



بسم الله الرحمن الرحيم

Sudan University for Science and Technology

College of Graduate Studies



**Prevalence and Risk factor of Brucellosis in Camels in North Kordofan State,
Western Sudan**

نسبة الإصابة وعوامل الخطر لمرض البروسيلا في الإبل في ولاية شمال كردفان , غرب السودان

**A Thesis Submitted to the College of Graduate Studies in Partial Fulfillment
of the Requirements for the Degree of Master in Preventive Veterinary
Medicine (M.P.V.M)**

By:

Younes Hassan Blal Hamed

B.V.M., 2016, University of Elbutana

Supervisor:

Prof. Galal Eldeen Elazhari Mohammed

January 2021

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قال تعالى:

(أَفَلَا يَنْظُرُونَ إِلَى الْإِبِلِ كَيْفَ خُلِقَتْ)

سورة الغاشية الآية (17)

DEDICATION

I would like to dedicate this work especially to my parents, Lovely sisters and brothers whom I owe them and to all my friends and Colleagues who supported and motivated me to continue work for the degree.

I would also like to dedicate this work to my deceased friend Dr. Baha_Alden .

ACKNOWLEDGEMENTS

Firstly I thank Allah alot for accommodate me to do this work. my thanks and appreciationto my supervisor **Prof. Gleeldin Mohammed Alazhari.** Who guided me throughout the work. A special thankful is also to my friend Dr.Yasir Juma, of ELObied veterinary Research laboratory.

List of Contents

Title	Page
Dedication	II
Acknowledgements	III
List of contents	IV
List of tables	VII
Abstract	VIII
Arabic abstract	IX
Introduction	1
Objectives of the study	4
LITERATURE REVIEW	5
1.1. Classification of Brucella	5
1.1.1 The Six Classical Brucella Species	5
1.1.2. The Novel Brucella species	6
1.2. Clinical signs of brucellosis	7
1.2.1. Clinical Findings of brucellosis in domesticated ruminants	8
1.2.1.1. Bovine Brucellosis	8
1.2.1.2. Brucellosis in Caprine	9
1.2.1.3. Brucellosis in Ovine	10
1.2.1.4. Brucellosis in Camel	10
1.3. The clinical signs of brucella in human	11
1.4 Global Distribution, Epidemiology and transmission of Brucellosis	11
1.4.1 Brucellosis in Africa	11
1.4.2. Brucellosis in livestock in the Sudan	12
1.5. Transmission of brucellosis	13
1.5.1. Transmission of brucellosis in domesticated ruminant	13

1.5.2. Zoonotic of brucellosis	14
1.6. Diagnosis of brucellosis	15
1.6.1. Bacteriological methods of brucellosis	15
1.6.1.1. Stained smears examination of brucellosis	15
1.6.1.2. Culture examination of brucellosis	16
1.6.2. Serological methods of examination of brucellosis	16
1.6.2.1. Rose Bengal plate test	17
1.6.2.2. ELISA tests	18
1.6.2.3. Serum agglutination test	18
1.6.2.4. Complement fixation test	18
1.6.3. Supplementary tests	19
1.6.3.1 Milk testing	19
1.6.3.1.1. Milk ring test	19
1.6.3.1.2. Milk ELISA	20
1.6.4. Fluorescence polarization assay	20
1.6.5. Intradermal test of examination of brucellosis	20
1.7. Prevention, control of animal Brucellosis	20
1.7.1. Prevention	20
1.7.2. Control	21
1.8. Test and isolation/slaughter policy	21
1.9. Hygiene	22
1.10. Control of animal movement	22
1.11. Vaccination	22
1.12. Eradication	24
1.13. Surveillance of brucellosis in animals	24
1.14. Intersectoral collaboration	25
MATERIALS AND METHODS	25
2.1. The Study area	26
2.2. Design of the study	26

2.3. Sample collection and Questionnaire survey	26
2.4. Buffered acidified plate antigen (BAPA)	27
2.4.1. Needed for the test	27
2.4.2. The test procedure	27
2.4.3. Reading of the results:	28
2.5. Rose Bengal plate test	27
2.5.1. Materials	28
2.5.2. The test procedure	27
2.5.3. Reading of the results	28
2.6. Data management and analysis	28
RESULTS	30
3.1. analysis of risk factors	30
3.1.1. Age factor	30
3.1.2. Sex factor	30
3.1.3. District factor	30
3.1.4. Breed factor	30
3.1.5. Body condition factor	31
3.1.6. Grazing factor	31
Discussion	34
Conclusion	36
Recommendations	36
Reference	37

List of Tables

Table No.	Title	Page
Table 2	Summary of univariate analysis for risk factors associated with camel brucellosis North Kordofan State, Sudan (n=230) using the Chi-squared test.(Rose Bengal Plate(RBP test))	32
Table 3	Summary of univariate analysis for risk factors associated with camel brucellosis North Kordofan State, Sudan (n=230) using the Chi-squared test. (Buffered Acidified Plate Antigen (BAPA test))	33

ABSTRACT

Brucellosis is a contagious zoonotic disease and important disease of animals worldwide (OIE, 2000). The disease causes a decrease in reproductive efficacy and an increased abortion rate in animals. The disease is transmitted from animals to humans by ingestion of infected food products, direct contact with an infected animal, or inhalation of infected aerosols. It is widely distributed in developing countries.

A cross-sectional study was conducted to determine the prevalence of brucellosis in camel in North Kordofan State. A total of 230 blood for serum samples were collected from camels, from December 2019 to March 2020 at north Kordofan state. The Rose Bengal Plate Test and Buffered Acidified Plate Antigen were used to detect brucellosis infection, the infected camels were 51 of 230 (22.2%) and 33 of 230 (14.8%) respectively. The breed was the significant risk factor associated with the disease ($\chi^2=10.557$; p -value= 0.001). The prevalence of brucellosis was 18.3% in Sheikan, 17.6 in Bara and 9.5% in Umkredem. The prevalence of the disease 18.7% in males and 10.3% in female. The prevalence of the disease 15.3% in adult and 14.3% in old and young. Prevalence of the disease 18.3% in good body condition animal and 13.7% in moderate and 4.2% in poor. It could be concluded that this study provided necessary information about prevalence and risk factors of the disease in the study area which help the decision makers to formulate control measures of disease .

المخلص

اجريت دراسة مقطعية لمعرفة مدى إنتشار داء البروسيلات في الابل في ولاية شمال كردفان وذلك في الفتره من 10 ديسمبر 2019 الى 3 مارس 2020. تم جمع عدد 230 عينة دم للامصال من الإبل، واستخدمت اختبار الروز بنغال ومستضد الصفائح الحمضية المخزنة للكشف عن الداء. كانت معدلات الإصابة 51 من 230 (22.2%) بإستعمال الروز بنغال الحمضي و 33 من 230 (14.8%) بإستعمال مستضد الصفائح الحمضيه المخزنه . كان الانتشار 18.7% في شيكان ، 17.6% في بارا ، 9.5% في ام كريدنم. في الذكور 18.7% وفي الإناث 10.3% وفي الحيوانات البالغة 15.3% والإنتشار كان 13.7% في الاجسام جيدة الصحة ، و13.7% في المتوسطه ، و4.2% في الضعيفه. كانت السلالة عامل المخاطره المعنوي للإصابة بداء البروسيلات (p.value=0.001)

INTRODUCTION

Camels (*Camelus dromedarius*) are important animals. They play an important role in sustainable agriculture for millions of people in the arid and semi-arid zones. Camels also provide milk, meat, wool and are used for water traction and for bearing burdens. Furthermore, The exportation of camels contributes to foreign currency earnings (Abd-Elmajid, 2000).

According to Food and Agriculture Organization statistics (FAO,2006), the approximate number of camel in the world is about 19 million head, of which 15 million are found in Africa and 4 million in Asia. Moreover, approximately 15 million dromedaries, representing two-thirds of the world camel population, are living in the arid areas of Africa, particularly in Northeast Africa.

Sudan has nearly five million camels, the second-largest national herd in the world, after Somalia. Tribal groups in Sudan breed distinctive types of camels, the well-known among these are the Anafi and Bishareen camels (Ali *et al.*,2017).

The camel farming is mainly traditional based on the mobility of the herd. The camel belt in the Sudan includes the states of North and South-Darfur, North and South-Kordofan, Khartoum, Gezira, Kassala, the Red Sea, the River-Nile, Northern Sudan, the White Nile, the Blue Nile and Sennar state (Ali *et al.*,2017). Like other livestock, camels are susceptible to different types of diseases. Animal diseases are responsible for great losses in the livestock sector. Cost-effective losses incurred by these diseases include reduced animal performance and weight gain, condemnation of whole carcasses or affected organs at slaughterhouses, costs of treatment and mortality in severe cases. Livestock diseases are divided according to pathogenic agent whether viral, bacterial, parasitic, and fungal in addition to metabolic diseases.

Bacterial infections of camel cause major problems in the developing world. These diseases are difficult to manage because in some cases they develop resistance to available commercial which is now a common problem worldwide.

One of the main bacterial disease causing severe losses in livestock industry nowadays is *Brucellosis* (undulant fever, Mediterranean fever or Malta fever in humans).

Brucellosis is a zoonosis and the infection is almost always transmitted by direct or indirect contact with infected animals or their products. *Brucellosis* is predominately a disease of domestic animals; however, it is highly transmissible to humans. Cattle, small ruminants, and pigs are among the primary hosts. The disease in these animals is characterized by abortion, orchitis, and chronic shedding of the bacteria. Humans are typically infected by consumption of unpasteurized dairy products or through occupational exposure. Some species of brucellae are extremely infectious with as few as 10 organisms capable of causing disease in humans (Godfroid *at el.*, 2011). 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. It is an important human disease in many parts of the world especially in the Mediterranean countries of Europe, north and east Africa, the Middle East, south and central Asia and Central and South America and yet it is often unrecognized and frequently goes unreported. There are only a few countries in the world (Canada, Australia, Japan, and nations of northern Europe (Corbel, 1997, European Commission, 2012), that are officially free of the disease although cases still occur in people returning from endemic countries (FAO,2006).

A disease of numerous manifestations, any organ system can be affected by hematogenous spread of bacteria, leading to the development of debilitating complications. Without proper treatment, chronic or latent infections can develop. Signs of disease nearly 30 years after infection have been documented (Ogredici *etal.*, 2010).

According to (OIE, 2006), *Brucellosis* remaining a public health hazard due to expansion of animal industries and urbanization, and the lack of hygienic procedures in animal husbandry and in food handling as well as expansions of international travel which stimulates the taste for exotic dairy goods such as fresh cheeses which may be contaminated, and the importation of such foods into Brucella-free regions, also contribute to the ever-increasing concern over human *Brucellosis*.

Despite advances in veterinary and human healthcare, *Brucellosis* remains an important disease worldwide. *Brucellosis* has not attracted the research and financial resources that other global diseases such as malaria, HIV, and tuberculosis have drawn. In this sense *Brucellosis* has been classified as a “neglected disease” by the World Health Organization (WHO). Economic losses result from *Brucellosis* infection because of the duration of the human illness and its long convalescence as well as a medical problem for the patient because of time lost from normal activities. In some areas, the animal disease remains a constant threat to human welfare, particularly for those in the most vulnerable socioeconomic sections of the population, in other words, there are many regions where effective diagnosis or treatment is not available and prevention procedures and sanitation measures for the detection and prevention of the infection in humans and animals are not adequately carried out.

The application of well-controlled laboratory procedures and their careful interpretation can assist greatly in this process because *brucellosis* may present in

many a typical form, for example, in many patients the symptoms are mild and, therefore, the diagnosis may not be even considered, moreover, even in severe infections differential diagnosis can still be difficult.

On the other hand, the prevention and control of *brucellosis* needs supportive action from various sectors, including those responsible for food safety and consumer education. Inter sectorial cooperation plays an important role in the control of *Brucellosis* and may contribute to the development of appropriate infrastructures in areas of animal production, food hygiene, and health care. The current study was thus; carry out to investigate the sero-prevalence of Camel *Brucellosis*, in different locality in North Kordofan state.

The objectives:

The objectives of this study were to investigate *brucellosis* in camels in North Kordofan State. Determine risk factors associated with *brucellosis* in North Kordofan state by using the RBPT and BABA tests.

Literature Review

Brucellosis is one of the most common zoonotic diseases worldwide (Pappas *et al.*, 2006). It is caused by bacteria of the genus *Brucella*. *Brucella* is gram negative coccobacilli and facultative intracellular organism. *Brucellosis* is predominately a disease of domestic animals; however, it is highly transmissible to humans. The disease in these animals is characterized by abortion, orchitis and chronic shedding of bacteria.

1.1 Classification of *Brucella*:

1.1.1 The Six Classical *Brucella* Species:

David Bruce (1887) isolated for the first bacteria of the genus *Brucella* from British soldiers found on the island of Malta who died of a disease then known as undulant fever, which was later named *Brucella melitensis*. Epidemiological association was noticed between the disease in humans and consumption of milk from infected goats. The incidence of the disease was drastically reduced among British soldiers due to the practice of boiling milk prior to consumption as recommended.

Bernhard Bang (1897) discovered the second member of the genus *Brucella* from cattle suffering from contagious abortion, later named *Brucella abortus*. It is also known as Bang's disease (Dalrymple-Champneys, 1950).

The differences in host preference and biochemical properties resulted in the division of the genus into the six classical *Brucella* species (Osterman & Moriyón, 2006): *B. melitensis*, *B. abortus*, *B. suis* (Huddleson, 1931), *B. ovis* (Buddle, 1956), *B. neotomae* (Stoenner & Lackman, 1957), and *B. canis* (Carmichael & Bruner, 1968). *B. canis* is typically considered to play a limited role in human disease, although evidence exists that the number of cases may be underestimated (Dentinger *et al.*, 2014, Lucero *et al.*, 2010). *B. ovis* infection has never been reported in humans.

Evidence is accumulating that host specificity may not be as stringent as previously believed. Nevertheless, the primary hosts of *B. melitensis* are sheep and goats, while *B. abortus* primarily infects cattle. *B. suis* has a broader host range. The different biovars of this species are known to infect swine, wild boar (*Sus scrofa*), European hare (*Lepus capensis*), reindeer (*Rangifer tarandus*), and rodents. *B. melitensis*, *B. abortus*, and *B. suis* are the most pathogenic in humans.

Based on antigenic components brucellae can be divided into smooth (S) and rough (R) strains, this distinction refers to the structure of the lipopolysaccharide (LPS) in the bacterial cell wall. Smooth strains involved natural virulent field strains of *B. melitensis*, *B. abortus*, *B. suis*, and *B. neotomae*, while *B. ovis* and *B. canis* are naturally rough strains. While S-LPS consists of three components, lipid A, core oligosaccharide, and O-antigen, in R-LPS the O-antigen is either absent or reduced to only a few sugar residues. Smooth strains are generally more pathogenic in humans (Rittig *et al.*, 2003).

1.1.2 The Novel Brucella species:

Recent isolation of novel *Brucella* species in wildlife and human hosts has led to considerable changes in *Brucella* taxonomy over the past decade. In 1994 brucellae were isolated from marine mammals, greatly expanding the genus' ecological range. Also, in 2007 two separate species from marine mammals were recognized, *B. ceti* and *B. pinnipedialis*, preferentially infecting cetaceans and pinnipeds, respectively (Foster *et al.*, 2007). The disease manifestations in marine mammals include reproductive lesions (Ohishi *et al.*, 2003), meningoencephalitis (Hernandez-Mora *et al.*, 2008), pulmonary and other abscesses (Cassle *et al.*, 2013), and asymptomatic infections (Nymo *et al.*, 2011).

(Scholz *et al.*, 2008) in 2008 recognized a novel *Brucella* species *B. microti* (*Microtus arvalis*) from voles suffered from a systemic disease characterized by edema of the extremities, skin abscessation, arthritis, lymphadenitis, orchitis, and

peritoneal granulomas during an epizootic affecting the wild vole population within a region of the Czech Republic in 1999-2003. In 2010 another *Brucella* species, *B. inopinata*, was added to the genus (Scholz *et al.*, 2010). Currently, *B. inopinata* is represented by a single isolate (strain BO1) and several “*B. inopinata*-like” bacteria that are yet to be officially classified. Strain BO1 was isolated from a breast implant infection of a woman from Portland, Oregon (De *et al.*, 2008).

The most recent organism to be added to the genus is *B. papionis* (Whatmore *et al.*, 2014), bringing the number of recognized *Brucella* species to eleven with five new species described in the past decade. *Brucella papionis* infection has been associated with two cases of stillbirth and retained placenta in baboons (*Papio* spp.) (Schlabritz-Loutsevitch *et al.*, 2009). The isolates were from cervical and uterine swabs from two baboons, one wild-caught, one colony-born, at a primate research center in Texas, USA following stillbirth in 2006.

1.2. Clinical signs of Brucellosis:

Brucellosis is a sub-acute or chronic disease which may affect many species of animals. In cattle, sheep, goats, other ruminants and pigs the initial phase following infection is often not apparent. In sexually mature animals the infection localizes in the reproductive system and typically produces placentitis followed by abortion in the pregnant female, usually during the last third of pregnancy, and epididymitis and orchitis in the male. Clinical signs are not pathognomonic and diagnosis is dependent upon demonstration of the presence of *Brucella* spp. either by isolation of the bacteria or detection of their antigens or genetic material, or by demonstration of specific antibody or cell-mediated immune responses (OIE, 2006).

1.2.1. Clinical findings of brucellosis in domesticated ruminants:

The severity of the disease depends upon many factors such as previous vaccination, age, sex and management such as herd or flock size and density.

1.2.1.1. Bovine brucellosis:

The most common clinical outcome of *B. abortus* infection in cattle is late-term abortion or full-term birth of weak offspring (Olsen & Tatum, 2010). Abortion typically occurs only during the first parturition following infection, with subsequent births often normal, although sometimes accompanied by bacterial shedding. Infection acquired by calves at birth may be temporary or develop into latent infection. Heifer calves that develop latent disease remain asymptomatic and serologically negative until first parturition at which time abortion and seroconversion are frequently observed (Wilesmith, 1978, Nicoletti, 1980). While shedding of bacteria in milk is an obvious sequela of infection, overt signs of mastitis are not typically present (Morgan, 1960). Quality of the milk remains high in terms of absence of visible particles and a low leukocyte count (Emminger & Schlam, 1943). Osteoarticular lesions are occasionally associated with *Brucella* infection in cattle. There is evidence suggesting that differences exist in the frequency of this disease presentation between different geographic locations, potentially due to the presence of different cattle breeds or *B. abortus* biotypes. For example, in western Sudan osteoarticular lesions are more commonly associated with *Brucella* infection; 92% of Zebu cattle (*Bos indicus*) with hygromas and 62% of Zebu cattle with arthritis were found to be seropositive for *Brucella* (Musa *et al.*, 1990).

In bulls, orchitis is the most common disease manifestation often with an associated seminal vesiculitis and epididymitis. Many bulls will remain asymptomatic, and infertility is not typically observed (Eaglesome & Garcia, 1992, Carvalho Neta *et al.*, 2010). *B. abortus* infection of other livestock including

buffalo, bison, yak, and elk resembles infection in cattle (Olsen & Johnson 2011; Kreeger *et al.*, 2000; Jackson *et al.*, 2014; Nicoletti, 1980).

1.2.1.2. Brucellosis in Caprine:

Brucella melitensis infection in goats has been reported to closely resemble disease in cattle infected with *B. abortus*. Sheep are more resistant to infection, and there is great variation in susceptibility between breeds (Alton, 1990). *B. melitensis* is associated with late-term abortion during the first parturition post-infection. Pregnancy can also go full-term with the birth of weak kids, heavily infected but healthy kids, or kids that escaped infection. Infection of kids may be temporary, as development of latent infection seems to be rare (Alton, 1970).

Following abortion or normal birth large numbers of brucellae are excreted; in goats shedding in uterine discharge can last 2-3 months and resume at subsequent parturitions (Alton, 1990). Sheep are less likely to abort, although breed differences likely exist. In sheep shedding in uterine fluid is of shorter duration and rarely reoccurs during succeeding pregnancies. However, shedding in milk over succeeding pregnancies has been observed in sheep (Tittarelli *et al.*, 2005). In male animals, especially in goats, orchitis appears to be a common manifestation of *B. melitensis* infection.

1.2.1.3. Brucellosis in Ovine:

B. ovis causes epididymitis and impaired fertility in male sheep (Buddle, 1956). Although experimental infection of goats is possible, it has not been reported to naturally occur (Burgess *et al.*, 1985, Ridler *et al.*, 2000). Among rams, only 30-50% of serologically or bacteriologically positive animals will have palpable lesions (Van Metre *et al.*, 2012). Shedding of brucellae in semen still occurs in asymptomatic rams, however, and these silent carriers disseminate infection throughout the herd. Fertility of asymptomatic animals may be normal or reduced. Infection is less common in ewes, but abortion or birth of weak lambs can occur (Poester *et al.*, 2006, Hartley *et al.*, 1955).

1.2.1.4. Brucellosis in camels:

Brucellosis in Camels causes considerable economic losses due to abortion and infertility.

Camels are susceptible to *Brucella* infection, exhibiting high seroprevalence in areas where *B. melitensis* or *B. abortus* are endemic. Disease in camels is reportedly more mild than in cattle, but orchitis, epididymitis, abortion, arthritis, hygromas, and shedding in uterine discharge and milk have all been recorded (Gwida *et al.*, 2012).

In pregnant camels, the bacteria localizes in the placenta and are most abundant in abortion material (Millar and Stack, 2012).

In a study conducted by Mohammed *et al.* (2015) from April to September 2012, to determine the seroprevalence and risk factors for brucellosis infection in camels in Khartoum State, Sudan. The prevalence by RBPT was 5.8% and further investigation for positive results by c-ELISA was 87.5%.

A serological study carried out in Egypt using 1126 blood samples collected from Dromedary camels. The modified Rose Bengal Plate Test (mRBPT) and competitive ELISA (cELISA) were used as screening and confirmatory tests,

respectively. The overall sero-prevalence of brucella antibodies was 4.17% and 3.73% as detected by the mRBPT and c-ELISA respectively (Hosein *et al.*, 2016). In Iran (Faham *et al.*, 2014), a total of 11.38% of blood samples as positive for *Brucella spp.* and 13.01% of the lymph node samples were positive for *Brucella pp.*

Also, Dawood (2008): carried out a study on the prevalence of camel brucellosis in Jordan during the years 2006 and 2007. The positive samples were subjected to confirmation by complement fixation test. The true prevalence of *Brucella* seropositive was 15.8%.

1.3. The clinical signs of brucellosis in human:

Brucellosis in human is usually marked by an intermittent or remittent fever accompanied by malaise, anorexia and prostration, and which, in the absence of specific treatment, may persist for weeks or months. Typically, few objective signs are apparent but enlargement of the liver, spleen and/or lymph nodes may occur, as may signs referable to almost any other organ system. The acute phase may progress to a chronic one with relapse, development of persistent localized infection or a non-specific syndrome resembling the “chronic fatigue syndrome” (Franco *et al.*, 2007).

1.4 Global Distribution, Epidemiology and transmission of brucellosis:

Brucellosis is the most common zoonotic infection worldwide with more than 500,000 people diagnosed each year. In livestock, the global disease burden is also immense. The disease is endemic in the Middle East, the Balkan Peninsula, Central Asia, and regions of Africa and Latin America.

1.4.1. Brucellosis in Africa:

Brucellosis is considered endemic in North Africa (Pappas *et al.*, 2006) with several studies recently published on disease prevalence in humans and livestock in Egypt (Holt *et al.*, 2011). The predominant *Brucella* species circulating in Egypt is

B. melitensis which is being responsible of infection in small ruminants, cattle, buffalo, and camels in this country and the *B. melitensis* biovar 3 were identifiable isolates in samples collected in 2007 with prevalence rates determined to be 3.6 - 5.4% in goats, sheep, buffalo, and cattle and (Samaha *et al.*, 2008), whereas Holt *et al.*, (2011) found that prevalence rates in cattle and buffalo were 11%.

Brucellosis has been reported in West Africa (Sanogo *et al.*, 2013), Central Africa (Dean *et al.*, 2012), and East Africa (Megersa *et al.*, 2011, Muendo *et al.*, 2012, Crump *et al.*, 2013, Kunda *et al.*, 2007). On a global scale sub-Saharan Africa thus may have the largest concentration of human and animal *Brucellosis*, a consequence of extensive disease burden and sheer number of people and animals on the continent (Racloz *et al.*, 2013).

In livestock, *Brucellosis* prevalence has been reported to be 10.6%, 2.2%, and 1.9% in cattle, camel, and goats respectively in Ethiopia (Megersa *et al.*, 2011) and 3.8%, 2.3%, and less than 0.5% in cattle, sheep, and goats respectively in Niger (Boukary *et al.*, 2013).

In North Africa, *B. melitensis* predominates even in cattle and buffalo (Samaha *et al.*, 2008). Both *B. melitensis* and *B. abortus* have been isolated from cattle in Kenya (Muendo *et al.*, 2012), while in West Africa and southern Africa *B. abortus* infection of cattle seems to predominate (Sanogo *et al.*, 2013).

The importance of *brucellosis* control, however, was underscored by findings of a study carried out by a group in Kenya. Of over 75 diseases affecting livestock, *Brucellosis* was determined to be one of the 10 most important in terms of impact on impoverished people (Perry, 2002).

1.4.2. Brucellosis in livestock in the Sudan:

Brucellosis is widely distributed in Sudan according to many studies.

Abu Damir *et al.*, (1984) reported that the prevalence rates were 4.9% while Abbas *et al.*, (1987), Bornstein and Musa (1987), Osman and Adlan (1987) found that the prevalence rates were found to be 3.0%, 5.9%, 8% respectively.

Agab (1993) reported that the prevalence rate was 30% whereas Musa and Shigidi (2001) found the prevalence rate of 1.4%. However Omer *et al.*, 2010 reported seroprevalence of 37.5% of *brucellosis* in camels. Solafa *et al.*, 2014 carried out prevalence of *brucellosis* among herds/flocks of cattle, camel, sheep and goats and the results were 76 %, 20%, 13% and 18% respectively.

1.5. Transmission of brucellosis :

1.5.1. Transmission of brucellosis in domesticated ruminants:

Most infections result from ingestion of bacteria either from diseased animals or contaminated feedstuffs. However, infection may also be acquired by respiratory exposure and by contamination of abraded skin and mucosal surfaces. Natural breeding transmits infection in swine and dogs, to a lesser extent, sheep and goats. (Crawford *et al.*, 1990)

In cattle, various routes can transmit *Brucellosis* namely: contact following an abortion, contaminated Pasture, inhalation, conjunctival inoculation, through broken skin contamination or udder inoculation from infected milking cups is also a possibility. Pooled colostrums for feeding newborn calves may also transmit infection. Sexual transmission usually plays little role in the epidemiology of bovine *Brucellosis*. However, artificial insemination can transmit the disease and semen must only be collected from animals known to be free of infection.

In sheep and goats, the mode of transmission of *B. melitensis* is similar to that in cattle but sexual transmission probably plays a greater role. Mixed grazing of flocks and herds belonging to different owners and purchasing animals from unscreened sources facilitated the transmission of the disease. The sharing of male breeding stock also promotes transfer of infection between farms. Transhumance of summer

grazing is a significant promoting factor in some areas as is the mingling of animals at markets (Tsend *et al.*, 2014).

1.5.2. Zoonotic of brucellosis :

Brucellosis is not considered a contagious disease in humans, although rare reports of sexual transmission, in utero infection, and nosocomial infection exists (Meltzer *et al.*, 2010, Mesner *et al.*, 2007). Except in these rare circumstances, *Brucellosis* is contracted via contact with infected animals or animal products. Most cases are caused by *B. melitensis* and *B. abortus*, with *B. suis* is also a highly zoonotic but less widespread. The disease in humans is occasionally caused by *B. canis* and infection by this species may be underreported (Dentinger *et al.*, 2014, Lucero *et al.*, 2010). Infection with marine mammal strains of brucellae has been diagnosed in four individuals (Sohn *et al.*, 2003; McDonald *et al.*, 2006). *Brucellosis* is typically a foodborne or occupational origin. Unpasteurized cow, small ruminant, and camel milk or milk products are most commonly associated with foodborne *Brucellosis*. Brucellae persist in soft cheeses, butter, and ice cream to a greater extent than hard cheeses and yogurt due to the low pH of the later products. If sufficiently cooked, muscle and organ meat from infected animals is not a source of human infection.

In some cultures, raw or partially cooked liver, spleen, and fetuses are consumed, however, these can be heavily contaminated with brucellae (Godfroid *et al.*, 2005). Foodborne exposure is the most common route of infection in travelers as well as in people of endemic countries where milk is not traditionally pasteurized or boiled before consumption. In other endemic areas unpasteurized products are not commonly consumed, and in these cases infection is primarily occupational. Herders/farmers, abattoir workers, leather makers, veterinarians, hunters, and laboratory personnel can be exposed to high disease of brucellae. Infection often occurs via inhalation or through skin lesions.

1.6. Diagnosis of brucellosis:

Diagnostic tests for *brucellosis* fall into two categories: those that demonstrate the presence of the organisms and those that detect an immune response to its antigens.

The isolation and identification of *Brucella* offers a definitive diagnosis of *Brucellosis* and may be useful for epidemiological purposes and to monitor the progress of a vaccination programme in animals.

Concerning the isolation of *Brucella*, not all infected animals give a positive culture and the methods and facilities that must be employed are not always readily available. Also, the detection of antibody or a hypersensitivity reaction provides only a provisional diagnosis. False positive reactions to serological tests can occur through a number of factors, including vaccination. Similarly, dermal hypersensitivity only indicates previous exposure to the organism, not necessarily active infection, and may also result from vaccination (CDC, 2006).

1.6.1. Bacteriological methods of brucellosis:

The isolation and identification of *Brucella* offers a definitive diagnosis of *Brucellosis* and may be useful for epidemiological purposes and to monitor the progress of a vaccination programme.

1.6.1.1. Stained smears examination of brucellosis:

Smears of placental cotyledon, vaginal discharge or fetal stomach contents may be stained using modified Ziehl-Neelsen (Stamp) or Koster's methods. The presence of large aggregates of intracellular, weakly acid-fast organisms with *Brucella* morphology is a presumptive evidence of *Brucellosis*. Care must be taken as other infectious agents such as *Coxiella burnetii* or *Chlamydia* may superficially resemble *Brucella* (Quinn *et al.*, 2002; Poester *et al.*, 2010)

1.6.1.2. Culture examination of brucellosis:

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 vaginal mucus, placenta, fetal stomach contents and milk using suitable selective culture media. It is of the utmost importance that faecal and environmental contamination of the material is kept to a minimum to give the greatest chance of successfully isolating *Brucella*. If other material is unavailable or grossly contaminated, the contents of the fetal stomach will usually be otherwise sterile and are an excellent source of *Brucella*.

In some circumstances it may be appropriate to attempt the isolation of *Brucella* post-mortem. Suitable material includes supramammary, internal iliac and retropharyngeal lymph nodes, udder tissue, testes and gravid uterus.

Milk samples should be allowed to stand overnight at 4 °C before lightly centrifuging. The cream and the deposit are spread on to the surface of at least three plates of solid selective medium. Placental samples should be prepared in the field by selecting the least contaminated portion and cutting off pieces of cotyledon. In the laboratory, the portions should be immersed in alcohol which should be flamed off before cutting with scissors or scalpel and smearing the cut surface on three plates of selective medium. Other solid tissues can be treated in a similar manner, or, ideally, they should be macerated mechanically following flaming before plating out. The tissues may be ground manually or 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.

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.

1.6.2. Serological methods examination of brucellosis:

The detection of specific antibody in serum or milk remains the most practical means of diagnosis of *Brucellosis*. The most efficient and cost-effective method is usually the screening all samples using a cheap and rapid test which is sensitive enough to detect a high proportion of infected animals. Samples positive to screening are then tested using more sophisticated, specific confirmatory tests for the final diagnosis to be made. It is absolutely essential that only internationally recognized tests using antigens standardized against the 2nd International anti-*B. abortus* Serum are used. Appropriate quality control sera should be included with each batch of tests, and tests should be repeated if the quality control criteria are not met.

Serological results must be interpreted against the background of the disease incidence, use of vaccination and the occurrence of false positive reactions due to infection with other organisms. As with all laboratory based diagnosis, it is imperative to correctly identify the “audit trail” of individual animal identity, sample number and test result so that there is complete certainty of the linkage between animal and result.

1.6.2.1. Rose Bengal Plate test (RBT):

The RBPT is one of a group of tests known as the buffered *Brucella* antigen tests which rely on the principle that the ability of IgM antibodies to bind to antigen is markedly reduced at a low pH. The RBPT plays a major role in the serological diagnosis of *Brucellosis* worldwide.

The RBPT is a simple spot agglutination test where 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. The procedure can be automated but this requires custom-made equipment (Godfroid *et al.*, 2010)

1.6.2.2. ELISA tests:

The ELISA tests offer excellent sensitivity and specificity whilst being robust, fairly simple to perform with a minimum of equipment and readily available from a number of commercial sources in kit form. They are more suitable than the CFT for use in smaller laboratories and ELISA technology is now used for diagnosis of a wide range of animal and human diseases. Although in principle ELISAs can be used for the tests of serum from all species of animal and man, results may vary between laboratories depending on the exact methodology used. Not all standardization issues have yet been fully addressed. For screening, the test is generally carried out at a single dilution. It should be noted, however, that although the ELISAs are more sensitive than the RBPT, sometimes they do not detect infected animals which are RBPT positive. It is also important to note that ELISAs are only marginally more specific than RBT or CFT (WHO, 2006).

1.6.2.3. Serum 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 that it should only be used in the absence of alternative techniques (Godfroid *et al.*, 2010).

1.6.2.4. Complement fixation test (CFT):

The sensitivity and specificity of the CFT is good, but it is a complex method to perform requiring good laboratory facilities and trained staff. If these are available and the test is carried out regularly with good attention to quality assurance, then it can be very satisfactory. It is essential to titrate each serum sample because of the occurrence of the prozone phenomenon whereby low dilutions of some sera from

infected animals do not fix complement. This is due to the presence of high levels of non-complement fixing antibody types competing for binding to the antigen.

At higher dilutions these are diluted out and complement is fixed. Such positive samples will be missed if they are only screened at a single dilution. In other cases, contaminating bacteria or other factors in serum samples fix or destroy complement causing a positive reaction in the test, even in the absence of antigen. Such “anti-complementary” reactions make the test void and a CFT result cannot be obtained (Godfroid *et al.*, 2010)

1.6.3. Supplementary tests:

1.6.3.1 Milk testing:

In dairy herds, milk is an ideal medium to test as it is readily and cheaply obtained, tests can be repeated regularly and give a good reflection of serum antibody. Milk from churns or the bulk tank 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 (WHO, 2006).

1.6.3.1.1. Milk ring test:

The milk ring test (MRT) is a simple and effective method, but can only be used with cow's milk. A drop of haematoxylin-stained antigen is mixed with a small volume of milk in a glass or plastic tube. If specific antibody is present in the milk it will bind to the antigen and rise with the cream to form a blue ring at the top of the column of milk. The test is reasonably sensitive but may fail to detect a small number of infected animals within a large herd (WHO, 2006). The same author mentions that non-specific reactions are common with this test, especially in *Brucellosis* free areas. The milk ELISA is far more specific than the MRT.

1.6.3.1.2. 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 (Sekiya, M. *et al.*, 2013)

1.6.4. Fluorescence polarization assay:

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

Evaluation has been limited however, and the procedure is not widely available. Further information is required before its overall value can be assessed.

1.6.5. Intradermal test examination of brucellosis:

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 reactions can occur in vaccinated animals.

1.7. Prevention and control of animal Brucellosis:

The justifications for prevention of the introduction of *Brucellosis* into populations represent in economic benefits and the protection of public health.

1.7.1. Prevention:

The measures of prevention for *Brucellosis*, involve:

Vigilant selection of replacement animals, where should originate from *Brucella*-free herds or flocks, as well as pre-purchase tests are necessary. Isolation of purchased replacements for at least 30 days followed by serological test prior to commingling is necessary.

Prevention of contacts and commingling with herds or flocks of unknown status or those with *Brucellosis* and laboratory assistance should be utilized to diagnose causation of abortions, premature births.

Testing of slaughtered animals with simple screening serological procedures such as the RBPT. And periodic milk ring tests surveillance for herds and flocks in cattle (at least four times per year), and Disinfection of contaminated areas should be performed thoroughly and proper disposal (burial or burning) of placentas and non-viable (OIE, 2006; Wernery, 2014).

1.7.2. Control:

The aim of an animal control programme is to reduce the risk of a disease on human health and the economic consequences. Control programs have an indefinite duration and will need to be maintained even after the “acceptable level” of infection has been reached, so that the disease does not re-emerge (Thrusfield, 2007).

Methods for the control of *Brucellosis* must be planned, monitored and applied by official authorities/legislation. In control Programme certain principles apply, i.e.: the reduction of exposure to *Brucella* spp. and the increase of the resistance to infection of animals in the populations. In another words these procedures represent in isolation/slaughter, hygiene, control of animal movement, vaccination.

1.8. Test and isolation/slaughter:

Serological tests are the usual method of identifying possible infected animals because there are no pathognomonic signs of *Brucellosis* in animals at individual level; one of a strong indicator of infection in naive herds/flocks is the occurrence of abortion storms.

In most cases, test and slaughter of positive animals is only successful in reducing the incidence if the herd or flock prevalence is very low (e.g. 2%).

The immediate slaughter of test-positive animals is expensive and requires animal owner cooperation. Compensation is usually necessary. Furthermore, the application of test and slaughter policies is unlikely to be successful with *Brucellosis* of sheep and goats where the diagnostic tests are less reliable than in

cattle. Test and slaughter is also unlikely to be successful in cattle if the remainder of the herd is unvaccinated, especially in large populations. Repeated herd or flock tests are necessary to further reduce the incidence of *Brucellosis* and to confirm elimination.

1.9. Hygiene:

Owners are often poorly informed about disease transmission and recommendations, so the classical procedure in disease control such as the methods of animal husbandry, patterns of commerce, prevalence of clinical signs, type of facilities, and degree of dedication of the owners of animals, is the goal in the application of hygienic methods to the control of *Brucellosis*.

Antibiotic treatment of known infected animals, or of those which are potentially exposed to them, has not been commonly used and it should be ruled out as an option in the control of *Brucellosis* (CDC, 2006).

1.10. Control of animal movement:

In practice, it is much more difficult to control the movement of camels and small ruminants kept under nomadic or semi-nomadic conditions than that of beef or dairy cattle kept under intensive conditions. The owners of herds and flocks may be accustomed to seasonal migrations which may cross national boundaries, but it is necessary in any programme to bound the spread of *Brucellosis*. Unauthorized sale or movement of animals from an infected area to other areas should be forbidden. As well as importations into clean areas must be restricted to animals that originate from *Brucellosis*-free areas, that have a herd/flock history of freedom from the disease and that have given negative reactions to recently performed diagnostic tests (OIE, 2006).

1.11. Vaccination:

Vaccination of animals usually results in elimination of clinical disease and the reduction in numbers of organisms excreted by animals which become infected.

Furthermore, animal owners are more likely to accept vaccination as a method of control since they are accustomed to this form of disease control. In many countries, vaccination is the only practical and economical means of control of animal *Brucellosis*.

There is general agreement that the most successful method for prevention and control of *Brucellosis* in animals is through vaccination. While the ideal vaccine does not exist, the attenuated strains of *B. melitensis* strain Rev.1 for sheep and goats and *B. abortus* strain 19 have proven to be superior to all others (Elberg and Faunce., 1957). The non-agglutinogenic *B. abortus* strain RB51 has been used in the USA and some Latin American countries, with encouraging results. The source and quality of the vaccines are critical. The dosages and methods of administration, especially with Rev.1, vary and these can affect the results.

Consequently, whole herd or flock vaccination can only be recommended when all other control measures have failed. When applied, the vaccinated animals must be identified by indelible marking and continually monitored for abortions resulting from the vaccine. Positive serological reactors and secretors must be removed from the herd on detection. It is often recommended that vaccination with strains 19 and Rev.1 should be limited to sexually immature female animals. This is to minimize stimulation of postvaccinal antibodies which may confuse the interpretation of diagnostic tests and also to prevent possible abortions induced by the vaccines. However, field and laboratory studies have demonstrated that conjunctival administration of these vaccines makes the vaccination of the herd or flock a practical and effective procedure. Rapid herd immunity is developed and application costs are minimized. The lowered dose results in lower antibody titres and these recede rapidly. Several diagnostic tests have been developed which are useful in differentiating antibody classes. Of these, the complement fixation test and ELISA are currently the most widely used (WHO/CDS/EPR/2006).

1.12. Eradication:

Eradication means the elimination of a pathogenic agent from a country or a zone. A highly organized effort is needed to reach eradication in either a territory and in a population. Eradication is conceptually very different from control, it's based on sanitary measures and on an organization of activities completely different from those implemented for a control programme (Thrusfield, 2007).

Crucial factors for the success of an eradication programme are the implementation of an effective surveillance system with adequate laboratory support, and the understanding and sharing of objectives for eradication by the decision-makers, farmers, and all other stakeholders. To keep an unaffected population free from an infection, prevention measures must be implemented to segregate an infectious organism from a geographical area and its human and animal populations. Adequate knowledge of the local human and animal populations and of the territory is essential (WHO, 2006).

On a long term basis, eradication programmes in general are more economically advantageous compared to control programmes. There is also little doubt that very often failures of control and eradication efforts are due to the absence of an adequate epidemiological surveillance system sustaining both technical and political decision-making (OIE, WHO, CDC, 2006).

1.13. Surveillance of brucellosis in animals:

The unit of reference for animal surveillance is usually the infected herd or flock rather than the individual animal.

Data from diagnostic laboratory findings could be used as an animal *Brucellosis* surveillance system as well as outbreak/case investigations and slaughterhouse or animal marketing tests, or specially commissioned local or national surveys. These data can be used to ascertain flock or herd prevalence of a given population or area, and in infected flocks or herds, the prevalence of the disease in the flock or

herd and to determine the incidence. An important use of incidence data is the evaluation of efforts to achieve control or elimination (OIE, 2006).

1.14. Intersectoral collaboration:

The zoonotic nature of *Brucellosis* necessitates close interaction between the public health authorities and the veterinary authorities; this collaboration is only the first step in establishing an effective control Programme. For a successful outcome, all sections of the community need to be involved in the process and to lend their support (OIE, 2006).

MATERIALS AND METHODS

2.1. The study area:

North Kordofan lies in the arid and semi-arid zones between latitude 11.15-16.45° N and longitude 27-32.15° E. It borders the Northern state in the north, Northern and Southern Darfur states in the west. West and South Kordofan states in the south, and The White Nile and Khartoum states in the east. Soil types are about 55% sand or gouze, 20% gerdud, 15% alluvial land and 10% clay land (Abdallah *et al.*, 2012).

2.2. Design of the study:

Cross sectional study was done for prevalence determination. Sample size was calculated according to the formula described by Thursfield (2007) based on previous prevalence (%) of *camelbrucellosis* in the Sudan reported by 200 sample with 95% confidence interval and 5% desired absolute precision. To carry out this survey multistage random sampling was used.

2.3. Sample collection and Questionnaire survey:

A total of 230 serum samples were collected from individual animals which selected randomly. These samples were kept on ice container and transported as soon as possible to the ElObied Veterinary Research Laboratory.

A questionnaire was designed to provide information about potential risk factors hypothesized to be associated with *brucella* in camel. The questionnaire included information about age, sex, locality, body condition score and breed of each camel sampled.

2.4. Buffered acidified plate antigen :

The test is prescribed by the OIE for international trade. It's a quick easy presumptive test to start with in order to exclude negative samples from further serological testing. it's a secondary binding qualitative plate agglutination test that uses a colored acidified antigen (pH3.8) to inhibit non-specific reactions due to IgM and enhance the agglutination ability of specific IgG1.

2.4.1. Material needed for the test are:

Standard BAPA test antigen, Control sera (negative, low positive and high positive)., Adjustable pippete, with disposable tips ,Minnesota testing box with glass plate, (illuminator with indirect light source, black back ground, and lid to prevent evaporation of test materials, Stirrer \spreader and Paper towel

2.4.2. The tests procedures:

The samples and antigen were allowed to come to room temperature. 20,40, and 80 μ l of each sample were measured on the center of the glass plate of the Minnesota testing box. Known high positive, control was included in each day's work. 30 μ l of BAPA antigen was added to each quantity of serum mixing the antigen bottle thoroughly by gentle shaking and inversion to ensure a homogenous suspension. The sample and antigen were mixed thoroughly using a stirrer enlarging the circle of the mixture to about 2cm in diameter. (the Spreaders was rinsed in water and wiped dry between samples). The glass plate was tilted in a circular motion for 4 rotations and were left for 4 min in the Minnesota box with the lid covered and were not switched on . The test was waited until reading. Rotated 4 times again, incubated for another 4 min in the box and finally rotated 4 further rotations.

2.4.3. Reading of the results:

The reactions were read immediately against the illuminated background of the Minnesota box. Any visible agglutination within 8 minutes was considered positive. No agglutination within 8 min was negative.

Results	Interpretation
No agglutination	-ve
agglutination	+ve

(CVRL, 2014)

2.5. Rose Bengal plate test:

The simple rapid test that prescribed by the OIE for international trade and control campaigns.

2.5.1. Materials:

Rose bengal antigen, Positive and negative control serum, Glass plate, polyethylene plate or enamel, Stirring sticks or tooth picks.

The serum samples were brought a room temperature.

2.5.2. The test procedure:

Equal volumes (20 µl) of RBPT colored antigen and the test serum were mixed on a clean glass slide with the help of a clean sterile

Shake for 4 minutes and read immediately.

2.5.3. Reading of the results:

No agglutination	-ve
agglutination	+ve

(CVRL, 2014)

2.6. Data management and analysis:

The data were entered into Excel spread sheets, statistical analysis of collected data were carried out with the computer application SPSS version 20 by using

Pearson's chi-square and to test total prevalence and the existence of differences in prevalence between risk factors.

RESULTS

Overall prevalence of brucellosis in camels by the RBPT was 51 of 230 (22.2%) serum samples positive by the RBPT (51), by the PABA were subjected to further confirmation using PABA were found positive with an overall prevalence 14.8% .

3.1. Analysis of risk factors:

3.1.1. Age factor:

The result of a different age groups of camels positive for brucellosis, 12 out of 84 (14.3%) young camel, 18 out of 118 (15.3%) adult camel and 4 out of 28 (14.3%) old camels. By the Chi-squared test, the result showed that there was no association between *camel brucellosis* and the age of animal ($\chi^2 = 0.043$; $P = 0.979$) (Table.3)

3.1.2. Sex factor:

Male animals had higher prevalence of 23 of 123 (18.7%) than the females 11 of 107 (10.3%).

There was no significant relationship in *brucella* infection between male and female ($\chi^2 = 3.219$; $P = 0.07$) (table.3)

3.1.3. District factor:

The highest prevalence reported in, Sheikan, Bara and Umkredem was followed 15 of 82 (18.3%), 13 of 74 (17.6%) and 6 of 36 (9.5%), respectively. However, camels in umrowaba were negative for brucellosis .No association was found between *brucella* infection and the origin camels ($\chi^2 = 4.549$; $P = 0.20$) (table.3).

3.1.4. Breed factor:

The prevalence of *camel brucellosis* in camels in North Kordofan state was 20 of 183 (10.9%), and 14 of 47 (29.8%) from Darfur breed were positive.

The Chi square test showed there was significant association between *camel brucellosis* infection and breed ($X^2 = 10.557$; $p\text{-value} = 0.001$) (Table.3)

3.1.5. Body condition score factor:

Regarding body condition score (BCS), 1 of 24(4.2%) camels of poor body condition, 14 of 102(13.7%) camels of moderate body condition and 19 of 104 (18.3%) camels good body condition were positive for brucellosis.

No statistical significant association was observed between categories of body condition and camel brucellosis ($\chi^2 = 3.241$; $P = 0.19$) (Table.3).

3.1.6. Grazing factor:

In relation to grazing, 27 of 178 (15.2 %) camels from mixed grazing were positive and 7 of 52 (13.5%) were positive for camel *brucellosis*. No association was observed between the grazing factor and camel *brucellosis* ($\chi^2 = 0.093$; $P = 0.76$) (Table.3).

Table: 2: Summary of univariate analysis for risk factors associated with camel brucellosis in North Kordofan State, (n=230) using the Chi-squared test. (RBPT test)

Risk factor	No. tested	No. positive (%)	Df	x²	p- value
Age					
Young	84	16(19.0)	2	0.879	0.64
Adult	118	29(24.6)			
Old	28	6 (21.4)			
Sex					
Female	107	21 (19.6)	1	0.753	0.38
Male	123	30 (24.4)			
Locality					
Sheikan	82	26 (31.7)	3	9.737	0.021*
Umrowaba	11	-			
Bara	74	16 (21.6)			
Umkredem	63	9(14.3)			
Grazing					
Mixed	178	35(19.7)	1	2.877	0.09
Non mixed	52	16(30.8)			
BCS					
Poor	24	3(12.5)	1	4.010	0.13
Moderate	102	19 (18.6)			
Good	104	29 (27.9)			
Breed					
Alarabi	183	36(19.7)	1	3.248	0.07
Darfour	47	15 (31.9)			

* = highly significant

Table: 3: Summary of univariate analysis for risk factors associated with camel brucellosis North Kordofan State, Sudan (n=230) using the Chi-squared test. (BAPA test)

Risk factor	No. tested	No. positive (%)	Df	x²	p- value
Age					
Young	84	12(14.3)	2	0.043	0.979
Adult	118	18(15.3)			
Old	28	4 (14.3)			
Sex					
Female	107	11 (10.3)	1	3.219	0.07
Male	123	23 (18.7)			
Locality					
Sheikan	82	15 (18.3)	3	4.549	0.20
Umrowaba	-	-			
Bara	74	13 (17.6)			
Umkredem	36	6(9.5)			
Grazing					
Mixed	178	27(15.2)	1	0.093	0.76
Non mixed	52	7(13.5)			
BCS					
Poor	24	1(4.2)	2	3.241	0.19
Moderate	102	14 (13.7)			
Good	104	19 (18.3)			
Breed					
Alarabi	183	20(10.9)	1	10.557	0.001*
Darfour	47	14 (29.8)			

* = highly significant

DISCUSSION

Despite the advances made in surveillance and control, the prevalence of *brucellosis* is increasing in many developing countries due to various sanitary, socioeconomic, and political factors (Pappas *et al.*, 2006). In camel, *brucellosis* is common and its prevalence is higher in intensive camel production systems (Abbas and Agab, 2002). The disease circulates in different species of animals including camels due to mixed herding practices, (Al-Majali *et al.*, 2008).

In this study the prevalence of the diseases by two serological camels using the RBPT was 22.2%, while it was 14.8% by use PABA. The RBPT is widely used in Sudan for *brucella* screening for regulatory control and for export requirements. Although the test is very sensitive and is suitable for screening herds, it can give false positive results due to vaccination with *B. abortus* strain 19 vaccine or for cross reactions with other bacteria (OIE, 2004).

Several factors were investigated as potential risk factors at individual level. Those included: locality, age, sex, BCS, breed and grazing. The questionnaire-based information collected during this study indicated that several factors could be considered as potential risk factors for the disease.

The prevalence of Brucellosis in this study was almost similar to that reported by Musa *et al* (2008), who reported a prevalence of 23.8% in camels kept with other ruminant species. Solafa Zein El abdin *et al.*,(2014) reported 20% prevalence in Jabel Aolia Locality. Dawood (2008) reported 19.4% prevalence in Jordan. However El-boshy (2009) in Egypt reported(7.3%) prevalence of brucellosis. Zewold and Haileselassie (2012) examined 768 camel serum samples for *brucellosis* and found 11.9% positive reactors by the RBPT. The differences in the prevalence of *brucella* in camels from different countries may be attributed to different husbandry and management practices.

According to the this study, higher seropositivity was recorded in camels with good BCS (18.3%) followed by moderate (13.7%) and poor (4.2%). This could be attributed to variation in sample sizes. In the present study, the prevalence of *brucellosis* was evident in the male more than female animals, and this contradicted with the result of Bayemi *et al.*, (2009). Females are generally kept for longer period of time than males and this is likely the cause of increased opportunity for exposure to brucella (Mekonnen *et al.*, 2010). Relatively higher vulnerability of female animals could also be that females are more physiologically nervous than male animals (Walker, 1999).

Brucellosis infection may occur in camels of all ages but more persistent in sexually mature animals (Abubakar *et al.*, 2010). The study reveals higher infection rate in adults (15.3%) followed by young and old with prevalence 14.3% in both. Similarly, younger animals have a tendency to be resistant to *brucellosis* and frequently clear infections although latent infections may occur. This can also be attributed to sex hormones that have a propensity to increase in concentration with age and sexual maturity and promote growth and multiplication of *Brucella*.

The camels in Sheikan locality were more seropositive (18.3%) than the camels in the other districts. This may be attributed to the close contact of livestock species, lack of herd health program, disorganized management system, frequent induction of high yielding animals without quarantine, higher population density of livestock and shared grazing and marketing along with poor management practices adopted narrow locality comparing to the rest locality.

In the present study, the animals in mixed grazing had higher prevalence rate (15.2 %) of *brucellosis* than non-mixed grazing (13.5%). It was well documented that the disease is transmitted between species (Dawood, 2008) and these findings are in accord with previous reports of higher prevalence levels in camels kept along with large and small ruminants (Abou-Eisha, 2000; Al-Majali *et al.*, 2008).

CONCLUSION

Based on the result an overall prevalence of *brucellosis* in camel infection in North Kordofan state by RBPT was 22.2% while by PABA test is 14.8.

Also the study showed high prevalence of *brucellosis* in camels in male than female.

Furthermore, the highest prevalence was recorded in Sheikan, Bara and Umkredem.

A significant correlation was observed between the *brucellosis* in camel and breed factor in this study.

Recommendation

- A study should be conducted for a longer period of time to confirm more facts.
- More studies should be done on *brucellosis* in different animals in different states to determine the rate of spread of disease.

REFERENCE

- Abbas, B.; Yassin T.T.M. and Elzubir A.E.A. (1987).** – Survey for certain zoonotic diseases in camels in the Sudan. *Rev. Elev. Méd. vét. Pays trop.*, 40 (3), 231–233.
- Abd-Elmajid, A. A. (2000).** "The one humped camels in the sudan" 1 edition ACSAD, Damascus.
- Abubakar, M.; Arshed, M.J.; Hussain, M.; Ehtisham–ul-Haq and Ali, Q. (2010).** Serological evidence of *Brucella abortus* prevalence in Punjab province, Pakistan a cross-sectional study. *Transboundry Emerg Dis.* 57(6): 443-447.
- Ali, R.M.E.; Ahmed, S.H.; Elbushra, A.A.; Elobied, H.A. (2017).** Camels Production in Sudan: Impact on the Food Security and Circumstances.
- Abou-Eisha, M.J. (2000).** Brucellosis in camels and its relation to public health. *Assiut Vet Med J.*, 44(87), 54-64.
- Abu Damir H., Kenyon S.J., Khalafalla A.E. and Idris O.F. (1984).** – *Brucella* antibodies in Sudanese camels. *Trop. anim. HlthProd.*, 16, 209–212.
- Agab, H.R.D. (1993).** *Epidemiology of camel diseases in Eastern Sudan with emphasis on brucellosis.* M.V.Sc. Thesis. University of Khartoum, Sudan.
- Alton, G. G., (1990):** *Brucellamelitensis*. In: K. Nielsen and J. R. Duncan (eds.), *Animal Brucellosis.* CRC Press, Boca Raton, Florida.
- Alton, G. G., (1990):** *Brucellamelitensis*, 1887 to 1987. In: K. Nielsen and J. R. Duncan (eds.), *Animal brucellosis.* CRC Press, Boca Raton.
- Bornstein S. and Musa, B.E. (1987).** Prevalence of antibodies to some viral pathogens, *Brucella abortus* and *Toxoplasma gondii* in serum from camels (*Camelus dromedarius*) in Sudan. *J. vet. Med., B*, 34, 364–370.

- Bayemi, P.H, Webb, E.C., Nsongka, M.V., Unger, H. and Njakoi, H. (2009).** Prevalence of *Brucella abortus* antibodies in serum of Holstein cattle in Cameroon. *Trop Anim Health Prod.* 41(2): 41-144.
- Buddle, M. B., (1956):** Studies on *Brucella ovis* (n. sp.), a Cause of Genital Disease of Sheep in New Zealand and Australia. *The Journal of Hygiene*, 54, 351-364.
- Burgess, G. W., Spencer, T. L. and Norris, M. J. (1985):** Experimental infection of goats with *Brucella ovis*. *Aust Vet J*, 62, 262-264.
- CDC:(2006),** Center for disease control
- CVRL: (2014),**Central veterinary research laboratory, animal researches resources corporation, Sudan
- Cassle, S. E., Jensen, E. D., Smith, C. R., Meegan, J. M., Johnson, S. P., Lutmerding, B., Ridgway, S. H. and Francis-Floyd, R. (2013):** Diagnosis and successful treatment of a lung abscess associated with *Brucella* species infection in a bottlenose dolphin (*Tursiops truncatus*). *Journal of zoo and wildlife medicine: official publication of the American Association of Zoo Veterinarians*, 44, 495-499.
- Crawford, R. P., Huber, J. D. and Adams, B. S. (1990):** Epidemiology and Surveillance. In: K. Nielsen and Duncan, J. R. (eds.), *Animal brucellosis*. CRC Press, Boca Raton.
- Crump, J. A., Morrissey, A. B., Nicholson, W. L., Massung, R. F., Stoddard, R. A., Galloway, R. L., Ooi, E. E., Maro, V. P., Saganda, W., Kinabo, G. D., Muiruri, C. and Bartlett, J. A. (2013):** Etiology of severe non-malaria febrile illness in Northern Tanzania: a prospective cohort study. *PLoS neglected tropical diseases*, 7,e2324.
- Dawood, A.H. (2008).***Brucellosis* in Camels (*Camelus dromedarius*) in the south province of Jordan. *Am J Agric Biol Sci.* 3(3): 623-626.

Dalrymple-Champneys, W., (1950): Undulant Fever A Neglected Problem. The Lancet, 255, 477-485.

Dentinger, C. M., Jacob, K., Lee, L. V., Mendez, H., A Chotikanatis, K., McDonough, P., LChico, D. M., De, B., KTiller, R.V., Traxler, R., MCampagnolo, E. R., Schmitt, D., Guerra, M. A. and Slavinski, S. A. (2014): Human *Brucella canis* Infection and Subsequent Laboratory Exposures Associated with a Puppy, New York City,2012. Zoonoses and public health.

De, B. K., Stauffer, L., Koylass, M. S., Sharp, S. E., Gee, J. E., Helsel, L. O., Steigerwalt, A. G., Vega, R., Clark, T. A., Daneshvar, M. I. , Wilkins, P. P. and Whatmore, A. M. (2008): Novel *Brucella* strain (BO1) associated with a prosthetic breast implant infection. *Journal of clinical microbiology*, 46, 43-49.

Dean, A. S., Crump, L., Greter, H., Schelling, E. and Zinsstag, J. (2012): Global burden of human *brucellosis*: a systematic review of disease frequency. PLoS neglected tropical diseases, 6, e1865.

Elberg, S. S. and Faunce, K. (1957): Immunization against *Brucella* infection. VI. Immunity conferred on goats by a nondependent mutant from a streptomycin-dependent mutant strain of *Brucellamelitensis*. *Journal of bacteriology*, 73, 211-217.

FAO,(2011).Food and Agriculture Organization of databases. (<http://faostat.fao.org/site>).

Faham, K., Ebrahim, R., Amir, S, Abbas, D, Hassan, M.,(2014): Molecular study of the prevalence of *BrucellaAbortus* and *brucellamelitensis* in the blood and lymphnode samples of slaughtered camels by polymerase chain reaction (PCR) in Iran :Acta Veterinaria-Beograd 2014, 64 (2), 245-256 UDK: 616.988:579.842 ; 579.842:577.2, DOI: 10.2478/acve-2014-0023.

Foster, G., B., Osterman, S., Godfroid, J., Jacques, I. and Cloeckaert, A. (2007): *Brucellaceti* sp. nov. and *Brucellapinnipedialis* sp. nov. for *Brucella* strains

with cetaceans and seals as their preferred hosts. *International journal of systematic and evolutionary microbiology*, 57, 2688-2693.

Franco, M. P., Mulder, M., Gilman, R. H. and Smits, H. L (2007): Human brucellosis. *The Lancet. Infectious diseases*,7, 775-786.

Gee, J. E., B. K. De, P. N. Levett, A. M. Whitney, R. T. Novak and T. Popovic, 2004: Use of 16S rRNA gene sequencing for rapid confirmatory identification of *Brucella* isolates. *Journal of clinical microbiology*, 42,3649-3654.

Godfroid, J., Nielsen, K. and Saegerman, C. (2010): Diagnosis of brucellosis in livestock and wildlife. *Croat Med J*,51, 296-305.

Godfroid, J., H. C., Scholz, T., Barbier, C., Nicolas, P., Wattiau, D., Fretin, A. M., Whatmore, A., Cloeckert, J., M.Blasco, I., Moriyon, C., Saegerman, J. B., Muma, S., Al Dahouk, H., Neubauer and J. J. Letesson, (2011):*Brucellosis* at the animal/ecosystem/human interface at the beginning of the 21st century. *Preventive veterinary medicine*, 102, 118-131.

Gwida, M., El-Gohary, A., Melzer, F. , Khan, I., Rosler, U. and Neubauer, H. (2012):*Brucellosis* in camels. *Research in veterinary science*, 92, 351-355.

Hartley, W. J., Jebson, J. L. and Macfarlane, D. (1955): Some observations on natural transmission of ovine brucellosis *N. Zeal. Vet. J.*, 3, 5-10.

Hernandez-Mora, G., Gonzalez-Barrientos, R., Morales, J. A., Chaves-Olarte, E., Guzman-Verri, C., Barquero-Calvo, E., De-Miguel, M. J., Marin, C. M., Blasco, J. M. and Moreno, E. (2008): Neurobrucellosis in stranded dolphins, Costa Rica. *Emerging infectious diseases*, 14, 1430-1433.

Holt, H. R., Eltholth, M. M. , Hegazy, Y. M., El-Tras, W. F., Tayel, A. A. and Guitian, J. (2011):*Brucella* spp. infection in large ruminants in an endemic area of Egypt: cross-sectional study investigating seroprevalence, risk factors and livestock owner's knowledge, attitudes and practices (KAPs). *BMC public health*, 11, 341.

Hosein, H., I, Rouby, S, Menshawy, Ghazy, A, (2016): Seroprevalence of camel *brucellosis* and molecular characterization of *Brucellamelitensis* recovered from dromedary camels in Egypt. *Res. J. Vet. Pract.* 4(1): 17-24.

Huddleson, I. F., (1931): Differentiation of the Species of the Genus *Brucella*. *American Journal of Public Health and the Nations Health*, 21, 491-498.

Kunda, J., Fitzpatrick, J., Kazwala, R., French, N. P., Shirima, G. , Macmillan, A., Kambarage, D., Bronsvort, M. and Cleaveland, S. (2007): Health-seeking behaviour of human brucellosis cases in rural Tanzania. *BMC public health*, 7, 315.

Megersa, B., Biffa, D., Abunna, F., Regassa, A., Godfroid, J. and Skjerve, E. (2011): Seroprevalence of brucellosis and its contribution to abortion in cattle, camel, and goat kept under pastoral management in Borana, Ethiopia. *Tropical animal health and production*, 43, 651-656.

Mohamed, E. , G., S., Elfadi, A.,A., M., and El Sanousi, E.,M. (2015): Epidemiological study of brucellosis in camels (*Camelusdromedarius*) in Khartoum State, Sudan. *Inter J Vet Sci*, 4(1): 39-43. www.ijvets.com.

Musa M.T. & Shigidi M.T.A. (2001). – Brucellosis in camels in intensive animal breeding areas of Sudan. Implications in abortion and early-life infections. *Rev. Elev. Méd. vét. Pays trop.*, 54 (1), 11–15.

Muendo, E. N., Mbatha, P. M., Macharia, J.,Abdoel, T. H., Janszen, P. V. , Pastoor, R. and Smits, H. L. (2012): Infection of cattle in Kenya with *Brucellaabortus* biovar 3 and *Brucellamelitensis* biovar 1 genotypes. *Tropical animal health and production*, 44, 17-20.

Nymo, I. H., Tryland , M. and Godfroid, J. (2011): A review of *Brucella* infection in marine mammals, with special emphasis on *Brucellapinnipedialis* in the hooded seal (*Cystophoracristata*). *Veterinary research*, 42, 93.

OIE, 2016 Terrestrial Manual Chapter 2.1.4 Brucellosis.

Osman A.M. & Adlan A.M. (1987). – Sudan. In *Brucellosis* in domestic animals: prevalence, diagnosis and control. Tech. series Off. int. Epiz., 6, 67–72.

Omer, M.M., Musa, M.T., Bakhiet, M.R. & Perret, L. (2010). – *Brucellosis* in camels, cattle and humans: associations and evaluation of serological tests used for diagnosis of the disease in certain nomadic localities in Sudan. Rev. sci. tech. Off. int. Epiz., 29 (3), 663–669

Ohishi, K., Zenitani, R., Bando, T., Goto, Y., Uchida, K., Maruyama, T., Yamamoto, S., Miyazaki, N. and Fujise, Y. (2003): Pathological and serological evidence of *Brucella*-infection in baleen whales (*Mysticeti*) in the western North Pacific. Comparative immunology, microbiology and infectious diseases, 26, 125-136.

Ogredici, O., Erb, S., Langer, I. Pilo, P. Kerner, A., Haack, H. G., Cathomas, G., Danuser, J. , Pappas, G. and Tarr, P. E. (2010):*Brucellosis* reactivation after 28 years. Emerging infectious diseases, 16, 2021-2022.

Osterman, B. and Moriyón, I (2006): International Committee on Systematics of Prokaryotes; Subcommittee on the taxonomy of *Brucella* : Minutes of the meeting, 17 September 2003, Pamplona, Spain. *International journal of systematic and evolutionary microbiology*, 56, 1173-1175.

Pappas, G., P. Papadimitriou, N. Akritidis, L. Christou and E. V. Tsianos, (2006): The new global map of human brucellosis. The Lancet. Infectious diseases, 6, 91-99.

Perry, B. R., (2002): Animal disease impact on the poor: study results. Investing in Animal Research to Alleviate Poverty. International Livestock Research Institute, Nairobi, Kenya.

Perry, Q. L., Hagius, S. D., Walker, J. V. and Elzer, P. H. (2010): Evaluating the virulence of a *Brucellamelitensis* hemagglutinin gene in the caprine model. *Vaccine*, 28 Suppl 5, F6-11.

Poester, F. P., Goncalves, V. S. , Paixao, T. A., Santos, R. L., Olsen, S. C., Schurig, G. G. and Lage, A. P. (2006):Efficacy of strain RB51 vaccine in heifers against experimental *brucellosis*. *Vaccine*, 24, 5327-5334.

Poester, FP, Nielsen, K., Samartino, LE, Yu, W.L. (2010). Diagnosis of *Brucellosis*. *Open Vet. Sci. J.*, 4:46.

Quinn, P.J., Markey, B.K., Carter, M.E., Donnelly, W.J. and Leonard F.C. (2002). *Clinical Veterinary Microbiology*. Harcourt Publishers Limited, Edinburgh, London. pp. 1-648.

Racloz, V., Schelling, E. , Chitnis, N.,Roth, F. and Zinsstag, J. (2013): Persistence of brucellosis in pastoral systems.*Revue scientifique et technique* (International Office of Epizootics), 32, 61-70.

Radostits, O.M., Gay, C.C., Blood, D.C., and Hinchcliff, K.W., (2007). *Veterinary Medicine*. 9th Ed., ELBS Bailliere Tindall, London, UK. pp. 870-871.

Ridler, A. L., West, D. M., Stafford, K. J., Wilson, P. R. and Fenwick, S. G (2000): Transmission of *Brucellaovis* from rams to red deer stags. *New Zealand veterinary journal*, 48, 57-59.

Rittig, M. G., A., Kaufmann, A., Robins, B., Shaw, H., Sprenger, D., Gemsa, V., Foulongne, B., and Dornand, J. (2003): Smooth and rough lipopolysaccharide phenotypes of *Brucella* induce different intracellular trafficking and cytokine/chemokine release in human monocytes. *Journal of leukocyte biology*, 74, 1045-1055.

Sanogo, M., Abatih, E., Thys, E., Fretin, D. Berkvens, D. and Saegerman, C. (2013): Importance of identification and typing of *Brucellae* from West African cattle: a review. *Veterinary microbiology*, 164, 202-211.

Samaha, H., Al-Rowaily, M., Khoudair, R. M. and Ashour, H. M. (2008): Multicenter study of *brucellosis* in Egypt. *Emerging infectious diseases*, 14, 1916-1918.

Sekiya, M., Zintl, A. and Doherty, M.L. Bulk (2013): milk ELISA and the diagnosis of parasite infections in dairy herds: a review. *Ir Vet J* 66, 14 (2013). <https://doi.org/10.1186/2046-0481-66-14>.

Solafa Zein El abdin, Tamador-ElkhansaaElnourAngara, AbdElhameed Ahmed Elfadil, Enaam Mohammed El Sanousi, Abdella Mohamed Ibrahim, Prevalence and Risk Factors of Ruminants *Brucellosis* in Jabel Aolia Locality, Sudan, *Sudan Journal of Science and Technology* (2014) 15(2): 60-72. *Journal homepage:* <http://jst.sustech.edu/>

Scholz, H. C., Al Dahouk, S., Tomaso, H., Neubauer, H., Witte, A., Schloter, M., Kampfer, P., Falsen, E., Pfeffer, M. and Engel, M. (2008): Genetic diversity and phylogenetic relationships of bacteria belonging to the Ochrobactrum-Brucella group by recA and 16S rRNA gene-based comparative sequence analysis. *Systematic and applied microbiology*, 31, 1-16.

Scholz, H. C., Nockler, K., Gollner, C., Bahn, P., Vergnaud, G., Tomaso, H., Al Dahouk, S., Kampfer, P., Cloeckert, A., Maquart, M., Zygmunt, M. S., Whatmore, A. M., Pfeffer, M., Huber, B., Busse, H. J. and De, B. K. (2010): *Brucellainopinata* sp. nov., isolated from a breast implant infection. *International journal of systematic and evolutionary microbiology*, 60, 801-808.

World health organization (WHO) (2006). *Brucellosis* in humans and animals. WHO/CDS/EPR/2006.7, p 1 – 102.

Schlabritz-Loutsevitch, N. E., Whatmore, A. M., Quance, C. R., Koylass, M. S, Cummins, L. B., Snider, C. L., Cappelli, D, Ebersole, J. L., Nathanielsz, P. W. and Hubbard, G. B. (2009): A novel *Brucella* isolate in association with two

cases of stillbirth in non-human primates - first report. *Journal of medical primatology*, 38, 70-73.

Tittarelli, M., Di, M., Ventura, De Massis, F., Scacchia, M., Giovannini, A., Nannini, D. and Caporale, V. (2005): The persistence of *Brucellamelitensis* in experimentally infected ewes through three reproductive cycles. *J Vet Med B Infect Dis Vet Public Health*, 52, 403-409.

Stoenner, H. G. and Lackman, D. B. (1957): A new species of *Brucella* isolated from the desert wood rat, *Neotomalepida* Thomas. *American journal of veterinary research*, 18, 947-951.

Tsend, S., Baljinnyam, Z., Suuri, B., Dashbal, E., Oidov, B., Roth, F., Zinstag, J., Schelling, E. and Dambadarjaa, D.(2014): Seroprevalence survey of *brucellosis* among rural people in Mongolia. *Western Pacific surveillance and response journal* : WPSAR, 5, 13-20.

Van Metre, D., Rao, C., S. Kimberling, C. V. and Morley, P. S. (2012): Factors associated with failure in breeding soundness examination of Western USA rams. *Preventive veterinary medicine*, 105, 118-126.

Verger, J.M., Grimont, F., Grimont, P. A. and Grayon, M. (1985):*Brucella*, a Monospecific Genus as Shown by Deoxyribonucleic Acid Hybridization. *International Journal of Systematic Bacteriology*, 35, 292-295.

Whatmore, A. M., Davison, N., Cloeckert, A., Al Dahouk, S., Zygmunt, M. S., Brew, S. D., Perrett, L. L., Koylass, M. S., Vergnaud, G., Quance, C., Scholz, H. C., Dick, E. J., Hubbard, Jr., G. and Schlabritz-Loutsevitch, N. E. (2014):*Brucellapapionis* sp. nov. isolated from baboons (*Papio* spp.). *International journal of systematic and evolutionary microbiology*.

WHO/CDS/EPR/2006.*Brucellosis* in humans and animals,

Walker, R.L. (1999). 'Brucella', In Dwright CH and Chunge ZY (eds.), *Vet Microbiol*. Blackwell Science, Massachusetts. pp. 196-203.

Wernery, U. (2014): Camelid *brucellosis*: a review: Central Veterinary Research Laboratory, P.O. Box 597, Dubai, United Arab Emirates E-mail: cvrl@cvrl.ae, Rev. sci. tech. Off. int. Epiz., 2014, 33 (3), 839-857