurized dairy products and undercooked meat products. For example, consumption of traditional Arabian / African delicacies such as raw liver can cause human infection (Malik, 1997). Infections by brucellosis are an occupational risk for farmerswho handle infected animals/carcasses and aborted fetuses or placentas, veterinarians, abattoir workers, laboratory personnel, ranchers, and meat –packing employees, and others who work with animals and consume their products(Smith and Cutler 2004.Tabak *et al* ., 2008**).***B .melitensis* infections occur more frequently in the general population while *B.abortus* and *B.suis* infection usually affect occupational groups (Acha *et al*.,2003;De Massis *et al* ., 2005).

Brucellosis causes more than 500,000 human infections per year worldwide. In the European Union, the highest prevalence of human brucellosis occurs in the countries of the Iberian Peninsula and the Mediterranean littoral or basin region (Portugal, Spain, Southern France, Italy and Greece) (WHO 1998b).

The most pathogenic and invasive species for human is *B.melitensis*, followed by B.suis.B.abortus and B.canis (Acha *et al*., 2003). *B.melitnsis B. suis* and B.abortus are listed as potential bio-weapons by the centers for disease control and prevention in the USA.This is due to the highly infectious nature of all these species , as they can be readily aerosolized.Inhalation of *Brucella* is not a common route of the infection, but it can be significant hazard for people in certain occupational such as those people working in laboratory acquiredpathogens (Robichaud *et al*., 2004).

Consumption of sheep or goat milk containing *B.melitensis* is an important source of human brucellosis worldwide and has caused by *B. melitensis* (De M assis *et al*., 2005., Wallach *et al* .,1997). Moreover, the prevalence of the human brucellosis acquired from dairy products in some countries is seasonal, reaching apeak usually after kidding and lambing (Dahouk *et al*., 2007).

An outbreak of brucellosis would be difficult to detect because the initial symptoms are easily confused with those of influenza (Chain *et al* .,2005).

However*Brucella*was proven recently that man to man infection is possible. This may be related to the continuous improvement in the diagnostic and epidemiological tools, or to the continuous adaptation of the organism to their hosts (Lucero *et al* .,2010;Al Dahouk *et al* ., 2017).

Hoover 1997 reported that the incubation period inhumans lasts from 5 to 60 days, but can also be longer. Clinical signs are not specific and can be acute or chronic.

According to Vrioni (2008)*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. *Brucellamelitensis* DNA persists in human blood for many years after infection despite appropriate treatment and apparent recovery.

Table (1): 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
Brucella ceti	Mild	Cetaceans
Brucella pinnipedialis	Mild	Seals
Brucella microti	Absent	Common voles

1.6. Epidemiology of Brucellosis

1.6.1. Aetiology

Brucellosis is caused by members of the genus *Brucella* more commonly by *Brucella* abortus in cattle, *B. melitensis* or *B. ovis* in small ruminants, *B. suis* in pigs and *B. canis* in dogs(The Center for Food Security and Public Health, 2009),which are facultative intracellular that can survive within host cells causing a chronic infectious disease in many species of animals and man, that may persist throughout the life of the animal, In addition to the "classical" *Brucellaspp*), the genus has recently been expanded to include marine isolates, which have zoonotic potential as well(Sohn *et al*., 2003; McDonald *et al*., 2006).

However, recent reports show that *Brucellamelitensis* is the most prevalent agent of brucellosis globally(Mantur and Amarnath, 2008; Seleem *et al*., 2010).Different *Brucella*strains have also been isolated from a great variety of wildlife species such as bison (*Bison bison*), elk (*Cervus elaphus*), feral swine

and wild boar (*Sus scrofa*), fox (*Vulpes vulpes*), hare (*Lepus capensis*), African buffalo (*Syncerus caffer*), reindeer (*Rangifer tarandustarandus*), caribou (*Rangifer tarandus groenlandicus*), chamois (*Rupicaprarubicapra*) and ibex (*Capra ibex*) and wildlife has to be considered as a reservoir for zoonotic brucellosis(Davis *et al*., 1990; Godfroid,2002; Rhyan,2000

Camels can be infected by *B. abortus and B. melitensis*. Different studies revealed that *B. abortus* and *B. melitensis* are most frequently isolated from milk, aborted fetus and vaginal swabs of diseased camels (Radwan *et al*., 1992; Gameel *et al*., 1993; Agab *et al*., 1994; Abou-Eisha, 2000; Hamdy and Amin, 2002). The spread of brucellosis depends on the *Brucella*species being prevalent in other animals sharing their habitat and on husbandry (Musa *et al*., 2008).

1.6.2. Species, biovarsof *Brucella*

Species identification and sub-typing of *Brucella*isolates are very important for epidemiologic surveillance (to know the species and/or biovar diversity) and investigation of outbreaks (to know the source of infection) in *Brucella*-endemic regions (Al Dahouk *et al.*, 2007; Marianelli *et al.*, 2007).

Although it has been proposed that the *Brucella*species should be grouped as biovars of a single species based on DNA hybridization studies and on the comparison of the genome of *B.melitensis* (Del Vecchio *et al*., 2002), and B. *suis* (Paulsen *et al*., 2002), the current classification of*brucellae* in species according to differences in host preference and in pathogenicity should be preferred (Moreno *et al*., 2002).

Since 1920, in addition to *B. melitensis*, *B. abortus* and *B. suis*, at least 7 new species have been identified as belonging to the *Brucella*genus with several additional new species under consideration for inclusion (Oslen and Palmer, 2014) as shown in Table (1).

Brucella melitensis biovar 3 is the most commonly isolated from China (Man *et al*., 2010), Egypt, Tunisia, Israel, Turkey and Jordan (Refai, 2002). But

Brucellamelitensis biovar 1 has been predominantly isolated from Libya (Refai, 2002), Iran (Zowghi *et al*., 2009) and Latin America (Lucero *et al.*, 2008).

Among the seven biovars of *B. abortus*, biovar 1 is most frequently isolated from cattle in countries where biovar prevalence has been studied, such as the USA (Bricker *et al.*, 2003), Latin America (Lucero *et al.*, 2008), Brazil (Poester *et al*., 2002), India (Renukaradhya *et al*., 2002) and Pakistan (Ali *et al*., 2014)

Through the modern molecular tools it was possible to prove that *B. melitensis*, *B. abortus, B. ovis* and *B. neotomae* represent 4 related clones of one organism while *B. suis* (including *B. suis* biovar 5) forms a distinct cluster from them but closely related to the marine mammals *Brucella* species isolated from dolphin, seal and porpoise. Meanwhile, *B. suis* biovars 3 and 4 seem to be evolved from *B. suis* biovar 1 and *B. canis*. These relationships were confirmed by the data delivered by whole genome sequencing (Whatmore et al, Foster *et al*., 2009).

At the time, at least 12 *Brucella* species are known. Due to its great economic and zoonotic importance, it is important to identify field isolates of *Brucella* not only at their species level but also their genotypes. This enables the detection of hidden foci of *Brucella* and to tract the sources of infection in the population. As an example, genotypic nalysis of different *B. abortus* field strains isolated from cattle, bison and elk showed that the cattle isolates are closely related to elkisolates but completely divergent from those of bison (Beja-Pereira *et al* .,2009).

Table (2): The species, biovars/biotypes, host preferences and zoonotic potentials of *Brucella*Species

Species	Biovars	Colony type	Host	First reported, country	Zoonotic Potential
B. melitensis	1-3	Smooth	Goat, sheep, camels, cows	Malta	High
B. abortus	1-6, 9	Smooth	Cattle, buffalo, camels, bison, elk, yaks	Denmark	High
B. suis	1-5	Smooth	Pigs (biotypes 1-3), wild boar and European hares (biotype 2), reindeer and caribou (biotype 4), wild rodents (biotype 5)	USA	High
B. neotomae	-	Smooth	Desert woodrat	USA	Unknown
B. pinnipedialis	-	Smooth	Seal	Scotland	Mild
B. ceti	-	Smooth	Dolphin, porpoise, whale	Scotland	Mild
B. microti	-	Smooth	Vole, fox, (soil)	Czech Republic	Unknown
B. inopianata	-	Smooth	Unknown	Australia	Mild
B. ovis	-	Rough	Sheep	New Zealand	No
B. canis	-	Rough	Dog	USA	Mild
Future species					
Brucella papionis sp. nov.	-	Smooth	Baboon	USA	Unknown
BO2	-	Smooth	Unknown	Austria	Mild
Frog isolate (exceptionally motile)	-	Smooth	Bullfrogs	Germany	Unknown

Source of table: (Rahman, AKMA (2015)

1.6.3. Ecology of *Brucellaspp*

Brucella spp. is Gram-negative, aerobic, coccobacilli, non-capsulated, non-motile and non-spore-forming (Bargen *et al*., 2012).

The *brucellae* are members of the α–proteobacteria (Moreno *et al*., 1990), and it is facultative extracellular intracellular parasites (Moreno and Moriyon, 2002).

The preferable organs and tissues of *Brucella* s*pp*. are placenta, mammary glands, and epididymis in animal reservoir host (Adams, 2002; Xavier *et al*., 2009; Neta *et al*., 2010).

The most important aspect of *Brucella*ecology is their ability to establish an intracellular replicative niche and remain protected from the host immune responses (Bargen *et al*., 2012).

1.6.4. Transmission

Brucellosis is a serious infectious disease affecting different mammalian species including man. Natural infection of farm animals occurs mainly through ingestion of food or water contaminated by uterine discharges, aborted feti or fetal membranes and even through licking the genitalia of diseased animals. In addition, infected males can also spread the infection among females through natural mating and artificial insemination. *Brucellae* can pass through intact or injured skin and through all mucous membranes (Pal M *et al* .,2017).

horizontal and vertical 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).

Brucellae transmission within hosts may occur via ingestion of the organismscontaminated feed or water or licking an infected calf ,placenta or fetus, or the genitalia of an infected animal soon after it has aborted or gave birth (Godfroid *et al*., 2004).Transmission within the natural hosts can occur through milk or via semen or genital secretions during mating.

Spread of the disease is due to movement of infected animals to disease free herds get the infection from infected herd at water points where a number ofherds come together.

Zoonotic Spread of the disease is due to unpasteurized milk products, while occupational exposure of farmers, veterinarians, or laboratory workers can result from direct contact with infected animals or tissues or fluids associated with abortion (Olsen and Palmer, 2014). Also, many infected camels are silent Carriers of brucellosis (Abbas and agab 2002) .

Only rare cases of vertical and horizontal (Wyatt, 2010) transmission between humans have been reported (Ruben *et al*., 1991; Mantur *et al*., 1996; Çelebi *et al*., 2007; Meltzer *et al*., 2010) and humans are generally considered to be incidental, or dead-endhosts for *Brucella*species (Meltzer *et al*., 2010).The transmission of brucellae from wildlife to domesticruminants is also possible (Mick *et al*., 2014).

The most common portals of entry for *Brucella*in animals and humans are mucous membranes of the respiratory (aerosol) (Franz *et al*., 2001)and digestive tracts, and in the natural host, also the conjunctiva and membranes covering the sexual organs.

The small ruminants act as extensive reservoir of *B. melitensis*, which constitutes a threat of infection to largeruminants including camels and man due to prolonged contact. (Dafni *et al.,* 1991) . The chance of transmission is higher during parturition and abortion when most of the *Brucella* contamination occurs (Abbas and Agab, 2002).

Venereal transmission is not a major route of infection under natural conditions, but artificial insemination with contaminated semen is a potential source of infection (Radostits *et al*., 2010; Chiebao *et al*., 2013).

The newly characterized *Brucella* species have a high genetic flexibility. Many of these isolates are mobile, fast growers, able to survive in the soil, more resistant to high acidity and unfavorable environmental condition and show high

capacity for adaptation to new non-mammals hosts such as amphibians and are high active metabolically. They can adapt themselves very quickly to their environment to extend their host range (Al Dahouk *et al* ., 2017),possible transmission of these unique properties of the atypical *Brucella* species to the widely spread typical *Brucella* spp. via mobile genetic elements (e.g. bacteriophages, transposons, pathogenicity islands, etc) will have a catastrophic effect on animal husbandry and public health world wide.

Possible potential role of the lungworms, cestodes and other parasites in transmission of marine brucellosis which will open the gate for new routes of transmission (Maio*et al* ., 2014). The role of some ectoparasites such as stomoxys in the transmission of terrestrial mammal *Brucella* was previously suspected (Baldacchino *et al* ., 2013). Climatic changes (global warming/ water scanty/dissertation) lead to the spread of insects/parasites (and therefore insect borne diseases) to new regions.

Airborne infections occur in animal pens, stables, laboratories and abattoirs (Schulze *et al* .,2020). Some cases have also occurred from accidental self-inoculation with live vaccines (Seleem *et al* .,2010; OIE, 2012). Moreover, it was also shown by Bradenstein *et al*. (Bradenstein *et al* .,2002), that Rev1 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.

1.6.5. Epidemiological factors

1.6.5.1. Virulence factors

Brucella has no classic virulence factors like toxins, fimbriae and capsules which raises the possibility that they might have unique and subtle mechanisms to penetrate host cells, evade host defenses, change intracellular trafficking to avoid degradation and killing in lysosomes and modulate the intracellular environment to allow long-term intracellular survival and replication (Delrue *et al*., 2004).

*Brucella*has developed mechanisms to avoid innate immunity by minimizing stimulation of pattern recognition receptors (PRRs) of the host.

The *Brucella*cell envelope has high hydrophobicity and itsLPS has a non-canonical structure that elicits a reduced and delayed inflammatory response compared with other Gram-negative bacteria (Rittig *et al*., 2001) and has lower stimulatory activity on TLR4 receptors (Rittig *et al*., 2003).

Experimental infection with *Brucella* strains isolated from frogs and cold blooded animals revealed high potential to invade and survive in mammalian host for about 3 months (Al Dahouk *et al* ., 2017; de Bagüés *et al* ., 2010).

Brucella is a robust pathogen, with a multiple routes of infection. It can resist inside and outside the mammalian hosts for a long time even under unfavorable conditions. It persists in the food up to 15 months even under unfavorable conditions as acidity and temperature between 11 and 14 °C. or for 2–3 days under 37 °C. *Brucella* may also survive in aborted infected feti and contaminated manure for more than 2 months in winter or few hours if exposed directly to sunlight (Lucero *et al* .,2010). The presence of functional glutamate decarboxylase dependent system (GAD system) in *B. microti* allows it survival at very low pH levels. The system is activated if the bacteria is exposed to very low pH values (≤2.5) in order to overcome the harmful effect of acid stress. The presence of GAD system has a great diagnostic importance as a PCR target for characterization of atypical *Brucella* species (Bastianelli *et al* .,2015).

1.6.5.2. Environmental Factors

The incidence of brucellosis in camel population appears to be correlated to breeding and husbandry practices. Herd sizes, density of animal population, and poor management are directly related to prevalence (Wernery and Kaaden, 2002).Seasons of the year may have influence on the management and contact of the infected and susceptible host.

The parturition in wet season (Schwartz and Dioli, 1992) increases the viability of the organisms in the environment, thus increasing the chance of infecting susceptible animals (Corbel, 1990).

As a result, the congregation of alarge number of mixed ruminants at water points facilitates disease spread. But higher brucellosis reactor rate in wet seasons than dry seasons (Baumann and Zessin, 1992).

*Brucella*may remain viable within the environment for a period of time. In general, the viability of *Brucella* spp. outside the mammalian host is enhanced by cool temperatures and moisture and decreased by high temperatures, dryness and direct exposure to sunlight. For example, *B. abortus* survives a couple of hours under direct sunlight but up to 185 days in the cold and shade. *Brucella Abortus*also survives in aborted fetuses, manure and water for periods of up to 150 to 240 days (Saegerman *et al*., 2010).

The newly discovered *Brucella* species in the last 20 years show great genetic diversity even more than that exists among thousands of isolates of the classical *Brucella* species discovered throughout the twentieth century. These atypical *Brucella* species have a close genetic relationship with soil bacteria. Genome analysis studies showed that B. microti lies in the midway between saprophytic soil bacteria and the pathogenic *Brucella* species. This enables them from gaining new genetic properties from the environmental soil bacteria (Al Dahouk *et al.*, 2017).

Role of soil as primary habitat for some *Brucella* types such as *B.microti* which has a nonliving natural reservoir outside its mammalian host. It can survive up to 6 months in the soil, which indicates an environmental niche shared by all members of family *brucellaceae*. Its frequent isolations from different animal species worldwide indicate that *B. microti* could possibly be an emerging pathogen and could release a pandemic of brucellosis. It is also possible that *B. microti* can multiply in the soil outside the mammalian host due to the presence of functional ketoadipate pathway (Audic *etal*., 2009;Scholz et*al*., 2008).

1.6.5.3. Host Factors

The reported animal level potential risk factors of brucellosis include age, breed, history of abortion, etc.(Al-Majali *et al*., 2009; Ibrahim *et al*., 2010; Boukary *et al*., 2013; Chand and Chhabra, 2013; Patel *et al*., 2014).

The herd level potential risk factors of brucellosis identified are large herd size, mixed farming, agroecological zones, contact with wildlife, new entry in the herd, artificial insemination, etc. (Muma *et al*., 2007; Al-Majali *et al*., 2009; Ibrahim *et al*., 2010; Chiebao *et al*., 2013; Chand and Chhabra, 2013; Patel *et al*., 2014).

Brucellosis 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 (Majid *et al*., 1999; Abou- Eisha, 2000).

Von Hieber (2010) found that 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.

The breeding camels had lower brucellosis infection rate than racing animals (Wernery and Wernery, 1990).

Some studies showed the equal distribution of *Brucella*antibodies among females and males Radwan *et al*, (1992). In other findings it appeared that males are less susceptible to the disease than females (Agab *et al*., 1997; Ajogi and Adamu, 1998). Higher susceptibility in female animals is attributed to physiological stresses (Walker, 1999).

The role of males in the spread of disease under natural condition is not important whereas Female animals have essential epidemiological importance not only in susceptibility but also in disseminating the disease via uterine discharge and milk. (Radostits *et al*., 1994) .

Although every *brucella* species is bound to a specific host, their pathogen-host relationship is not exclusive. The growing population lead to intensive breeding of farm animals and it is common to have mixed livestock farming strategy which facilitates cross species infections (Ducrotoy *et al*., 2015; XavierI *et al*., 2009).Also the urbanization and the alteration of human socio demographics.

The human population increases worldwide, the human and the domestic animals are coming in closer contact with wild animals. As no vaccination policy is applied in *Brucella* free countries, the domestic animals are very susceptible to brucellosis. The reintroduction of the disease through contact with infected wild animals will have a catastrophic effect and causes storms of abortion.

In general, and according to Millar . & Stack (2012) 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 localise in the placenta and are most abundant in abortion material (up to bacteria) including the fetal stomach, vaginal discharge and colostrums.

1.7. Pathogensises of brucellosis

The digestive tract is the main route of *Brucella*entrance*;*some studies investigated possible virulence factors involved on successful infection through the digestive tract(Paixão.*et al* 2009; Bandara.*et al* 2007; Delpino.*et al* 2007). The bacteria penetrate intact mucosal surface. In the alimentary tract after exposure to infection, the epithelium covering the ileal Peyer's patches are the preferred sites of entry**.**

Bacteria are eventually taken up by phagocytic cells (macrophages, dendritic cells, etc.) and reach the regional lymph nodes, leading to subsequent systemic dissemination (Salcedo *et al*., 2008).

The *Brucella*LPS O-polysaccharide appears to be a key molecule for cellular entry, to prevent complement-mediated bacterial lysis and to prevent apoptosis

of the macrophages within which they reside allowing them to extend their longevity (de Bagüés *et al*., 2004; Lapaque *et al*., 2005).

Following entering into the host cell, smooth *Brucella*quickly traffic through the early endosomal compartment and depart the phagosome to form the modified phagosome (termed brucellosome). *Brucella*initially localize within acidified phagosomes (Rittig *et al.*, 2001),where they are exposed to free oxygen radicals generated by the respiratory burst of phagocytes. Localization of*Brucella* in an acidified environment induces expression of the VirB operon (virB 1–10), which controls expression of genes associated with a typeIV secretion system. The VirB operon interacts with the endoplasmic reticulum to neutralize the pH of the phagosome (Anderson *et al*., 2008). The *Brucella*-induced modifications of the phagosome prevent fusion with the lysosome.

Little is known about the pathological changes in camels. Gross lesion may be found in the predilection sites uterus, udder, testicles, lymph nodes, joint bursa and placenta. Hydrobursitis was often observed in brucellosis positive dromedaries causing swelling of the bursa (Werney and Kaaden, 2002).

The abortion in farm animals may be due to placentitis, direct effect of endotoxins or inflammatory response in fetal tissue (Walker, 1999).

Brucellae are stealth microbes which tend to chronicity rather than causing acute fatal infection. *Brucella* keeps its victims alive to maintain their survival. Throughout their evolution, *Brucella* developed dynamic strategies to escape recognition and attacks by the immune system, to modulate the acquired immune response of the host, and to escape intracellular inactivation. This makes the treatment of brucellosis very difficult.

The presence of functional glutamate dec-arboxylase dependent system (GAD system) in*B . Microti*allows itsurvival at very low pH levelsIn addition, the GAD system enables oral infection (survival in the stomach) and the later survival when being engulfed by macrophages (Lamontagne *et al* .,2009; Bastianelli *et al* .,2015).

1.8. Clinical symptoms and signs

Bovine brucellosis is associated with abortion during the last trimester of gestation, and production of weak newborn calves, and infertility in cows and bulls (xavier *et al*., 2009). Bovine brucellosis may also be responsible for retention of placenta and metritis and results in 25% reduction in milk production in infected cows (Acha and Szyfres, 2003; FAO, 2006). In some parts of Africa, hygromas and abscess in carpal joints are the major clinical signs in nomadic or semi-nomadic cattle herds infected with *B. abortus* biovar 3 (FAO, 2006; Bankole *et al*., 2010; Boukary *etal*., 2013).

Brucellosis in goats is characterized by late abortion, stillbirths, decreased fertility and low milk production (Lilenbaum *et al*., 2007). Mastitis is also commonly observed feature of caprine brucellosis compared with bovine brucellosis. The affected mammary gland maybe characterized by multinodular firmness with watery, clotted milk (Cutler *et al*., 2005).

In humans, Brucellosis is life threatening and presents with nonspecific symptoms, including intermittent fever, weight loss, depression, hepatomegaly, and splenomegaly. Arthritis, spondylitis, osteomyelitis, epididymitis, and orchitis, as well as other more severe complications such as neurobrucellosis, liver abscesses, and endocarditis, are common in some patients (Bingo̤l A, Yücemen N, Meço O (1999))

1.9. Diagnosis

Brucella may be diagnosed definitively by isolation and identification of the causative organism.

Brucellosis has many Diagnostic tests which applied for the purposes of: confirmatory diagnosis, screening or prevalence studies, certification, and, surveillance in order to avoid the reintroduction of brucellosis (in countries where brucellosis is eradicated) through importation of infected animals or animal products (Godfroid *et al*., 2010).

The diagnostic methods include direct tests, involving isolation of organism or DNA detection by polymerase chain reaction (PCR)-based methods and indirect tests, which are applied either in vitro (mainly to milk or blood) or in vivo (allergic test). Isolation of *Brucellaspp*. or detection of *Brucellaspp*. DNA by PCR is the only method that allows certainty of diagnosis.

1.9.1. Bacteriological methods

It should be noted that all infected materials present a serious hazard, and they must be handled with adequate precautions during collection, transport and processing, precautions to be taken include use of safety cabinet inlaboratory; wearing gloves, protective cloth and facemask, autoclaving materials incontact with the organism and disinfecting contaminated surfaces FAO(2006).

1.9.1.1. Stained smears

Smears of vaginal discharge, fetal stomach contents or placental cotyledon may be stained using modified Ziehl-Neelsen (Stamp) method. The presence of large aggregates of intracellular, coccobacillus red organisms is presumptive evidence of brucellosis. It is still often used, even though this technique is not specific as other abortive agents such as *Chlamydophila abortus* or *Coxiella burnetii* are alsoresemble *Brucella* FAO (2006).

1.9.1.2. Culture

Bacterial culture has an important role in confirmation of the disease and it is essential for antimicrobial susceptibility, biotyping and molecular characterization which provide valuable epidemiological information to know the sources of infection in outbreak scenarios and the strain diversity in endemic regions (Kattar *et al*., 2008; Álvarez *et al*., 2011). Evidence of *Brucella* is provided by the demonstration of *Brucella*-like organisms in abortion material or vaginal discharge using modified acid-fast staining, and is considered presumptive, especially if supported by serological tests.

*Brucella*may most readily be isolated in the period following an infected abortion or calving, but isolation can also be attempted post-mortem.

*Brucella*are excreted in large numbers at parturition and can be cultured from a range of material including fetal membranes, vaginal secretions, colostrum, milk, aborted fetuses (stomach, spleen, and lung), semen, and hygroma fluid. *Brucella*may also be isolated post-mortem from supra-mammary, internal iliac and retropharyngeal nodes, spleen, udder tissue, testes and gravid uterus, using suitable selective culture media.

1.9.1.3. Commonly used basal media include

Serum dextroseagar (SDA) (Agab *et al*., 1994), glycerol dextrose agar (GDA), and trypticase or tryptose soya agar (TSA) . SDA is usually preferred for observation of colonial morphology. Skirrow agar is a satisfactory medium for both *Brucella*species and *Campylobacterfetus* Terzolo *et al*., (1991). a non-selective, biphasic medium, known as Castañeda's medium, is recommended for the isolation of *Brucella*from blood and other body fluids or milk, where enrichment culture is advised. Castañeda's medium is used because *brucellae* tend to dissociate in broth medium, and this interferes with biotyping by conventional bacteriological techniques (OIE 2018). Contamination is prevented by use of selective media containing actidione (30 mg/l), bacitracin (25mg/l), polymixin B (5mg/l) and vancomycin (20mg/l)(Walker, 1999).

The Farrell medium (FM) is the most commonly medium used For the isolation of *Brucella*spp., which contains antibiotics able to inhibit the growth of other Bacteria present in clinical samples. However, due mainly to the nalidixic acid and bacitracin contained in its formulation, FM is inhibitory for *B. ovis* and also for some *B. melitensis* and *B. abortus* strains (Marin *et al*., 1996).

Milk samples should be allowed to stand overnight at 4 °C before lightly centrifuging.The cream and the deposit are spread on to the surface of at least three plates of solid selective medium Placental samples should be prepared in the field by selecting the least contaminated portion and cutting off pieces of cotyledon. In the laboratory, the portions should be immersed in alcohol which should be flamed off before cutting with scissors or scalpel and smearing the cut

surface on three plates of selective medium. Other solid tissues can be treated in a similar manner, or, ideally, they should be macerated mechanically following flaming before plating out. The tissues may be ground manually or homogenised in a blender or stomacher with a small proportion of sterile water. Fetal stomach contents are collected, after opening the abdomen, by searing the surface of the stomach with a hot spatula and aspirating the liquid contents with a Pasteur pipette or syringe (FAO, 2006).

Bacterial colonies may be provisionally identified as *Brucella*on the basis of their cultural properties and appearance, Gram staining, and agglutination with positive antiserum. If available, a PCR-based molecular identification method may be used. Although blood and tissue culture remains the "gold standard" for diagnosis, culture has low sensitivity, is time consuming, and is a risk for the laboratory personnel (Bricker, 2002; Navarro *et al.*2004).

1.9.2. Serological Methods

Diagnosis of brucellosis in different animal species depends largely on isolation of the causative agent, or detection of the antibodies through a battery of serological tests with varying levels of sensitivities and specificities.The detection of specific antibody in serum or milk remains the most practicalmeans of diagnosis of brucellosis.

The Brucella has two typesof colony named ‘smooth’ and ‘rough’ according to the lipopolysaccharide (LPS) in their outer cell wall (Baldwin and Goenka,). The ‘smooth’ phenotype has complete lipopolysaccharide consisting of lipid A, a core oligosaccharide and an O-side chain polysaccharide (S-LPS/OPS) while LPS of ‘rough’ strains lack the O-side chain (R-LPS). *B.ovis and B. canis* are both naturally ‘rough’ species and all the others are ‘smooth’ species (Cardoso *et al*., 2006). The S-LPS or *B. abortus* whole cells and R-LPS are usually used as antigen for the diagnosis of brucellosis caused by smooth and rough species respectively (Nielsen and Duncan, 1990; OIE, 2008).For example, the RBT uses *B. abortus* biotype 1 (Weybridge 99) whole cells as antigen, which will be able

to detect antibody against *B. melitensis* also as both share common epitopes in an O-side chain polysaccharide. Eventhough the serology is a standard technology for the epidemiological surveillance of brucellosis, but cross-reactions between *Brucella*species and other Gram negative bacteria such as *Yersinia enterocolitica* O: 9, *Francisella tularensis*, *Escherichia coli* O:157, *Salmonella urbana* group N, *Vibriocholerae* and *Stenotrophomonas maltophilia* are a major problem(Muñoz *et al.,* 2005).

False-positive serological results caused by *Y. enterocolitica* O: 9 may affect up to 15% of the cattle herds in regions free from brucellosis, generating considerable additional costs for surveillance programs (Muñoz *et al.,* 2005).

There are two procedures for the serologicaltests, the conventional tests and primary binding assays. All conventional tests base on the antibody performing a secondary function, for instance fixation of complement while in primary binding assays the only function of the antibody is attachment to its antigen. (Neilsen and Wu, 2010) .

Table (3). Sensitivity and specificity of serological tests for brucellosis

Serological test	Sensitivity in %	Specificity in %
SAT	81.5	98.9
CFT	90–91.8	99.7–99.9
RBT	87	97.8
cELISA	95.2	99.7
iELISA	97.2	97.1–99.8
FPA	96.6	99.1
MRT	88.5	77.4

cELISA: complement enzyme-linked immunosorbent assay

CFT: complement fixation test

FPA: fluorescence polarization assay

iELISA: indirect enzyme-linked immunosorbentassay

MRT: milk ring test

RBT: Rose Bengal test

SAT: serum agglutination test

1.9.2.1. Conventional Tests

1.9.2.1.1. Agglutination tests

Slow tests requiring incubation from 8 to 24 hours:

Standard tube agglutination test (SAT)

The SAT has been used extensively for brucellosis diagnosis and, although simple and cheap to perform, its lack of sensitivity and specificity mean thatit should only be used in the absence of alternative techniques (FAO, 2006).

Because of the specificity problems associated with the original tube agglutination test, large number of modifications was made to inactivate IgM agglutinine. Of this modification, the acidified antigen, rivanol precipitation and the use of 2- mercaptoethanol are in common use in various laboratories, while

most of the other modifications (heating the serum, addition of antiglobulin, antigen with increased sodiumchloride,

•SAT with added reducing agents such as 2-mercaptoethanol or dithiothreitol

The 2-mercaptoethanol (2ME) test is a confirmatory serological test that allows Selective quantification of IgG anti-brucella due to inactivation of IgM in the test sample . Production of IgG is usually associated with chronic infection and therefore, apositive result with this test is a strong indicator of brucellosis. However, this test hassome drawbacks including the toxicity of mercaptoethanol, which requires a fumehood for its manipulation, and the possibility of IgG degradation caused by the 2-mercaptoethanol, which may result in false negative results.

Sensitivity of the 2-mercaptoethanol test varies from 88.4 and 99.6%,and its specificity from 91.5 and 99.8% (Nielsen *et al*., 2004). The test does noteliminate vaccinal antibodies, therefore is not recommended for international trade.The 2-MET is, however, used extensively for national control and or eradicationprogrammes (Nielsen, 2002).

• SAT with addition of rivanol to precipitate glycoproteins

In rivanol precipitation test, rivanol (2-ethoxy-6, 9-diaminoacridine lactate) is added to serum, the precipitate is removed by centrifugation. Serum (diluted or undiluted) can be further tested by aquick plate agglutination test or a tube test. As the precipitation tests are laborious, therefore, usually used as confirmatory tests (Nielsen, 2002).

• SAT with addition of ethylene diamine tetraacetic acid to reduce IgM Binding (EDTA)

SAT with addition of ethylene diamine tetraacetic acid to reduce IgM binding (EDTA), the SAT detects IgG less efficiently, especially IgG1, resulting in low assay specificity. Addition of EDTA has improved it specificity significantly and reduce the level of fals positive result. (OIE 2004)

• SAT with antiglobulin added to enhance agglutination

The antiglobulin test (AGT) was developed to detect antibodies which, although they combine with the antigen, donot give rise to agglutination. The presence of these so –called "incomplete agglutinins" can be detected by using an antibody directed against the IgG fraction of the species of animal being tested. These reagents can be o btained commercially.

The test is performed in two phases. First, the conventional SAT is carried out, and after reading, those tubes that do not show any agglutination are centrifuged and the deposited cells washed thoroughly.The presence, if any, of the nonagglutinating *Brucella* antibody on the *Brucella* antigenis detected by adding the antiglobulin reagent at its working dilution and either reincubating the tubes and reading after 24 -h o f incubation.

• Milk ring test

An adaptation of agglutination test uses hematoxylindisolved in ethanole stained whole cell antigen added to milk (OIE 2004), if antibody is present in the milk, aportion will be attached to the milk fat globules via the Fc portion of the antibody. These antibodies will agglutinate with the antigen and as they as globules rise in the milk, apurple band will appear at the top of the milk .if no antibody is present the fat band will remain buff colored .The test consists of mixing colored *Brucella*whole-cell antigen with fresh bulk milk. In the presence of anti-*Brucella*antibodies, antigen-antibody complexes form and migrate to the cream layer, forming a purple ring on the top of the column of milk. In the absence of antigen-antibody complexes, the cream remains colorless this test is prescribed by the OIE for use only with cow milk. Bulk milk can be screened to detect the presence of infected animals within the herd, which can then be identified by blood testing. This method of screening is extremely effective and is usually the method of choice in dairy herds(OIE, 2009).In contrast to cattle milk, Van Straten *et al* (1997) reported that camel milk cannot be used to detect

lacteal brucellosis antibodies using the conventional milk ring test (MRT), because camel milk lacks the agglutinating substance required to cluster fat globules , It is also known that camel milk fat globulins are tiny micelles which, therefore, do not cream up to produce a surface fat layer.so musa(1996)used the MRT to detect antibodies in camel milk and the sensitivity increase 300%. The researchers named this test a modified MRT because *Brucella*-negative cow milk is added to the camel milk, producing a typical blue-coloured creamy ring when antibodies to *Brucella*bacteria are present.

Milk testing

This method of screening is extremely effective and is usually the method of choice in dairy herdsmilk from the bulk tank can be screened to detect the presence of infected animals within the herd which can then be identified by blood testing.

Rapid agglutination tests performed in minutes:

Rose BengalPlate Test

The RBT is one of a group of tests known as the buffered *Brucella*antigen tests which depend on the principle that the ability of IgM antibodies to bind to antigen is markedly reduced at a low pH. The low PH used reduces non-specific reactions because it prevents some agglutination by IgM and encourages agglutination by IgG1.In the RBPT drops of stained antigen and serum are mixed on a plate and any resulting agglutination signifies a positive reaction. The test is an excellent screening test but may be oversensitive for diagnosis in individual animals, particularly vaccinated ones. False negative reactions may occur, due to pro-zoning with sera containing very high levels of antibody. In addition, false positive serological reactions (FPSR) may occur due to some cross reacting antibodies and antibodies resulting from *B. abortus* S19 vaccination (OIE, 2009).

Modified Rose Bengal

Rose Bengal test is a simple, fast, reliable with high sensitivity and is considered by the OIE as one of the prescribed tests for international trade. It has been used for several decades, proving to be successful for eradicating bovine brucellosis in some countries, and is one of the official tests currently used in the EU for the eradication of B. melitensis infection in small ruminants. In order to increase the sensitivity of RBT in small ruminants and to minimize the discrepancies with the CFT result, two volumes of serum and one volume of antigen, approximately 80 µl of serum and 40 µl of antigen areused and the test is termed the modified mRBT(OIE 2016).

Buffered antigen plate agglutination

According to OIE manual 2018 this test is very sensitive ,especially in cattle for detection of vaccine-induced antibody, and positive samples should be retested using a confirmatory or complementary test(s) because false-negative reactions may occur, usually due to prozoning, which may be overcome by diluting the serum or retesting after a given time. While the BPAT has been extensively used with apparent good results in small ruminants and pigs in some countries, its diagnostic value in these species has not been reported at international level.

- **Card**

This test is essentially the same as the R B P T . All materials used in the card test are disposable and provided as field or laboratory kits. These kits provide plasma collection equipment that allows rapid separation of plasma using phytohemagglutinin and an anticoagulant. Thirty microliters each of plasma and Rose Bengal-stained antigen are placed on ateardrop area of acard, mixed, and spread. The test is read immediately after rocking for 4 min.

- **Antigen with rivanol added**
- **Heat treatment of serum**

The incubation of serum-antigen mixture at 56 ° C reduces titers, especially the so –called "nonspecific" titers. This reduction rarely happens with high-titered

sera. The effect on titer is more pronounced with sera from vaccinated than sera from nonvaccinated animals.

• Addition of 10% sodium chloride

1.9.2.1.2. Precipitation tests

Agar gel immunodiffusion

An antigen, polysaccharide B (compose of acyclic glucose molecule and a small amount of OPS, the active part of prepration), derived from B. melitensis was used ,either incorporated in to the agar matrix in SRD ,followed by addition of test serum to awell cut in the agar matrix or in the AGID added to well in the agar matrix adjacent to another containing test serum , if antibody was present ,aring of precipitation would appear within a couple of hours or after longer incubation period with sera containing less antibody . Similarly , in the AGID test,opposing well in the agar matrix,filled with antigen and test serum would produce aprecipitin band if the serum containing antibody while these tests provided data not available by other means, the sensitivity was insufficient for wide scale diagnostic use and neither test is recommended by the OIE .

Radial immunodiffusion

1.9.2.1.3. Complement fixation tests

Complement fixation test is the most prominent test to detect antibodies in different animal species including camels. CFT has been and is a widely used as a confirmatory test in control/eradication programs.This test is prescribed by the OIE The technique is performed as follows using standard 96-well microtitre plates with round (U) bottoms. 25 µl of diluted inactivated test serum is place in the well of the first, second and third rows. The first row is an anti – complementary control for each serum. 25 µl of CFT buffer is add to all wells except those of the second row. Serial doubling dilutions are then prepared by transferring 25 µl volumes of the serum from the third row. Onwards; 25 µl of the resulting mixture in the last row is discarded. 25 µl of antigen of working dilution is add to each well except in the first row. 25 µl of complement, diluted

to the number of units required, is added to each well. Control wells containing diluent only, complement and diluent and antigen, complement and diluent, are set up to contain 75 µl in each well. The plate is covered and incubated at 4°C overnight The plate from the firstday is prewormed at 37°C for 30 min. in an incubator,50 µl of the freshly prepared haemolytic system is added into each well and shaked carefully. The plate is incubated at 37°C for 15 -30 min. The incubation is stopp when the complement controls with 2 and 1 units complement show complete haemolysis. The plate is centrifuged for 5 min at 2,000 rpm. The haemolysis is compared with standards corresponding to 0, 25, 50, 75 and 100% lysis. The absence of anti-complementary activity is check for each serum in the first row.

- **Warm**
- **Cold**
- **Haemolysis in gel**
- **Indirect haemolysis**

1.9.2.2. Primary Binding Assays

1.9.2.2.1. Radioimmunoassay

1.9.2.2.2. Fluorescence immunoassay

1.9.2.2.3. Particle counting fluorescence immunoassay

1.9.2.2.4. Indirect enzyme immunoassay

The IELISA was first developed by Carlsson in the year 1976 for the diagnosis of human brucellosis. Since then alarge number of variations have been described (reviewed by Neilsen and Gall , 1994), however the most common formate uses SPLS antigen coated passively on to a polystyrene matrix. Diluted serum is added (the diluting buffer usually contain a detergent such as tween 20 and divalent cation chelating agent ,ethylene diamine tetraacetic acid (EDTA) and ethylene glycol tetraacetic acid(EGTA) ,to reduce non specific binding of serum protein(Nielsen *et al* ., 1994) followed by an antiglubulin reagent specific for cross reacting with the test species immunoglobulin,conjugated with an

enzyme ,usually horseradish piroxidase or alkaline phosphatase ,by including a strong positive , a week positive and negative serum control,assay performance and quality control are easily assest. An approved version of this test uses purified SLPS as the antigen, serum diluted 1:50 and a mouse monoclonal antibody specific for bovine IgG1 conjugated with horseradish peroxidase (OIE Manual, 2000a).

This is an OIE prescribed test for international trade,however, it has not been standardized (OIE Manual, 2000a).

The iELISA is very sensitive but lacks the capability to fully resolve the false positive serological reactions (FPSR) problem and the problem of differentiating between antibodies resulting from cross-infection and S19 vaccination (OIE, 2009).

1.9.2.2.5. Competitive enzyme immunoassays

Since both the conventional serological tests and the iELISA cannot distinguish vaccinal antibody ,competitive enzyme immunoassay was developed .The main rationale for this assay was the vaccine induced antibody of lower affinity due to the shorter exposure to antigen due to immune elimination compared to field infection in which antigen persisted ,resulting in increased antibody affinity (MacMillan *et al* ., 1990).The most used format of the cELISA utilizes SLPS from *B.abortus* as antigen passively attached to polystyrene matrix ,followed by incubation with competing antibody and appropriately diluted test serum .After mixing and incubation ,a reagent for detecting bound monoclonal antibody ,labeled with an enzyme usually horseradish peroxidase or alkaline phosphatase ,is added followed by substrate or chromogen after a suitable incubation period . A wash procedure is performed between each step. Aseries of controls, including strongly positive, a weakly positive , a negative serum as well as abuffer (no serum) controls must be included . Result are calculated as percent inhibition against the buffer control (0% inhibition).The cELISA is aprescribed test by the OIE for international trade and an alternative test for swine

brucellosis (OIE Manual,2000a,b).It is used for the diagnosis of brucellosis in most mammalian species through incorporating SLPS passively immobilized in 96 well polystyrene plates. Competitive ELISA and fluorescence polarization assay have been reported to circumvent the False positive serological reactions|(FPSR) problems due to cross-reacting antibodies and S19 vaccination (Nielsen and Ewalt, 2010).

The test based on competition between a monoclonal antibody specific for a common epitope of OPS and the test serum. By selecting a monoclonal antibody with slightly higher affinity for the antigen than most of the vaccinal/cross-reacting antibody but with lower affinity than most antibodies arising from infection, thus the most cross-reacting antibodies could be eliminated in the majority of cases.

1.9.2.2.6. Fluorescentpolarization assay

The fluorescence polarization assay (FPA) was developed as the test that could be performed outside the diagnostic laboratory, allowing for rapid and accurate diagnosis (reviewed by Nielsen and Gall, 2001). The basis of the test is that amolecule in solution rotates randomly at a rate inversely proportional to its size if the molecule is labeled with a fluorescent marker and is examined by plane polarized light , a small molecule will rotate through agiven angle faster than alarger molecule . The time of rotation may be measured using horizontal and vertical measurement (Nielsen et al., 2000) For diagnosis of brucellosis , a fluorescence polarization analyzer is used to obtaine background measurement of fluorescence of diluted serum. Antigen consisting of OPS fragment , approximately 22KDa in size ,labeled with fluorescein isothiocyanate is added and incubated for 2 min, followed by final reading in the analyzer which automatically subtracts the background reading . The net result is presented in millipolarization units. The FPA can be performed almost anywhere using a portable analyzer which receive power from a laptop computer, using serum ,milk or EDTA anti coagulated blood (Nielsen and Gall , 2001).

This technique, which requires special reagents and reading equipment, is claimed to have advantages in sensitivity and specificity over other methods.

1.9.3. Supplementary tests

Milk ELISA

The ELISA may be used to test bulk milk and is extremely sensitive and specific, enabling the detection of single infected animals in large herds in most circumstances.

Intradermal test

The Brucellin allergic skin test is an allergic test that detects the specific cellular immune response induced by *Brucella* infection.This procedure, using a standardized antigen preparation such as Brucellin INRA or Brucellergene OCB, can be used for monitoring the status of herds in brucellosis-free areas. It is sensitive and specific but false positive reactionscan occur in vaccinated animals.

Brucellosis skin tests have been tried by some researchers, particularly on Bactrian camels in the former USSR, using different allergens. (Ten V.B. & Cejdachmedova 1993) . The skin test is highly specific but its sensitivity is low, making it a good herd test. The antigen does not sensitise the animal's immune system and therefore will not induce interference in the diagnosis of the disease.

Better approaches used more than one serological test, accompanied by molecular detection and culture, for the best diagnosis and control (El-Sharkawyet al., 2019, El-Diasty.,*et al* 2018).

1.9.4. Molecular method

Recently progress has been made in applying new molecular and genetic diagnostic methods to improve the diagnosis of brucellosis and nucleic acid amplification techniques might circumvent the diagnostic window being presented before production of specific antibodies (Bricker,2002; Ghorbani .,*et al*.,2013; Gwida*et al*.,2011).

Nucleic acid amplification methods, such as polymerase chain reaction (PCR), are rapid, sensitive, and highly specific and can counteract limitations of conventional detection methods (Elfaki *et al*.,2005; Wareth *et al*.,2015). PCR considered an additional means for detection of the presence of *Brucella* DNA in a sample, furthermore can provide a complementary identification and typing method based on specific genomic sequences. (OIE2018)

Several PCR based methods have been developed. The best-validated methods are based on the detection of specific sequences of *Brucella spp*., such as the 16S-23S genes, the IS711 insertion sequence or the BCSP31 gene encoding a 31-kDa protein(Baddour and Alkhalifa, 2008). These techniques were originally developed on bacterial isolates and are now also used to detect *Brucella*spp. DNA in clinical samples . PCR-based assays are rapid and highly sensitive and specific, with some assays detecting down to 10 cells in less than two hours (Bounaadja *et al*., 2009).

Molecular diagnostic techniques represent an important breakthrough in thediagnostic practice. A number of genus or species-specific conventional PCR assays using primers derived from different gene sequences from the *Brucella*genome have been established. These assays were adapted for *Brucella*detection in different clinical specimens. In the majority of studies, conventional Polymerase chain reaction(PCR)proved to be a good means to detect *Brucella*DNA from clinical specimens (Leal-Klevezas *et al.,*1995).

The real-time PCR assays which using different detection chemistries have already been established and improved sensitivity, specificity and speed of performance compared with conventional PCR. Moreover, some of them were evaluated with various clinical samples of human and animal origins. Most of the authors confirmed that real-time PCR was a very sensitive method for clinical samples (Debeaumont *et al.,*2005; Queipo-Ortuño *et al.* 2005;Queipo-Ortuño *et al.* 2006). The polymerase chain reaction, (PCR) as a diagnostic tool in brucellosis is advancing and will be soon at the point of replacing actual

bacterial isolation. It is rapid, safe and cost effective. The PCR, including the real-time format, provides an additional means of detection and identification of *Brucellaspp*. (Bricker and Halling, 1994; Bricker *et al*., 2003; Lopez-Goňi *et al*., 2011) . The major advantages of real-time PCR are that it can be performed in a very short time, does not require electrophoretic analysis and overcomes the cross reaction with gram-negative bacteria.

(PCR) assays can be applied to detect *Brucella*DNA in pure cultures and in clinical specimens, i.e. serum, whole blood and urine samples, various tissues, cerebrospinal, synovial or pleural fluid, and pus(Colmenero *et al*., 2010; Debeaumont *et al*., 2005; Queipo-Ortuño *et al*., 2006,).

The PCR is more sensitive than blood cultures and more specific than serological tests (Al Dahouk *et al*., 2013).

some bacterial infections, such as *Chlamydophila abortus,* interfere with brucellosis in small ruminants (Bhandi*et al*., 2019) . A new PCR method shows high specificity and sensitivity for brucellosis diagnosis (Kaden*et al*., 2017; Singh*et al*.,2010).

The first species-specific multiplex PCR assay for the differentiation of *Brucella* was described by Bricker and Halling (1994). The assay, named AMOS-PCR, was based on the polymorphism arising from species specific localization of the insertion sequence IS711 in the *Brucella* chromosome, and comprised five oligonucleotide primers that can identify *B. abortus*, biovars 1, 2 and 4, *B. melitensis*, *B. ovis* and *B. suis*.

Also a new multiplex PCR assay has been used for rapid and simple one-step identification of *Brucella which* can identify and differentiate in a single step most *Brucella*species as well as the vaccine strains *B. abortus* strain 19 (S19), *B. abortus* RB51 and *B. melitensis* Rev.1, this assay named Bruce-ladder, (Lopez-Goňi *et al*., 2011).

Table (4). List of some available tests for the diagnosis of brucellosis

Tests	Agglutination tests	Primary Binding Assays
Slow	Slow Agglutination (SAT)	Radioimmunoassay
	SAT with added reducing agents such as 2-mercaptoethanol or dithiothreitol	Fluorescence immunoassay
	SAT with addition of rivanol to precipitate glycoproteins	Particle counting fluorescence immunoassay
	SAT with addition of ethylene diamine tetraacetic acid to reduce IgM binding (EDTA)	Indirect enzyme immunoassay
	SAT with antiglobulin added to enhance agglutination	Competitive enzyme immunoassay
	Milk ring test	Fluorescence polarization assay
Rapid	Rose Bengal	
	Modified Rose Bengal	
	Buffered Plate agglutination	
	Card	
	Heat Treatment of serum	
	Addition of 10% sodium chloride	
Tests	Precipitation Tests	Compliment Fixation Test
	Agar gel immunodiffusion	Warm
	Radial immunodiffusion	Cold
		Hemolysis in gel
		Indirect hemolysis
Tests	Allergic tests	
	Skin test	

1.10. Puplic health and zoonotic importance

Brucellosis remains to be the main zoonosis and is found globally. Furthermore, it seems that the issue of *Brucella* in the camel has potentially important implications for public health and implementation of brucellosis controlprograms**.**

Brucellosis is a well documented zoonosis worldwide posing serious public health problems and extensive economic losses (Neta *et al*., 2010). the disease has a limited geographic distribution, but it remains a major public health problem in the Mediterranean region, western Asia, parts of Africa and Latin America.Brucellosis in camels has a great impact on public health. Particularly, the custom of drinking raw camels milk is prevailing particularly in nomadic regions in the Middle East countries, as it is believed to have an aphrodisiac effect and a cure many human illness (Hamdy and Amin, 2002).

Brucellosis causes severe economic losses as result of stormy abortions or reproductive failure, sterility and reduced milk production rates, besides to that, it adds to the burden shouldered by the farmers; the costs of control and management. Also brucellosis of animals reduces the foreign exchange earnings (FEE) by denying exportation of animals to international markets (McDermott, Grace and Zinsstag , 2013)(Angara and Ali 2014). Furthermore, it seems that the issue of *Brucella* in the camel has potentially important implications for public health and implementation of brucellosis control programs. Primarily, the camel may act as a reservoir for the dissemination of contaminated secretions to other domestic animals and humans. Secondly, in several nations, no formal surveillance and eradication programs for camel brucellosis have been proposed.

The landscape of the disease is influx with emergence or reemergence of new foci (Pappas *et al*., 2006; Seleem *et al*., 2010) and that existing *Brucella species* adapt to changing social and agricultural environments (Godfroid *et al*., 2005).

Brucellosis is considered a reemerging disease of special importance in north and east Africa, the Middle East, the Mediterranean countries, south and central Asia and Central and South America (Corbel and WHO, 2006).

Climatic conditions and increasing international travel and trade as well as sanitary factors play an important role in the prevalence and geographic redistribution of the disease (Pappas *et al*., 2006;Gul and Khan, 2007).

In man,transmission occurs as a result of ingestion of milk, contact via skin abrasion, mucous membranes and inhalation (Seifert, 1996; Radostits *et al*., 2007)

Higher prevalence were recorded among butchers and people who habitually consume raw milk(Masoumi *et al.,*1992).Camel keepers consume camel milk as well as liver without heat treatment(Gameel *et al.*, 1993).

The isolation of the two major pathogenic *Brucella*species *B. melitensis* and *B. abortus,* from milk and other samples of camel origin (Gameel *et al.,* 1993;Agab *et al.,* 1994; Hamdy and Amin,2002)clearly indicated 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 (Abou-Eisha, 2000; El-Ansary *et al.,* 2001).

The situation is even more grave as farmers from rural areas think that raw camel milk has a healing effect on the digestive system(Gwida et al .,2011).The annual incidence of brucellosis in people in the Mediterranean and Middle East countries varies from 1 to 78 cases per 100.000 (OIE, 2000,El Sherbini *et al*., 2007). Infection with *Brucella spp*. continues to pose a human health risk globally despite strides in eradicating the disease from domestic animals (Mantur *et al*., 2007).

Brucella is an ideal bioterrorism/biological weapon due to its low infectious doses, persistence in the environment/host, rapid transmission via different routes including aerosols, and difficult treatment by antibiotics. Any scape of

the organism from military storage or use in terroristic attack will have catastrophic effect.

Brucella vaccinal strains may accidentally induce human outbreaks. Human brucellosis caused by *Brucella* RB51 vaccinal strain shed in cow's milk was reported by CDC in September 2017 in Texas state (CDC Report published in 2017).

1.11. Economic impact of brucellosis

Brucellosis remains widespread in the livestock populations and presents a great economic and public health problem in African countries (Faye B *et al*.,2005).

The *Brucella* causes severe disease in livestock and has an enormous impact on the economy of developing countries (Radostits.,*et al*., 2007).

From economic viewpoint losses in livestock commonly result from reproductive disturbances, mainly abortion; weak newborns; infertility and low milk yield. Economic impacts could be direct and indirect costs.The disease can also have an impact on export and import of animals constraining livestock trade,and reduces meat and milk productionand causes neonate losses and infertility.Non-viable or slow growing animals may also be an outcome of the infection. Abortions often lead to a drop in milk yield of up to (50%), whereas, late abortions lower milk production by 20% - 30%. Moreover; carrier animals suffer a milk drop of 7% –10% following a normal parturition.Agriculture and livestock comprise about 70% of the economic activity in Kurdufan(Shuaib 2011, 2014) .

A mixture of farming systems are practised in the region including nomadic, sedentary and semi-sedentary animal production systems.

Animal producers in the West Kurdufan State depend on the sale of male animals for their various needs. A herder can hardly sell female animals as these make productive assets. Abortions; weak offspring; permanent infertility and prolonged gestation intervals due to temporary infertility; will greatly change herd structure and decrease income of a herder. A shortage of male animals

ready for sale in a herd enforces the sale of females and consequently the depletion of the productive assets, all these reasons affect the producers indirectly and they could be trapped in poverty because of successive sales of females for various reasons and no doubt the shortage of male animals available for sale is one reason.

Moreover, at the macro level; brucellosis constrains access to export trade. As we mentioned earlier; West Kurdufan has the big role in national camel and other animal species trade and exports. Acommitment of disease freedom is required by the importing countries, which strictly precondition *Brucella* testing to each individual animal to remove positive cases. In many instances whole consignments were rejected in importing countries under the claims of *Brucella* positive cases and the consignments were turned back to the port of origin. This entails immense costs to all stakeholders along the value chain in addition to the national economy.

To satisfy the export requirements; the quarantine authorities in Elkhiwey (West Kurdufan) and Elrahad (North Kurdufan) where some export facilities are placed; have to do immense work to sort out and dispose of all physically unfit animals.Blood samples are to be collected from each individual animal intended for export to test for *Brucella*. All these measures make extra expenses to exporters who often complain of their inability to compete in the forign markets and suffered great losses that put the majority of them out of the business. The contraction in the export business will no doubt lead to a recession in local livestock markets. This will have far reaching impact on animal producers and the state economy at large. It is worth noting that *Brucella* positive cases that denied access to export trade are categorized as rejects.

They are often thrown back to pasture; thus becoming a significant health concern.(WSRMP .2007).

1.12. Prevalence of camel brucellosis in different countries

Brucellosisis considered to be one of the most important zoonotic diseases of camels and other domestic animals in some countries of northern Africa and Asia.

Camels are frequently infected with *Brucella*organisms, especially when they are in contact with infected large and small ruminants (Radwan *et al* ., 1992).

Camel brucellosis was recorded to be caused by *B.* abortusand *B.melitensis* with a prevalence of 1.9-20% (Abbas and Agab 2002). Several published literature regarding the prevalence of camel brucellosis from different countries

Sudan:

Yagoub *et al.*(1990)stated that the prevalence rate of *B. abortus*tested by the RBT was 6.54, 5.79, 9.32, 5.03 and 8.06%, respectively, from 1985 to 1989 in 1,502 serum samples collected .

Agab *et al.,*1994 investigated 38 serum samples by RBT. They reported that 32 (84.2%) were positive for *Brucella*and they isolated *B. abortus* biovar 3 from 3 samples.

Musa (1995) collected 416 samples from seven herds of camels in Western Sudan. The prevalence was 7.9, 9.32, 5.03 and 8.06 %, respectively ,The author suggested that camels are the second most affected animal species besidescattle.

EL-Ansary *et al*.(2001)examined 64 camel serum samples from 5 herds. which were screened for *Brucella*antibodies by the slide agglutination test and the seroprevelance was 0%.

Musa and Shigidi (2001) investigated 3413camel serum samples in Sudan. Of which 3,274 camels were examined by conventional serological tests and 256 (7.82%) were positive. The remaining 29 sera were examined by RBT and competitive ELISA (cELISA). Four (13.8%) out of the 29 serasamples examined by cELISA were positive, while only 3 (10.3%) were positive by RBT.

Yagoub (2005)investigated 756 camel serum samples.*Brucella*was not isolate from the herd. Only 12 (1.6%) showed high agglutination titres**.**

Omer *et al.,* 2007collected 14,372 serum samples from camels in Kassala during 2004 to 2006. All samples were examined by the RBT. The percentage of the positive sera during 2004, 2005 and 2006 was found to be 12.3, 15.5 and 30.5% (mean 19.4%), respectively.

Atotal of 83 samples collected from a field outbreak ofbrucellosis (21 camels mixed with cattle, sheep and goats and 62 apparently healthy camels from the abattoir in Darfur)and examined for brucellosis, Out of 21 camelsmixed with cattle , 5 (23.8%) were serologically positive and only three camels exhibited clinical signs of brucellosis. From the abattoir samples, 6 (9.7%) were serologically positive for brucellosis .Musa *et al.,* 2008.

Omer *et al*., 2010 investigated brucellosis in 2225 camels in certain nomadic regions in Sudan, using serum and milk samples. Serum samples were examined by Rose Bengal plate test (RBPT), modified RBPT (mRBPT), serum agglutination test (SAT) and competitive enzyme-linked immunosorbent assay (cELISA). Overall seroprevalence in camels (milk and serum samples) was 37.5%. The seroprevalence in males was 28.2% and in females 40.1%.

Mahammed, (2016).collected252 samples camels from four selected localities in Gadarrif State and examined by the RBPT. Among these, 23 were positive giving an individual prevalence rat of 9.2%. This Study show that the occurrence of the disease was slightly higher in Algadarrif 89.o %, Butana 89.2%, and Alshwak 92.1%.

Abdallah & Baleela, (2017)detected 41 out of 100 camels positive for brucellosis in Darfour by using PCR.

Taha *et al* .,2020 reported seroprevalence of 36.2%, 42.6 and 19.8% of brucellosis in camels with the RBT, CFT and cELISA, respectively, in Elgadaref State, Eastern Sudan.

In the study of Mohamed et *al* .(2015) atotal of 415 camels in 39 herds were collected from four localities in Khartoum State and blood samples were collected and screened by RBPT. Twenty four samples tested positive giving a prevalence of 5.8%

Eritrea:

Omer *et al*.(2000)examined samples from 98 camels by RBT and CFT. They Reported that the prevalence rate of *Brucella*infection in camels was 3.1% .

Ethiopia:

Teshome *et al*.(2003) tested 1442 camels by RBT and CFT,the result of their study indicated 4.2% prevalence of brucellosis in the tested camels.

In the studycarried out by Hadush *et al*.,2013in the Afar region of Northeast Ethiopia, an overall seroprevalence of 4.1% (95% CI: 2.9 to 5.3%) was recorded in camels using both RBPT and CFT.

Libya:

Azawi *et al.,* 2001tested 520 serum samples from camels of both sexes and from different regions for *Brucella*antibodies by RBT, SAT, iELISA and cELISA. They found that seropositivity varied from 7 (1.4%) by RBT, 6 (1.2%) by SAT 16 (3.0) % by cELISA and 18 (3.5%) by iELISA.

Egypt:

Abou-Eisha (2000) collected 592 sera samples from apparently healthy camels from North Sinai Province, Egypt by card test and standard tube agglutination test. 6 (1.01%) and 10 (1.7%) samples were positive with SAT and card test, respectively. *B. melitensis* biovar 3 was isolated from the milk of two seropositive animals. The author mentioned that most cases of infected camels were in close contact to or grazing with sheep and goats.

EL-Boshy *et al.,* 2009 investigated 340 dromedary camels from Nobaria cityusing agglutination and complement fixation tests. 25 (7.35%) were positive by both tests; 14 (4.12%) for *B. abortus*and 11 (3.23%) for *B. melitensis.* They

mentioned that *B. abortus* provoked more clinicopathological changes than *B. melitensis*.

Somalia:

Ahmed *et al*.,2017reported that Out of the 11 camel herds tested by using SAT and cELISA, the results revealed that 5 herds (45.5%) and 4 herds (36.4%) were seropositive to *Brucella*antibodies respectively.

Saudi Arabia:

Alshaikh *et al.,*2007examined 859 serum samples collected from housed and free ranged camels. All samples were tested by RBT, STA, cELISA and CFT. They revealed that 16 samples (1.86%) were positive by RBT, 27 (3.14%) by STA, 26 (3.03%) by cELISA and 34 (3.96%) by CFT, also they were examined the sera positive in CFT by PCR and they reported that all were *B. abotus.*

Table (5) . *Brucella* species isolated from camelids in different countries

Country	Author	Year	Species	Organs
Jordan	Al-Majali	2006	*B. melitensis* biovar 3	Aborted fetuses, vaginal swab
Libya	Gameel *et al.*	1993	*B. melitensis* biovar 1	Milk
Saudia Arabia	Gameel *et al.*	1993	*B. melitensis* biovar 1	Milk, vaginal swab, aborted fetuses
	Radwan *et al.*	1992	*B. melitensis* biovars 1 and 2	Milk
	Radwan *et al.*	1995	*B. melitensis* biovars 1, 2, 3	Milk
	Ramadan *et al.*	1998	*B. melitensis*	Carpal hygroma
	Al Dubaib	2007	*B. melitensis*	n/a
Sudan	Agab *et al.*	1996	*B. abortus* biovar 3	Teats, lymph nodes, vaginal swab, testis
	Musa *et al.*	2008	*B. abortus* biovar 6	Lymph nodes
	Omer *et al.*	2010	*B. abortus* biovar 6	Lymph nodes, testis
UAE	Wernery *et al.* (camels from Sudan)	2007	*B. melitensis* biovars 1 and 3	Milk, lymph nodes, placenta
	Moustafa *et al.*	1998	*B. melitensis*	Milk
Egypt	El-Seedy *et al.*	2000	*B. abortus* biovars 1 and 7 *B. melitensis* biovar 3	Organs

n/a: information not available UAE: United Arab Emirates

1.13. Control and eradication of brucellosis in domestic ruminants

Blasco and Molina-Flores(2011)considered that Before establishing a national control strategy, the impact of brucellosis on the livestock economy and human health, adequacy of national veterinary service organization to carry out the strategy, collaboration between public health and veterinary services and the costs of the different control or eradication strategies must be evaluated as part of this strategy.

Some of the developed countries successfully eradicated animal brucellosis by combined vaccination and test and- slaughter programs (Pappas *et al.*, 2006), along with effective disease surveillance and animal movement control .

In order to eradicate brucellosis, combined test and slaughter program is usually implemented initially by compulsory vaccination, then vaccination is gradually restricted and eventually prohibited during removal of seropositive animals or herd depopulation (when the herd or flock prevalence is low: for example <2%). More than a decade is usually needed to complete the brucellosis eradication program by a "test-and-slaughter" policy and key forsuccess is a sufficient financial compensation scheme for farmers for their culled livestock (Corbel, 2006).Other than expense,a good record keeping, infrastructure, cooperation between all related stakeholders and epidemiologic surveillance are also essential for successful eradication program.

Both S19 and Rev1 interfere with the serological diagnostic testing. However, conjunctival vaccination with reduced doses before the age of 4 monthsavoids the serological interference and abortions and udder infections (Godfroid *et al*., 2011) .

For example, the original dose of S19 vaccine is 2.5–12x10^{10} CFUs for calfhood vaccination, but the United States and some other countries switched to a reduced dosage of 3–10x10^{9} CFUs in the 1980s in an effort to reduce the number of calfhood vaccinates having retained antibody titers (Olsen and Tatum,2010).

Control

Until now, there are no studies on eradication strategies or vaccination of camel brucellosis (Gwida*et al*., 2012).

Eradication of brucellosis by test-and-slaughter is unfeasible in developing countries because of limited resources to compensate farmers whose animals are slaughtered during such screening programs (Godfroid *et al*., 2011).However, a mass vaccination strategy (avoiding pregnant animals in mid-gestation) may be applied to control brucellosis in developing countries.

The herd/flock level control of animal brucellosis may be achieved using some general principles: reducing the exposure to *Brucella*spp. and increasing the resistance to infection of animals in the populations.

Brucellosis has been eradicated in most developed countries that implemented a tight control program like test and slaughter (Mwebe *et al*.,2011).

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).

1. The reduction of exposure to *Brucella* spp

Farm sanitation and biosecurity: Aborted fetuses, placentae and contaminated litter should be disposed by incineration or deep burial mixing with lime at sites away from water courses. Any area in which an abortion or infected parturition has occurred should be washed down with an approved disinfectant.

Dung should be cleaned daily and stored in a isolated area until rendered safe by natural decay (this will probably require about one year) or else burnt or soaked in disinfectant before disposal.

Premises that have held *Brucella*-infected animals should not be re-stocked until at least four weeks have elapsed between cleaning and disinfection. Rodent control measures should be enforced and insect infestation kept to a minimum by the use of fly screens, light traps and insecticides. The use of maternity pens

to isolate animals during and post-parturition is essential as these animals shed the most *Brucella*.

Isolation of post-parturient animals reduces the spread of infection to the rest of the herd or flock(Corbel, 2006).

Control of animal movement: The control of animal movements between herds, and especially from farms or regions with a high prevalence of disease is a basic principle of animal disease control and is a necessary and highly effective measure. The control of animal movement within a country is sometimes impossible without regulatory/legislative support.

The permanent, individual identification of animals is also very important to identify the interstate/division/district and cross-border movements and market chain interactions of livestock within the country and the region is also necessary. Unauthorized sale or movement of animals from an infected area to other areas should be forbidden. Replacement stock should be procured from brucellosis free herds/flocks. The application of pre-movement testing will reduce the risk of spread of brucellosis between herds/holdings and provides additional assurance for the purchaser in this regard. Isolation of purchased replacements for at least 30 days and a serological test prior to entering the herd/flock is necessary. In case of porous borders with neighboring countries, a regional control strategy should be developed to prevent illegal trafficking of livestock.All imported livestock should be monitored in quarantine stationsbefore entered into the country (Corbel, 2006; Loth *et al*., 2011; Islam *etal*., 2013b; Mondal and Yamage, 2014).

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.

2. Increasing the resistance to infection of animals in populations

Vaccination: The most successful method for prevention and control of brucellosis in animals is through vaccination. While the ideal vaccine does not

exist, the attenuated strains of *B. melitensis* strain Rev.1 for sheep and goats and *B. abortus* strain 19 have been used widely.

Strain 19 vaccine

A widely used vaccine for the prevention of brucellosis in cattle is *B. abortus* S19, which remains the reference vaccine with which any other vaccines must be compared. It is used as a live vaccine and is normally given to female calves aged between 3 and 6 months as a single subcutaneous dose of 5–8 × 10^{10} viable organisms. A reduced dose of from 3 × 10^{8} to 5 × 10^{9} organisms can be administered subcutaneously to adult cattle, but some animals can develop persistent antibody titres and may abort and excrete the vaccine strain in the milk.

***B. melitensis* strain Rev.1 vaccine**

Brucella melitensis Rev.1 is the most widely used vaccine for prevention of brucellosis in sheep and goats.This vaccine is used as a freeze-dried suspension of live *B. melitensis* Rev.1 strain for the immunisation of sheep and goats. It should be given to lambs and kids aged between 3 and 5 months as a single subcutaneous or conjunctival inoculation**.**

Young (three months) dromedaries received a full dose of the vaccine and adults (10 years) a reduced dosage. Both groups developed *Brucella* antibodies with titres 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 ×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.

CHAPTER TWO
MATERIALS AND METHODS

2.1. Study Area:

The study was carried out in three localities in West Kurdufan State (WKS)in sudan namely, Alnehood, Elkhwai and Ghubaish.

West Kurdufan State located between latitudes 27°-29°N, and longitudes 14°-20°E. The state borders North Kurdufan, South Kurdufan, East Darfur and North Darfur. Its area is 14400 square kilometers extending from low rainfall savanna to high rainfall and hill catena and its vegetation varies greatly (Alshareef, 1994; IFAD, 2003;),The climate of the WKSwas thoroughly described by IFAD (2003). The northern part is dry with an average Annual rainfall concentrated in a single relatively short summer season during June to September and the region enjoys an annual rainfall of 300mm per annum.

The far most southern part of the state is characterised by a high annual rainfall up to 400mm on the average and high vegetation density. As has been reported by Hunting Technical Services in 1963, the state comprises a number of soil types, the two major and most extensive types being the sandy (qoz) soils (70% of arable lands) in the northern part and the clay soils (30% of arable lands) dominating in the southern part.

The variation of climatic zones and different soil types is reflected in the main economic activities of the inhabitants in the study area, which are based on integration of agriculture and animal production.

Kordofan falls in the grass-land and wood-land savannahs; it has abundant fodder and grazing areas in rainy seasons during which animals are trekked by pastoralists to the northern part of the region while in the dry seasons animals are trekked to the southern part of the region up to the Bahar Al-ghazal River in South Sudan**.**

Geographical map of West Kurdufan State

2.2. Study Population

Five hundreds (500) apparently healthy camels with no history of vaccination against brucellosis were randomly sampled from 60 camel herds in three localities. Tow camel production systems were tested in this study. These animals were sampled in the period between September 2018 and April 2019.

2.3. Design of study

Multistage random sampling was designed based on, locality, herd and animal. Selection between localities, herds and individual animals based on simple random sampling.Three localities selected randomly during the study namely, Alnehood, Alkhewai, and Gubaish.

2.4. Sample Size

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

$$n = \frac{(1.96)^2 \, Pexp \, (1- Pexp)}{d^2}$$

Where: n = required sample size ,Pexp = expected prevalence and d = desired absolute precision (Thrusfield 2007)

The expected prevalence in the present study was estimated as 50%. This was based on a previous study with prevalence rates ranging from 1.4 to 89.5% (Musa and Shigidi., 2001**)**.The average was 45% and inflated to 50% to widen the chances of observation and estimate the distribution of brucellosis in WKS. So the sample size was calculated as follows:

$$n = = \frac{(1.96)^2 \times 0.5 \times 0.5}{(0.05)^2} = 400$$

The total calculated sample size was 400 however, 500 camels were screened from the study area.

2.5. sample collection

In the period between September 2018 and April 2019, approximately a total number of (500) blood samples were collected aseptically from apparently healthy camels and devided between plain tubes with serum clot activator for serological examinationand anticoagulant tube(EDTA) for molecularexamination ,About 7 to 10 ml of blood was collected aseptically from the jugular vein of camles,the samples in plain tubes were kept in an upright position at 25 ºC for about 2 hours, the serum samples were separated by centrifuging at 6000 x g for 10 min. The separated serum samples were collected in a screw capped plastic vials and transported to the laboratory of the Veterinary Research Institute (VRI)in El-Obeid where they were stored at -20ºC till used.Whole Blood samples were kept in the refrigerator (2–8 °C) and one

day later convenient amount of whole blood droped in a screw capped plastic vials and transported to the Central Veterinary Laboratory for molecular examination.Livestock farmers of the selected areas were informed about the survey. However, to encourage their participation in this study and facilitate the process of sampling, the author administered anthelmintic and multivitamin injections to their animals during sampling period.

Questionnaire

At the time blood samples were collected, questionnaires were filled by the aid of the owner of each sampled herd.

Aquestionnaire included possible location, age, sex(male and female),herd size (<20,20-40 and> 40) mangment type(extensive and semi-intensive), history of abortion (yes and no),sharing of breeding male (yes and no) , whether reared individuallyor contact with other ruminant species (yes and no), contact with other camel herds (yes and no), herd,s man awareness of brucellosis (yes and no).

From the total camels tested 28.4% (142/500) were males while 71.6 %(358/500) were female.

In this study camels tested were divided According to the Age factor to three age groups .young group were considered under 5 years, medium age group from 5years to 10 years old and old age group over 10 years old, Out of the 500 camels 18.8 % (n=94) were young, 73.4 %(n=367) were medium age and 7.8%(n=39) were old camels.

In this work camel herds tested was classified into three categories according to Herd size (small <20, medium 20 – 40 and large > 40 animals) Individual camels were 7.2 %(n=36) in small herds, 12% (n=60) in medium herds and 80.4% (n=404) in large herds.

Of the total camels tested 67 %(n=335) were reared under nomadic system (extensive) whereas 33% (n=165) were kept in small groups around the localities (semi-intensive).

The number of serum samples tested were 21.8% (109/500) from herds with history of abortion and 78.2% (391/500) from herds didn't show any abortion case.

Of the total camels sampled 20.4 % (102/500) belong to herds that sharing breeding males whereas 79.6 % (398/500) didn't share males for breeding.

In the present study 32.6 % (163/500) of the total camels sampled kept with other ruminants whereas 67.4 % (337/500) kept away from other ruminants.

Of the 500 camels sampled 30.4 % (152/500) were in contact with other camel herds, while the unaccompanied other camel herdscontributed 69.6 % (348/500).

During the study a questionnaire survey was conducted among camel herds men to assess their attitude-practice and awareness towards brucellosis. The first group of herds men found to be knowledgeable on what brucellosis, its clinical signs, transmission**,** zoonotic impact and control measures were 7.4% (37/500) and the others with absence of knowledge were 92.6% (463/500).

2.6. Diagnostic Techniques

Serological test

(A) Rose Bengal Plate Test

This test was carried out at the laboratory of the Veterinary Research Institute, El-Obeid, Sudan.The test depends on early detection of *Brucella*specific agglutinins by using antigen stained with Rose Bengal and buffered to a low pH, usually 3.65 ± 0.0. All serum samples collected and RBT antigen obtained from Veterinary Research Institute, Soba.Were brought at room temperature before the test. The test was performed according to the OIE manual (2016).

A30µl of RBPT antigen quantities were dispensed to each circle on the plate and 30 µl of the antigen was added after the antigen bottle was shaken to ensure

homogenous suspension, the antigen and each serum samples were mixed thoroughly by a wooden spreader. The plate was shacked gently for 4 minutes and the degree of agglutination reactions were noted immediately after 4 minutes.

(B)Serum Agglutination Test (SAT)

All camel serum samples were tested by the SAT, This test was performed according to the method used by OIE manual (2016).

Test procedure

The test was done in glass tubes suitable volumes (1ml), araw of seven Wassermann tubes were used per sample at least. An amount of 0.8 ml of phenol saline ((0.85% sodium chloride and 0.5%phenol)) was placed in the first tube and 0.5 ml was placed In each of the other tube using an automatic pipette. To the first tube ,0.2 ml of the serum samples was added and thoroughly mixed with phenol saline ,then 0.5 of the mixture was transferred to the second tube ,from which (after mixing) 0.5 ml of diluted serum dilution was transferred to the next tube and so on . This process was continued until the last tube, from which, after mixing, 0.5 of diluted serum was discarded this result in tow fold dilution of serum (1/5, 1/10, 1/20, and so on)by using an automatic pipette, 0.5 ml of diluted standard antigen was added to each tube.

The contents of each tube was thoroughly mixed by shaking the rackes this gives final dilutions of 1/10, 1/20, 1/40,…..etc. The tubes were incubated at 37c overnight before recording the results and then examinedagainst a black background with the light coming from above and behind the tubes .Complete agglutination and sedimentation with water-clear supernatant wasrecorded as ++++, nearly complete agglutination and 75% clearing as +++,marked agglutination and 50% clearing ++, some sedimentation and 25%clearing as +, and no clearing as zero.

Conversation of serum agglutination test results in to IU Ab/ml

Due to variation in density of antigens, agglutinatios tests in different countries may result in different titres even for the same positive serum, Adoption of an international unitage system was done as recommended by the OIE (2004)

Moleculer Technique

A total of 500 whole blood samples were examined byPCR. For DNA extraction a DNA Purification Kit (Analytikajena) was used according to the manufacturer's instructions. Briefly, 200 µL of the blood sample was placed in a sterile Eppendorf tube (1.5 mL size).Then 200 µL of lysis solution and 20 µL of proteinase K were added. The mixture was vortexed for 10 min and incubated for 10 min at 60c°. 4 µL RNase was added and incubated for 5min after vortex then centrifuged using a refrigerated Eppendorf centrifuge at 16,000 rpm for 1 min to remove condensation. Three hundred and fitty (350) µL of BL was added to the resuspended white pellet and pipetted for 3-5 times to lyse the white blood cells and centerfugated (12000 rpm)for one min after addition of the sample to spin filter .400 µL washing C was added in anew reciver tube and centrifuged for one min ,then 600 µL of buffer saline was added and centrifuged (12000) for one min at 2000 rpm.Then the filtrate was discarded and spin filter was added to receiver tube and centrifuged at max speed ,3min.for elution the spin filter was added to elution tube and 200 µL elution buffer (60°c) was added and incubated for 2 min and then centrifuged. Extracted DNA was kept at −20°C until use in PCR analyses. Concentration and purity of DNA were confirmed spectrophotometrically. Furthermore, agarose gels with 5 µL of DNA were used to examine the quality and quantity of DNA. Finally, sheep glyc-eraldehyde 3-phosphate dehydrogenase was also used to confirm the quality of extracted DNA (Unver. *et al* 2006).

Multiblex Polymerase Chain Reaction (PCR)

PCR and other genetic techniques are broadly used for the rapid detection of brucellosis.Diagnosis of brucellosis by PCR using blood as a sample is applicable. In the present investigation, further effort was made for diagnosis of brucellosis from blood samples (Singh*et al*., 2010; Zerva*et al*., 2001).

Multiblex PCR was used for the detection of *B. abortus*, the following set of primers was used with an expected product size of 494bp: forward 5'GACGAACGGAATTTTTCCAATCCC-3'and reverse:

5'-TGCCGATCACTTAAGGGCCTTCAT-3'. For the detection of B. *melitensis* the following set of primers was used with an expected product size of 733bp: forward

5'-AAATCGCGTCCTTGCTGGTCTG-3'and reverse 5' - TGCCGATCACTTAAGGGCCTTCAT-3'

According to Khamesipour *et al*.(2014) For atotal volume of 20 µl, 1 µl primer, 5 µl template DNA and 11µl distilled water were added to iNtRON Maxime™ PCR premix(Intron, South Korea). The DNA was amplified in thermo- cycling conditions using PCR machine (primus 96 advance,Germany) as follow: preheated to 110°C, 95°C for 5 min; 35 cycle were performed: 95°C for 1 min, 65 °C for 1min, 72°C for ,1 min extension at 72°C for 7 min, with a final hold at 4°C. The product was then visualized using a 1.5% (w/v) agarose gel dissolved in 1× TBE buffer (743 mMTris–HCl (pH 8), 87 mM boric acid, and 5.3mM Na2EDTA), stained with Ethidium bromide.

Appendix

5x TBE Buffer

27 gm Tris base

14gm Boric acid

0.369 gm EDTA

250 ml H_2O

Sodium Tris EDTA (STE) Buffer

100 m M Na Cl

10 m M Tris –HCL PH.8.0

1 m M EDTA

Proteinase K

Stock solution is 100 mg/ml

Working solution 10 mg/ml

1.5 % Agarose gel

0.75 gm agarose

40 ml d H_2 O

10 ml 1x TBE buffer

Heated to dissolve and wait for a while before a ddition of 4 µl Ethidium Bromide

Sodium Didocyle sulphate (SDS)

Used as 20% solution, not refrigerated, or autoclaved

Guanidine hydrochloride

57.2 gm dissolve in 100ml water

Ammonium acetate 7.5M

57.8gm in 100ml water

2.7.Statistical analysis

Data of questionnaire and tested blood samples were transferred into Microsoft Excel spreadsheet database, then imported to the Statistical Package for Social Sciences (SPSS) version 26.0 software for windows (SPSS Inc., Chicago, IL, USA) for performing appropriate statistical analyses.

Descriptive statistics, frequencies and cross-tabbing were obtained for each risk factor. Univariate and multivariate analyses by means of the 2-tailed chi-square test and logistic regression model were performed to test the hypothesized variation of the potential risk factors between test-positive and test-negative animals.Significant risk factors in the univariate analysis were subjected to Multivariate analysis using logistic regression.All risk factors with $p \leq 0.05$ were considered significant.

CHAPTER THREE

RESULT

The result of examination of camels for brucellosisin WKS is presented in thetable (6).

Of the total camels chosen in the analysis 49% (245/500), 8.6% (43/500) and 42.4% (212/500) fromAlnehood, Elkhwai and Ghubaish localitiesrespectively, the seroprevalence of the disease was higher inElkhwai 16.3(N=7),Alnehood 3.7 %(n=9), Ghubaish1.4 %(n=3). There was statistical significance differences between three localities (p.value=0.000).

The overall prevalence of brucellosis in camels examined was 3.8 %(19/ 500) and in the herds examined was 25 %(15/60). All serum samples were subjected furthermore to the SAT test.Seventeen camels were positive and showed prevalence of 3.4% (17/500).

Table (6): Seroprevalence of camel brucellosis at herd and individual levels using RBPT and SAT test .

Localities	**No of tested samples**	**RBPT Positive (%)**	**Sat Positive (%)**	**No of tested herds**	**RBPT Positive (%)**	**Sat Positive (%)**
Alnehood	245	9(3.7%)	9(3.7%)	37	7(19%)	7(19%)
Elkhwai	43	7(16.3%)	5(11.7%)	7	5(71.5%)	5(71.5%)
Ghubaish	212	3(1.4%)	3(1.4%)	16	3(18.75%)	3(18.75%)
Total	**500**	**19(3.8%)**	**17(3.4%)**	**60**	**15(25%)**	**15(25%)**

The prevalence of Brucellosisin femalecamels was3.9% (14/356) and in the males 3.5 % (5/144) . There was no statistical significance differences between male and femalecamels (p.value=0.807).

The prevalence of Brucellosiswas 3.2 % (3/94) in young, 4.1 % (15/367) in medium and 2.6 %(1/39) in old camels . There was no statistical significance differencesbetween 3 age groups (p.value=0.843).

The prevelance of brucellosis in small herds was 2.7 % (1/36), 3.3 % (2/60) in medium herds and 4% (16/404) in large herds. There was no statistical significance differences between herd size (p.value=0.920).

The seroprevalence of brucellosis in nomadic herds was 3.9% (13/335) higher than that in semi intensive herds 3.6 %(6/165)There was no statistical significance(p.value=0.893)**.**Although there was no statistically significant difference (P=0.293) The seroprevalenceof the disease in the herds with history of abortinwas 5.5% (6/109) and in herds without abortion history was3.3 %(13/391) .The occurrence of the disease was higher in individual of herds sharing males 5.9 % (6/102) compared to that didn't share males 3.3 % (13/398).

The association between the two categories did not show significant statistical difference (P value=0.514).

Camels with other ruminants showed seroprevalence 5.5 %(9/163) higher than that in camels kept alone 3 % (10/337). There was statistical significance between the two categories (p.value.0.011).

The seroprevalence of the disease in the herds in contact with other camel herds was 7.2%(11/152) andin the herds unaccompanied other camel herds was 2.3 % (8/348).There was statistical significance between the two groups (p.value.0.000).The higher seroprevalence of brucellosis was seen in camels reared by nomads without knowledge towards brucellosis 3.9% (18/463) ,and in the camels reared by nomadsknowledgeable on what brucellosis was 2.7 % (1/37).

There was no statistical significance between two groups (p.value=0.717).

Table (7) distribution of camels tested for brucellosis according to therisk factors

Risk factors	Frequency	Percent%	Cumulative percent%
Localities			
Alnehood	245	49.0%	49.0%
Ghubaish	212	42.4%	91.4%
Elkhwai	43	8.6%	100.0%
Sex			
Male	144	28.8%	28.8%
female	356	71.2%	100.0%
Age			
Small <5Y	94	18.8%	18.8%
Medium age 5-10Y	367	73.4%	92.2%
Old >10Y	39	7.8%	100.0%
Herd size			
<20 Small	36	7.2%	7.2%
20-40 Medium	60	12.0%	19.2%
>40 Large	404	80.0%	100.0%
Management			
extensive	165	33.0%	33.0%
semi intensive	335	67.0%	100.0%
Sharing of breeding male			
yes	102	20.4%	20.4%
no	398	79.6%	100.0%

Table (7): continue

Risk factors	Frequency	Percent%	Cumulative percent%
contact with other species			
yes	178	35.6%	35.6%
no	322	64.4%	100.0%
contact with other camel herds			
yes	163	32.6%	32.6%
no	337	67.4%	100.0%
Herdman brucellosis awarnes			
yes	37	7.4%	7.4%
no	463	92.6%	100.0%
History of abortion			
yes	109	21.8%	21.8%
no	391	78.2%	100.0%

Table(8):The prevalence of brucellosis and associated risk factors in 500 camels examined by RBPT in WKS

Risk factors	No of tested samples	No of positive samples	Percentage (%)
Localities			
Alnehood	245	9	3.7%
Elkhwai	43	7	16.3%
Ghubaish	212	3	1.4%
Sex			
Male	144	5	3.5%
female	356	14	3.9%
Age			
Small <5Y	94	3	3.2%
Medium age 5-10Y	367	15	4.1%
Old >10Y	39	1	2.6%
Herd size			
<20 Small	36	1	2.8%
20-40 Medium	60	2	3.3%
>40 Large	404	16	4%
Management type			
extensive	335	13	3.9%
semi intensive	165	6	3.6%
Sharing of breeding male			
yes	102	5	4.9%
no	398	14	3.5%

Table (8): continue

Risk factors	No of tested samples	No of positive samples	Percentage (%)
Contact with other species			
yes	178	12	6.7%
no	322	7	2.2%
contact with other camel herds			
yes	163	15	9.2%
no	337	4	1.2%
Herds men brucellosis awarnes			
yes	37	1	2.7%
no	463	18	3.9%
hitory of abortion			
yes	109	6	5.5%
no	391	13	3.3%

Table(9):Univeriate Analysis for the Association between brucellosis in camels and risk factor in West Kurdufan State by using the Chi square test.

Risk factors	No of tested	No of positive	Percentage (%)	$_x$2	P value
Localities				21.627	.000
Alnehood	245	9	3.7%		
Elkhwai	43	7	16.3%		
Ghubaish	212	3	1.4%		
Sex				.059	.807
Male	144	5	3.5%		
female	356	14	3.9%		
Age				.341	.843
Small <5Y	94	3	3.2%		
Medium age 5-10Y	367	15	4.1%		
Old >10Y	39	1	2.6%		
Herd size				.167	.920
<20 Small	36	1	2.8%		
20-40 Medium	60	2	3.3%		
>40 Large	404	16	4%		
Management				.018	.893
extensive	335	13	3.9%		
semi intensive	165	6	3.6%		

Table (9): continue

Risk factors	No of tested	No of positive	Percentage (%)	$_x$2	P value
Sharing of breeding male				.426	.514
yes	102	5	4.9%		
no	398	14	3.5%		
contact with other species				6.542	.011
yes	178	12	6.7%		
no	322	7	2.2%		
contact with other camel herds				19.309	.000
yes	163	15	9.2%		
no	337	4	1.2%		
Herdsmen brucellosis awarnes				.132	.717
yes	37	1	2.7%		
no	463	18	3.9%		
History of abortion				1.108	0.293
yes	109	6	5.5%		
no	391	13	3.3%		

There were three variables for risk factors showed P<0.05 in analysis by Chi-square (contact with other camels herds P=0.000, localities P=0.000 and contact with other ruminant species P=0.011),furthermore the three variables were analyzed by using the logistic model and revealed statistical significant with the occurrence of the disease (p<0.05) .

Tabe (10): Analysis for the Association between brucellosis in camels and risk factor in West Kurdufan State by using the logistic regression.

Risk factors	**No of tested**	**No of Positive**	**Exp(B)**	**95%C.I.forExp(B)**	**P-value**
Contact with other species					
yes	178	12	-	-	-
no	322	7	0.307	0.119 – 0.796	0.015
Contact with other camel herds					
yes	163	15	-	-	-
no	337	4	0.119	0.039 – 0.363	0.000
Localities					
Alnehood	245	9	-	-	0.000
Elkhwai	43	7	13.546	3.347 - 54.822	0.000
Ghubaish	212	3	5.099	1.788 - 14543	0.002

Out of 500 whole blood sample tested by (PCR) none of them was found positive for *Brucella spp.*

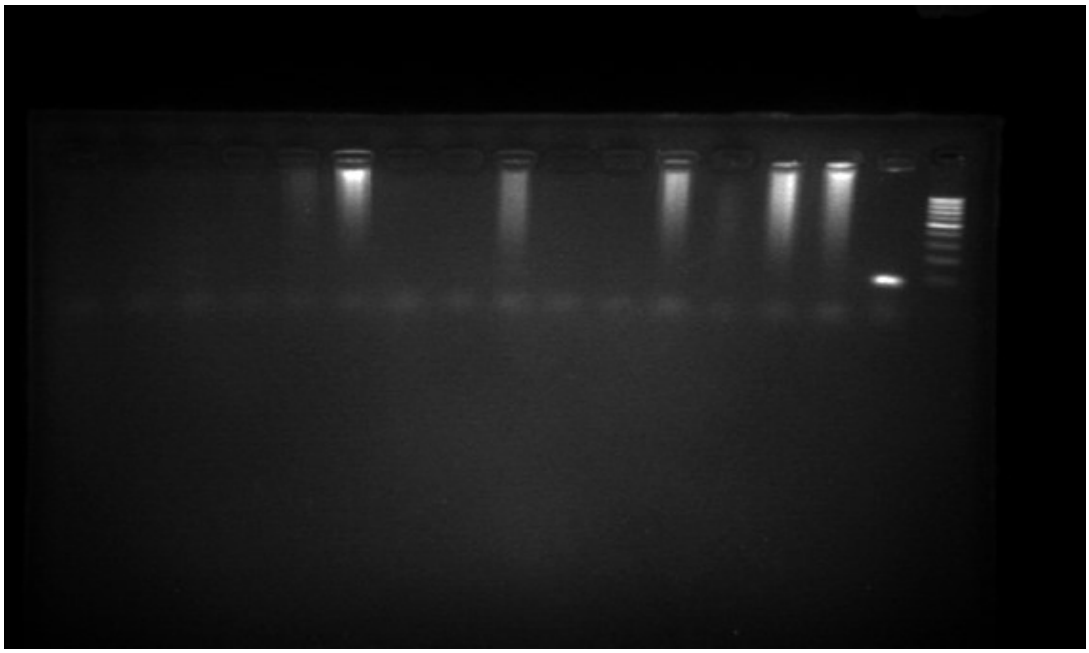

Figure 1. Ethidium bromide-stained agarose gel electrophoresis of PCR products (100 bp)for the detection of *Brucella*spp. in camel samples after PCR amplification.

Lane 1: 100 bp DNA ladder (Fermentas, Germany); lane2: positive control ;lanes 3to 16 negative sample; lane 17: Negative control.

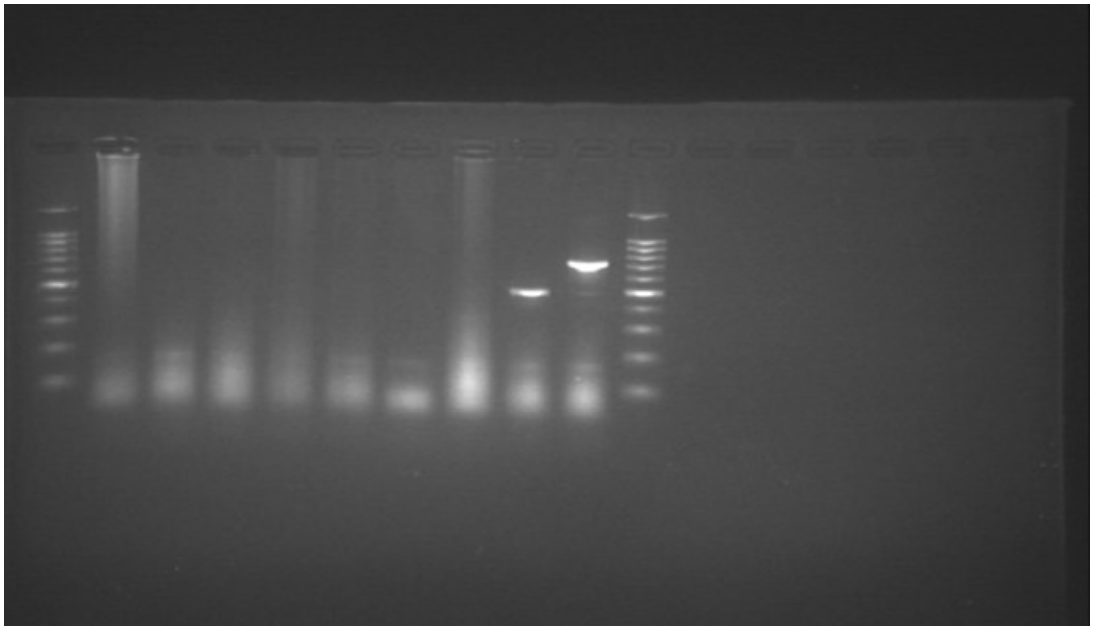

Figure 2. Ethidium bromide-stained agarose gel electrophoresis of PCR products (100 bp) for detection of *B. abortus and B. melitensis* in camel samples after PCR amplification.

Lane 1 and 11 : 100 bp DNA ladder (Fermentas, Germany); lanes 2 and 3 positive control (lane 2: *B. melitensis* in camel; lane 3: *B. abortus* in camel); lane 4: Negative control;lanes 5 to 10 negative samples

CHAPTER FOUR

DISCUSSION

In this study and according to the results of RBPT, the prevalence of *Brucellosis* in camels was (3.8 %).This was in agreement with the studies of other investigators in Sudan who found the prevalence of the disease range between 1.8% to 43.9%.Yagoub *et al*.(1990) examined 1502 camels and found 1.82% seroprevalence in 79 young camels, 6.95% in adult males and 13.77% in adult females, Musa (1995) reported 7.76% in 1,314 camels in Darfur, Western Sudan, Majid *et al.,*1999 found the prevalence of 13.9% and 43.9% in camels in the Sudan. Higher prevalence was recorded in Sudan (Musa and Shigidi 2001, Omer *et al*., 2010 ,Taha*et al.*, 2020 reported 36.2% in Elgadaref State, Eastern Sudan).

Radostits *et al*.(2007) attributed the differences in the prevalence of camel brucellosis from different countries to varying husbandry and management practices, the number of susceptible camels, the virulence of the organisms, presence of reactor animals in the region, absence of veterinary service, lack of awareness about the disease in camels and continuous entry of infected camels into a susceptible camel herds .

In the present study the overall prevalence of brucellosis in camels examined was 3.8% and 3.4% withRBPT and SAT test, respectively, this finding in the line with the study of Nasir *et al.* (2004) which indicated thatRBPT detected higher percentage of sero-positive animalsas compared to SAT.

this study revealed statistical significance differences in prevalence of brucellosis between three localities (p.value=0.000) ,in Elkhwai 16.3(N=7), Alnehood 3.7%(n=9),Ghubaish 1.4%(n=3) ,this result is similar to the finding of Mohamed*et al* ., 2015 which showed significant association between the prevalence of *Brucella* infection and the location of camel rearing (locality).

The prevalence of brucellosis in camels reared with other ruminants (cattle, sheep and goats) was in significant statistically (P=0.011), Camels reared with

other ruminants showed prevalence of 6.7% (n=12) higher than those camels kept separately in which theprevalence was 2.2 % (n=7). This finding agreed with that reported by other investigators(Teshome *et al*., 2003, Al-Majali *et al*., 2008 , Mohammed *et al*., 2011 andMohamed*et al* .,2015).

The present result is in accordance with that recorded by Radwan *et al*.(1995) Frequent isolation of *Brucella melitensis* from camels further suggests the role of small ruminants in the occurrence of camel brucellosis. Abbas *et al*., 2000 and Al-Majali *et al*., 2008 also suggested the role of small ruminants in dissemination of infection with *Brucella*to camels.

Inspite that there was no significant relation between the herd size and brucellosis in camels in the study (P=0.920). The results revealed that the herd size appears to be a risk factor for brucellosis in camels, In large herds with more than 40 camels ,the prevelance was 4% (n=16), in medium sized herds with 20-40 camels was 3.3 % (n=2) in small sized herds with less than 20 camels was 2.8 % (n=1). This result was in agreement with that previously studies reported by (Gameel *et al*., 1993; Abbas and Agab 2002; Abou-Eisha, 2000; Wernery and Kaaden, 2002; Bati 2004; Al-Majali *et al*., 2008 and Mohammed *et al*., 2011). It was suggested that more contacts between camels may occur in large herds than smaller ones.

In this study the prevalence of *Brucella*was 3.2 % (n=3) in young camels and 4.1 % (n=15) in medium age and 2.8 % (n=1) in older age camels. these findings are similar to those reported by other inestigators(Musa and Shigidi, 2001;Bati ,2004; Al- Majali *et al*., 2008; Dawood ,2008;Omer *et al*., 2010 and Swai *et al*., 2011. Usually young animals are protected by maternal immunity until when the immunity disappears, thus the susceptibility seems to be low among them. Similarpatterns were found in cattle, and Oloffs *et al.* (1998) reported that30% of the positive animals in Uganda were younger than threeyears of age and within them was a 2-year-old bull, which was not introduced for service.

Adult camels are more exposed to infection, The prevalence was lower among the elder animals in this study compared to the medium age camelsandthis is attributed to the presence of few number of elder camels within the herds.

The overall prevalence was reduced from 3.8% to 3.4% when SAT test was used. This variation could be due to the lowsensitivity and specificity of the SAT test compared with theRBPT.

There was significant association between the prevalence of *Brucella*among camels in different localities(P=0.000). The prevalence of the disease was higher in Elkhwai locality 16.3 % (n=7), Alnehood 3.7% (n=9) and Ghubaish11.4% (n=3).This result is in agreement with that recorded by Al-Majali *et al*. (2008) and Swai *et al*.(2011).Teshome *et al*., 2003attributed the effect of locality on *Brucella*infection to husbandry, management practice, absence of veterinary service, lack of awareness, and uncontrolled movement ofcamels from place to another.

The present study showed prevalence 3.5% (n=5) inthemale camels and 3.9% in females with no significantassociation (p=0.807).This observation was dissagreed with that reported by Radwan *et al*. (1992)

Although no statistically significant difference (P=0.893) observed between the two management systems, the present study found lower seroprevalence in semi-intensive camel herds (3.6%) than the nomadic camels (3.9%). This finding is also supported by Radostits *et al*.(2007) who stated that the movement may worsen the epizootic situation of brucellosis.

In this study the seroprevalence of Brucellosiswas 4.9% (n=5) in camelherds shared camels for breeding and 3.5 % (n=14) in herds not shared breeding camels. Moreover the prevalence of the disease in camels reared by nomads aware with brucellosis is 2.7(n=1) lower than those rared by unaware nomads3.9 %(n=18).Although there was no significant statistical association ($p<0.05$) these findings can potentially play a major role of transmission of the disease in camels.

Camels within herds contacted other camels in pasture and water points showed prevelance of the disease (9.2%) higher than that showed by other in herds not in contact (1.2%) with statistically significant difference (P=0.000).Spread of the disease is due to movement of infected animals to disease free herds get the infection from infected herd at water points where a number of herds come together.

Detection of brucellosis in camel serum samples by PCR has been described by Alshaikh *et al.,*2007 in Saudi Arabia. This is a very reliable diagnostic tool, which can even differentiate between *B. melitensis* and *B. abortus* brucellosis.Whereas no positive samples detected by PCR from blood of 500 camels in this study. This result in agreement of the finding of Ullah*et al.,* 2015 who detect 0.00% PCR positive cases from RBPT positive camles blood .This might beattributed to presence of antibodies in healthy camels or oscillating of immunoglobulin titers (Gwida *et al*., 2011). It is noteworthy to mention that all serologically positive camels were clinically normal at the time of sampling.

Also our findings in agree with those of Abdelgawad *et al*.(2017) who found five camels where positive for serological tests (BAPAT ,RBPT and CFT) but negative for PCR. different result were obtained by (Abdallah & Baleela, 2017) who detected 41 out of 100 camels were positive for *Brucellaspp* by PCR.

Kaushik*et al*.(2006) found only 18 samples were positive by PCR compared to 62 by RBPT and 41 by i-ELISAand attributed the Wide variation in samples detected to many factors. PCR detects DNA, which may be in low quantity in blood samples even though antibody titer is quite high.

Recommendations

1- We need more investigation to study the effect of other risk factor on brucellosis distribution between camels in the WKS
2- EffortS should be made to control the disease at this low level of prevalence.
3- The best way to stop the spread of the disease is to share knowledge and further molecular test towards brucellosis control in Sudan.
4- Control and eradication of brucellosis. Infection seems to be necessary in camels and the disease should be considered because of its impact on human health.

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Apendex

Table 1: distribution of camels tested for brucellosis according to the localities

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Alnehood	245	49.0	49.0	49.0
	Ghubaish	212	42.4	42.4	91.4
	Elkhwai	43	8.6	8.6	100.0
	Total	500	100.0	100.0	

Table 2: distribution of camels tested for brucellosisaccording the sex of camels

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	144	28.8	28.8	28.8
	Femal	356	71.2	71.2	100.0
	Total	500	100.0	100.0	

Table 3: distribution of camels tested for brucellosis according the age group

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Young	94	18.8	18.8	18.8
	Medium age	367	73.4	73.4	92.2
	old age	39	7.8	7.8	100.0
	Total	500	100.0	100.0	

Table 4: distribution of camels tested for brucellosis according the herd size

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Small	36	7.2	7.2	7.2
	Medium	60	12.0	12.0	19.2
	Large	404	80.8	80.8	100.0
	Total	500	100.0	100.0	

Table 5 : distribution of camels tested for brucellosis according the management type

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Semi-intensive	165	33.0	33.0	33.0
	Extensive	335	67.0	67.0	100.0
	Total	500	100.0	100.0	

Table 6 : distribution of camels tested for brucellosis according the history of abortion in the herd

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	109	21.8	21.8	21.8
	No	391	78.2	78.2	100.0
	Total	500	100.0	100.0	

Table 7 : distribution of camels tested for brucellosis according to the sharing of breeding male

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	102	20.4	20.4	20.4
	No	398	79.6	79.6	100.0
	Total	500	100.0	100.0	

Table 8 : distribution of camels tested for brucellosis according the contact with other ruminant

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	178	35.6	35.6	35.6
	No	322	64.4	64.4	100.0
	Total	500	100.0	100.0	

Table 9 : distribution of camels tested for brucellosis according the contact with other camel herds

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	163	32.6	32.6	32.6
	No	337	67.4	67.4	100.0
	Total	500	100.0	100.0	

Table 10 : distribution of camels tested for brucellosis according the awareness of herdmen about brucellosis

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	37	7.4	7.4	7.4
	No	463	92.6	92.6	100.0
	Total	500	100.0	100.0	

Table 11: The prevalence of brucellosis and associated risk factors in 500 camels examined by RBPT in WKS

Risk factors	No. tested	No. positive	Percentage (%)
Localities			
Alnehood	245	9	3.7%
Elkhwai	43	7	16.3%
Ghubaish	212	3	1.4%
Sex			
Male	144	5	3.5%
female	356	14	3.9%
Age			
Small <5Y	94	3	3.2%
Medium age 5-10Y	367	15	4.1%
Old >10Y	39	1	2.6%
Herd size			
<20 Small	36	1	2.8%
20-40 Medium	60	2	3.3%
>40 Large	404	16	4%
Management type			
extensive	335	13	3.9%
semi intensive	165	6	3.6%
Sharing of breeding male			
yes	102	5	4.9%
no	398	14	3.5%
Contact with other species			
yes	178	12	6.7%
no	322	7	2.2%

Table 11: continue

Risk factors	No. tested	No. positive	Percentage (%)
contact with other camel herds			
yes	163	15	9.2%
no	337	4	1.2%
Herdman brucellosis awarnes			
yes	37	1	2.7%
no	463	18	3.9%
hitory of abortion			
yes	109	6	5.5%
no	391	13	3.3%

Table12: Univeriate Analysis for the Association between brucellosis in camels and risk factor in West Kurdufan State by using the Chi square test.

Risk factors	No. tested	No. positive	Percentage (%)	$_x2$	P value
Localities				21.627	.000
Alnehood	245	9	3.7%		
Elkhwai	43	7	16.3%		
Ghubaish	212	3	1.4%		
Sex				.059	.807
Male	144	5	3.5%		
female	356	14	3.9%		
Age				.341	.843
Small <5Y	94	3	3.2%		
Medium age 5-	367	15	4.1%		
10Y	39	1	2.6%		
Old >10Y					
Herd size				.167	.920
<20 Small	36	1	2.8%		
20-40 Medium	60	2	3.3%		
>40 Large	404	16	4%		
Management				.018	.893
extensive	335	13	3.9%		
semi intensive	165	6	3.6%		

Table12: continue

Risk factors	No. tested	No. positive	Percentage(%)	$_x2$	P value
Sharing of breeding male				.426	.514
yes	102	5	4.9%		
no	398	14	3.5%		
contact with other species				6.542	.011
yes	178	12	6.7%		
no	322	7	2.2%		
contact with other camel herds				19.309	.000
yes					
no	163	15	9.2%		
	337	4	1.2%		
Herdman brucellosis awarnes				.132	.717
yes	37	1	2.7%		
no	463	18	3.9%		
History of abortion				1.108	0293
yes	109	6	5.5%		
no	391	13	3.3%		

Tabe13: Analysis for the Association between brucellosis in camels and risk factor in West Kurdufan State by using the logistic regression.

Risk factors	No. tested	No. Positive	Exp(B)	95% C.I.forExp(B)	P-value
Contact with other species					
yes	178	12	-	-	-
no	322	7	0.307	0.119 – 0.796	0.015
Contact with other camel herds					
yes	163	15	-	-	-
no	337	4	0.119	0.039 – 0.363	0.000
Localities					
Alnehood	245	9	-	-	0.000
Elkhwai	43	7	13.546	3.347 - 54.822	0.000
Ghubaish	212	3	5.099	1.788 - 14543	0.002

Questionnaire

EPIDEMIOLOGY OF BRUCELOSIS IN CAMELS

IN WESTE KURDUFANSTATE-SUDAN

1/Date: …… Serial No: ……..

2/ **Owner Name: …… Phone No:…….**

3/ Location

(Locality)…….

4/ sex:

Male ☐ Female ☐

5/ Age:

5 years < ☐ 5-10 years ☐ >10 years ☐

6/Herd size:

<20 ☐ 20-40 ☐ >40 ☐

7/ Managementtype:

Extensive ☐ semi-intensive ☐

8/ History of abortion in herd:

Yes ☐ No ☐

9/sharing Breeding camel

Yes ☐ No ☐

10/ Contact with other ruminant:

Yes ☐ No ☐

11/- Contact with other camel herds:

Yes ☐ No ☐

12/ A wariness of Brucellosis

Yes ☐ No ☐