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Assessment of Serological Tests Used for Diagnosis of Brucellosis in Camels and Goats Intended for Exportation in Sudan

تقييم الاختبارات المصليه المستخدمه لتشخيص الحمي المالطيه فى الابل والماعز المعده للتصدير فى السودان

A Thesis Submitted in Fulfillment of the Requirement for the Degree of Doctor of Philosophy in Veterinary Medicine (Microbiology)

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DEDICATION

I dedicate this work to my beloved parents Soul with love, brother, Sister My Son Abdella And my husband who always supports me

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ABSTRACT

Prevalence of brucellosis in camels and goats in the Sudan is high and the disease affects their productivity and is a main constraint to their export to the Arabian Peninsula. This study was conducted from September 2017 to September 2020. to compare between the serological tests, Rose Bengal Plate test (RBPT), Buffered Acidified Plate Antigen (BAPA), Standard Tube agglutination Test (SAT) and Enzyme- Linked Immune Sorbent assay (ELISA) to diagnosis brucellosis from 3000 samples, 1500 samples from exported camels and 1500 from goats in Suakin quarantine mager in Port Sudan locality, Red Sea State, Sudan to control the disease among this animals. One hundred eighty five out of 1500 sera samples from camel were positive (12.3%) by RBPT and (180/1500) sera sample from goats were positive (12%).This positive samples from each species were retested by BAPA test, the results was found 77.3% (143/185) positive in camel and 75% (135/180) in goats. When the SAT was used in camel the result was 39.4% (73/185) and 20% (36/180) in goats. c-Elisa test in camel reveled result of 55.1% (102/185) in camel and 25.6% (46/180) in goat were positive. Statistical analysis using kappa program showed relation between RBPT and BAPA tests, the result showed that all positive samples of camel by RBPT showed prevalence of 77.3%, 39.5%, 55.1% by BAPA, SAT and c-ELISA respectively, and 75%, 20% and 25.6% positive in goats respectively. These results indicated that Rose Bengal Plate Test was the best test for diagnosis of brucellosis in exported animals followed by BAPA, c-Elisa and SAT in camel. As for goats the Rose Bengal Plate Test gave higher positive results followed by BAPA, I-ELISA and SAT this study has shown that the disease was prevalent in exported camels and goat.

ملخص البحث

تنتشر البروسيلا فى الجمال والماعز فى السودان بنسبة عالية جدا مما يؤثر على الانتاجية وهذا يعوق تصدير الابل والماعز الى شبه الجزيره العربية .صممت هذه الدراسه في الفتره مابين 2017-2020 للمقارنه بين الاختبارات السيرولوجية اختبار روز بنقال الصحنى والبابا والسات والاليزا فى فحص البروسيلا لعدد 3000 عينة ، 1500 عينة من الابل و1500 عينة اخرى من الماعز من محجر سواكن بمدينه بورتسودان لاغراض السيطره على المرض والتصدير. تم فحص عدد 1500 عينة من الابل و1500 عينة من الماعز باختبار روز بنقال الصحنى وكانت النتيجة عدد 185عينة موجبة بنسبة (12.3%) فى الابل وعدد 180 موجبة بنسبة (12%) فى الماعز وهده العينات الموجبة لكل من الابل والماعز تم فحصها بواسطه اختبار البابا وكانت النتائج عدد143 عينة موجبة للاختبار و42 عينة سالبة بنسبة (77.2%) فى الابل و135 عينة موجبة للاختبار و45 سالبة للاختبار بنسبة (75%) فى الماعز اما عند استخدام اختبار التراص الانبوبى (السات) فى الابل كانت 73 عينة موجبة للاختبار و112عينة سالبة بنسبة (39.4%) وفى الماعز36 عينة موجبة للاختبار و144 سالبة للاختبار بنسبة (20%) اما اختبار الاليزا فى الابل اعطى عدد 102 عينة موجبة و83 عينة سالبة بنسبة (55.1%) بينما كانت النتيجه للاليزا فى الماعز عدد 46 عينة موجبة و136 سالبة للاختبار بنسبة 25.6%. اظهر التحليل الاحصائى باستخدام كابا وجود علاقه بين كل من اختبار روزبنقال الصحنى والبابا حيث كانت النتائج فى الابل كالاتى: كل العينات الموجبه لروز بنقال وجدت موجبة للبابا بنسبة 77.3% ونسبة 22.7% اعطت نتيجة سالبة للبابا . العينات الموجبة لروز بنقال كانت موجبة للسات بنسبة 39.5% و60.5% منها اعطت نتائج سالبة للسات وكذلك العينات الموجبه لروز بنقال اعطت نسبة 55.1% موجبة للاليزا ونسبة 44.9% سالبة للاليزا. اما التحليل الاحصائى فى النتائج الموجبة لروز بنقال الصحنى للماعز اعطى نسبة 75% موجبة لاختبار للبابا و25% سالبة للبابا و 20% موجبة للسات ونسبة 80% سالبة للسات وكذلك نسبة 25.6% موجبة للاليزا ونسبة 74.4% سالبة للاليزا. ومن هذه النتائج والمقارنات نرى ان اختبار الروز بنقال الصحني كان اكثر حساسيه لمرض البروسيلا واعطى نتائج ايجابية اعلى ويليه البابا ثم الاليزا ثم السات في الابل والماعز . اظهرت هذه الدراسه انتشار المرض فى صادر الابل والماعز.

Introduction
Introduction

Brucelloss is a contagious bacterial zoonotic disease of veterinary and public health importance in developing countries. The disease affects domestic animals (cattle, sheep, goats camels and pigs), humans and wildlife. It is caused by various *Brucella* species such as *B. melitensis* in small ruminants, *B. abortus* in cattle, *B. suis* in swine and *B. canis* in dogs, while all the species are known to zoonotic importance. *Brucella* species are slow-growing, Gram negative, small cocobacilli and intracellular bacteria that is capable to survive and multiply within epithelial cells, placental trophoblasts, dendritic cells and macrophages (Gorvel, 2008). *Brucella melitensis* is considered to have the highest zoonotic potential followed by *B. abortus* and *B. suis*. According to the Office for International des Épizooties (OIE, 2009), the disease is also classified as one of the neglected zoonoses with a serious veterinary and public health importance throughout the world (WHO, 2006; OIE, 2009). Globally, it is estimated that nearly 500,000 cases of brucellosis would occur in humans every year (Pappas *et al*., 2006), and often persists in the poorest and most vulnerable populations (FAO, 2003).

The economic and public health impact of brucellosis remains of concern in developing countries (Roth *et al*., 2003). The disease poses a barrier to trade of animals and animal products, an impediment to free animal movement (Zinsstag *et al*., 2011). It also causes losses due to abortion or breeding failure in the affected animal population, diminished milk production and in human brucellosis causing reduced work capacity through sickness of the affected people (FAO, 2003).

In Africa and central Asia, the incidence of brucellosis is generally considered higher in pastoral setting. However, because of the difficulty to access pastoral communities, the occurrence and the control of brucellosis is poorly understood both in humans and their animals in the pastoral settings of the sub-Saharan Africa where the burden of the disease could be high (Mcdermott and Arimi,2002).

Sudan is the largest Arab and African Countries; it is bordered by 7 countries (Egypt, Libya, Ethiopia, central Africa, South Sudan, Eretria and Chad). And it is divided into 26 States one of them is Red sea state is separates Sudan from Saudi Arabia at the eastern side (Anon a 2019).

Suakin Veterinary Quarantine (SVQ) is located in the south west .an part of Suakin city, about 10 km from the Port Sudan, and occupies area of 339.3 acres (dimensions of 1500x950 meters).(Anon a 2019).

Sudan exports Camel, cattle and small ruminants go to the gulf countries, in particular to Saudi Arabia, which accounts for 13,921,674 of all camel, Cattle, Sheep and goats exported by Sudan during three years ago (2017-2019).(Anon a 2019)

According to reports of the General Directorate of Veterinary Quarantine and Meat Hygiene, the Ministry of Live stock, Fisheries and the Rangeland, there are some of the infectious diseases that have an impact on exports of animals in Sudan. Brucellosis is one of the most common zoonotic diseases in the present time in both developed and developing countries alike according to the classification of global organizations WHO, FAO and OIE (Mohamed, 2011).The importance of this disease is due to the high economic losses as well as the danger to human health and safety (General Administration of Live stock, kassala state, 2012)

Total number of the Camels and goats exportation during three years ago in (2017) the positive samples of Brucellosis were 699 out of 37109 (1.9%),in (2018) the positive samples of Brucellosis were 1055 out of

59851 (1.8%) and in (2019) the positive were 247 out of 12240 (1.7%) in camels, in (2017) the positive samples of Brucellosis were 219 out of 202399 (0.1%) , (2018) the positive were378 out of 199115 (0.2%) and in (2019) the positive were 3481 out of 147476 (2.3%) in Goats (Anon b 2019). The disease in camels is the main constraint to their exportation to the Arabian Peninsula for breeding purpose, and every year many consignments are rejected because of detection of brucellosis despite their screening prior to shipment with the RBPT.

The objectives of this study were:

- Evaluation of the occurance of brucellosis by RBPT in Camel and Goat intented for exportation in Suakin Quarantine.
- Conduction of comparative study among different serological tests commonly used for detection of brucellosis in exported animals.
- Conformation of *Brucella* detected in RBPT Positive sera by use of (BAPA, SAT and ELISA test).
- Comparative analysis for different test (RBPT, BAPA, SAT and ELISA) used for Brucella diagnosis.

Chapter one

Literature Review

1.1 Historical background

1.1.1 History of brucella infection in animals

Carpenter and Hubbert (1963) stated that in central Europe where abortion was rampant in cattle, the disease was considered infectious even in the sixteenth century. In a book "the Complete Farmer" it was stated that: "the foetus and foretal membrane were considered contagious and when it happens the abortion should be immediately buried and the cow kept as widely apart as possible from the herd." (Cited by Bang, 1897). This proves that contagious abortion was a worry to cattle breeders for a long time. As a result, Nocard was commissioned to study the epizootic abortion which prevailed among cattle at that time (Bang, 1897). In this investigation, Nocard, described two morphologically different microbes in uterine exudates of an aborted cow and distinguished them as micrococci and thick bacilli. The investigator cultivated the two microbes in gelatin peptone or bouillon and obtained cultures similar to those he had described from uterine exudates. Unfortunately, Nocard could not reproduce the disease with either of the two isolate. Taking advantage of Nocard's inconclusive work, Bang (1897) continued the investigations using cover glass preparations from the yellowish exudates of the allantoic fluid, stained them with methylene blue and demonstrated small bacteria in pure form, some of which were found intracellular. then concluded that the epizootic abortion was a specific uterine catarrh caused by definite bacterium. Furthermore, Bang (1897) recovered the bacterium in a pure form using serum-gelatin agar and could always demonstrate and cultivate typical abortion bacilli from

different abortion material. The investigator noticed that the organism remained viable in the uterus and could cause abortion for the second time in previously aborted cows. Moreover, Bang was able to reproduce the disease by injection of pure cultures into the vaginas of pregnant cows, thus proving that the organism he discovered was the cause of epizootic abortion. Further experiments on ewes and mares proved the occurrence of the disease in those species, and noticed that goats kept in contact with aborted cows also aborted. It in recognition of Bang's work that brucellosis is often named Bang's disease.

1.1.2. History of brucella infection in human

Malta fever was earlier known by other names such as adeno typhoid, intermittent typhoid, gastric and bilious remittent fever (Bruce, 1887). The disease had a wide distribution in the Mediterranean area. Bruce described it as having along duration, a fever which often runs high and continuous, remittent and intermittent in type. The patient suffer rise in temperature, profuse sweating and the spleen is often enlarged. There are always rheumatic or neuralgic pains, Joints involvement and orchitis. " David Bruce isolated the etiologic agent of the fever in (1887) from the spleen of four fatal human cases on the Island of Malta. Bruce named the bacterium *Micrococus melitensis* and the same worker renamed the species a *Brucella melitensis* in 1887 to honor the work of Bruce (Smith, 1979). The disease was recognized as a zoonotic by (Zammit 1905) who proved that man acquired Malta fever by consumption of infected goats' milk. Troum (1914) isolated *Br. abortus* from a guinea pig inoculated with a tonsil material from a child, and that was the first instance in which the organism was isolated from a human source. Traum (1914) isolated *Br. suis* from aborted swine. Kaeer (1924) was able to isolate *Br. suis* from blood of a patient with undulant fever. The disease was thus identified as an animal disease transmissible to man. Bevan (1921) in

Rhodesia demonstrated by culture and serological tests that *Br. abortus* could cause "undulant fever" in man. Evans (1918) pointed out that *Micrococcus melitensis* described by Bruce and *Bacillus abortus* isolated by Bang were morphologically and antigenically similar. Later, such organisms were grouped in one genus; *Brucella*. Meyer was the first to suggest that the generic name *Brucella* in the family Bacteriaceae (Corbel, 1989).

1.2 Characteraization of the genus brucella

Brucella are coccobacilli or short rods 0.6 to 1.5 μm long by 0.5 to 0.7 μm in width. They are arranged singly and less frequently in pairs or small groups. The morphology of Brucella is fairly constant except in old cultures, where pleomorphic forms may be evident. *Brucella*are non-motile. They do not form spores, flagella, or pili. True capsules are not produced. Brucella are Gram-negative and usually do not show bipolar staining. They are

Not truly acid-fast but resist decolouration by weak acids, thus stain red by the Stamp's modification of Ziehl-Neelsen method,

Which is sometimes used for the microscopic diagnosis of brucellosis from smears of solid or liquid specimens (Table 1)

Table 1: Differential characteristics of brucella compared to some other Gram- negative bacteria (Alton *et al* 1988)

Test	Brucella	Bordetella	Yerisins enterocolitica 0:9
Morphology	Small coccobacilli	Small coccobacilli	Rod
Motility at 37°C	-	+	-
Lactose fermentation on Mac Conkey agar	-	-	-
Acid production on agar Containing lactose	-b	-	+
Haemolysis on blood agar	-	+	-
Agglutination with:Sbrucella Antiserum	+f	-	+
R-brucella Antiserum	+g	-	-

a : Positive and negative species within the genus

b: B. neotomae may show some fermentation.

c :Except B. ovis, B. neotomae and some strains of B. abortus

d : Except B. ovis and some strains of B. abortus

e: Except B. ovis

f : Except B. ovis , B. canis and R-forms of other species

g : B. ovis , B. canis and R-forms of other specie

1.2.1 Culture and biochemical characteristics:

The organism is aerobic but some strains require CO_2 for primary isolation. Growth is slow and is usually visible after 48 hours of incubation at 37C°. Colonies are about 0.5 mm is diameter and appear round, convex with smooth glistering surface. The recommended enriched media for primary isolation and optimum growth include serum agar, liver infuion, dextrose potato, glycerol potato and Brucella agar (Buxton and Fraser,1977) .On blood agar, colonies are usually 0.5-1.0 mm in diameter, raised and convex, with an entire edges and smooth shiny surface.

1.2.3 Polymerase chain reaction:

The PCR technique is a very useful tool for the diagnosis of brucellosis because of its simplicity, higher degree of sensitivity and specificity together with its speed, versatility in sample handling and risk reduction for laboratory personnel, (Morata *et al*., 2001). Serum sample should be used preferable over whole blood for the molecular diagnosis of Brucellosis, (Zerva *et al*., 2001). The test was used to diagnose caprine brucellosis and it
was shown to be more sensitive than the RBPT and culture techniques (Leal – Klevezas *et al*., 2000)

1.2.4 Biochemistry:

The metabolism of brucella species is oxidative and usually shows little
Fermentative action on carbohydrates in conventional media. Many strains require supplementary (5-10%) carbon dioxide for growth especially on primary isolation.

Although brucella neotomae, *Br.ovis* and some strains of *Br. Abortus* are catalase positive and oxidase negative, other brucellae are catalase positive and oxidase positive. The bacterium reduces nitrates, produces hydrogen sulfide and hydrolyzes urea to variable extent. Member of the

genus brucella do not produce indole, liquefy gelatin, lyses blood, produce acetyl-methyl carbinol or utilize citrate. The methyl red reaction is negative and litmus milk is either unchanged or rendered alkaline (Scientific Committee on Animal Health and Animal Welfare, July 2001, Raga, 2006).

1.2.5 Antigenic relatedness:

All smooth brucella strains show complete cross-reaction with each other in agglutination test with unabsorbed polyclonal antisera, across-reaction which does not extent to non-smooth variants. Cross reactions between non-smooth strains can be demonstrated by agglutination tests with unabsorbed anti-R sera. Lipopolysacchride (LPS) comprise the major surface antigens of the corresponding colonial phase involved in agglutination. The S-LPS molecules carry the A and M antigens, which have different quantitative distribution among the smooth brucella strains. This is of value in differentiating biovars of the major species using absorbed mono specific A and M anti sera. Serological cross reactions have been reported between smooth brucella and various other gram negative bacteria, e.g. Escherichia coli 0:116 and 0:157, Salmonella group N (0:30) of Kaufmann-White, Pseudomonas multophilia, Vibrio choleras and especially Yersinia enterocolitica 0:9. These organisms can induce significant levels of antibodies which cross-react with S-LPS brucella antigens in diagnostic tests (Scientific Committee on Animal Health and Animal Welfare, July 2001).

1.2.6. Susceptibility to Phages

There are about 40 phages which are lytic and specific to the genus *Brucella* and they are not known to be active against any other bacteria that have been tested. Therefore, lysis by *Brucella* phages is a useful test

to confirm the identity of *Brucella* species and for speciation within the genus (Anon a, 2011). *Brucella* phages currently in use for typing are Tbilisi (Tb), Weybridge (Wb), Izatnagar (Iz) and Rough Culture (R/C) (Alton *et al*., 1988). The first three phages are used for differentiation of smooth *Brucella* and R/C is used for rough B*rucella* (*B. ovis*, *B. canis*) (Corbel *et al*., 1988). (Table 2).

Table 2: Biovar differentiation of the species of the *brucella* according to Alton *et al* (1988)

Species	Biovar	Co2 requirement	H2S Production	Growth on Dyes		Agglutination		
				Thionine	Basic Fuchsin	A	M	R
Br.melitensis	1	-	-	+	+	-	+	-
	2	-	-	+	+	+	-	-
	3	-	-	+	+	+	+	-
Br.abortus	1	+c	+	-	+	+	-	-
	2	+c	+	-	-	+	-	-
	3	+c	+	+	+	+	-	-
	4	+c	+	-	+d	-	+	-
	5	-	-	+	+	-	+	-
	6	-	-	+	+	+	-	-
	7	+ or -	+	+	+	-	+	-
Br.Suis	1	-	+	-	-e	+	-	-
	2	-	-	+	-	+	-	-
	3	-	-	+	+	+	-	-
	4	-	-	+	-f	+	+	-
	5	-	-	+	-	-	+	-
Br.neotomae		-	+	-g+	-	+	-	-
Br.ovis		+	-	+	-f	-	-	+
Br.canis		-	-	+	-f	-	-	+

a= dye concentration, 20ug/ml in serum dextrose medium (1:50000).
A=Amono-specific antiserum; M=M mono-specific antiserum; R=rough brucella antiserum.
C= usually positive on primary isolation
d= some strain do not grow on dyes
e=negative for most strain
f=negative for most strain
g=growth at 10ug/ml (1:100000) thionin

1.2.7 Susceptibility to dyes and antibiotics

Susceptibility to the dyes, thionin and basic fuchsin (20ug/ml), which varies between biovars, is one of the routine typing tests of brucella. On primary isolation, brucellae are usually susceptible in vitro to gentamicin, tetracyclines and rifampicine. Most strains are also susceptible to the following antibiotics: ampicillin, chloramphenicol, cotrimoxazole, erythromycin, kanamycin, novbiocin, spectinomycin and streptomycin, but variation in susceptibility may occur between species, biovars and stains. Most strains are resistant to B-lactamins, cephalosporin, polymyxin, nalidixic acid, amphotericin B,bacitracin, cycloheximide, clindamycin, lincomycin,nystatin and vancomycin at therapeutic concentration.(Alton *et.al*., 1988).

1.3 Taxonomy of the genus brucella

Considering their high degree of DNA homology (> 90 % for all species), Brucella have been proposed as a monospecific genus in which all types should be regarded as biovars of *B. melitensis* (Verger *etal* 1985). Since this proposal has not yet met with complete agreement, the old classification of the genus (and relevant nomenclature) into six species, i.e *B.melitensis ,b.abortus, B.suis, B.neotomas, B.ovis and B.canis* (Corbel and Brinley Morgan, 1984), is the classification used world-wide. The first 4 species are normally observed in the smooth form, *where as* B.ovis and b.canis have only been encountered in the rough form. Three biovars are recognised for *B. melitensis* (1-3), seven for b. abortus(1-6 and 9), and five for B. suis (1-5) . Species identification is routinely based on lysis by phages and on some simple biochemical tests.

Brucella is taxonomically placed in the alpha-2 subdivision of the class Proteobacteria. There are 10 species of *Brucella* based on preferential

host specificity: *B. melitensis* (goats), *B. abortus* (cattle), *B. suis* (swine), *B. canis* (dogs), *B. ovis* (sheep), *B. neotomae* (desert wood rats), *B. cetacea* (cetacean), *B. pinnipedia* (seal), *B. microti* (voles), and *B. inopinata* (Whatmore, 2011). *B. melitensis* (small ruminants), *B. abortus* (cattle), *B. suis* (swine), and *B. canis* (dogs) are known to cause human disease. *B. neotomae* (desert wood rats) and *B. ovis* (sheep) are not pathogenic to humans. The majority of human cases worldwide areattributed to *B. melitensis* (Pappas, 2006). In general, *B. melitensis* and *B. suis* are more virulentfor humans than *B. abortus* or *B. canis* (WHO, 2006). *B. melitensis*, *B. abortus*, and *B. suis* have 3, 7, and 5 biotypes, respectively (Alton *et al*., 1988; Lindquist *et al*., 2007). Sequencing andannotation of the genomes of *B. suis*, *B. melitensis*, and *B. abortus* has been completed; themajority of the open reading frames share greater than 99 percent sequence similarity between species (Paulsen *et al*., 2002; Halling *et al.*, 2005).

1.4 Definition of Brucellosis

Brucellosis is a contagious disease of animal, which is transmitted to man (anthropozoonosis), (Carpenter and Hubbert, 1963) and characterized by inflammation of the genital organs and foetal membrane, abortion at the late stage of pregnancy with retained placenta, sterility and formation of localized lesions in the lymphatic system and joints (Cadmus et al., 2006).

1.4.1 Aetiology

Brucellosis is caused by Gram negative coccobacilli of the genus *Brucella* which are facultative intracellular that can survive within host cells causing a chronic infectious disease that may persist throughout the life of the animal. In addition to the "classical" *Brucella* spp. (*B.abortus*, *B. suis*, *B. melitensis*, *B. canis*, *B. ovis*, and *B. neotomae*) the genus has recently been expanded to include marine isolates, whichhave zoonotic

potential as well (Sohn *et al*., 2003; McDonald *et al*., 2006). Camels can be infected by *B. abortus and B. melitensis*. Different studies showed 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**)** and the spread of brucellosis depends on the *Brucella* species being prevalent in other animals sharing their habitat and on husbandry (Musa *et al*., 2008).

1.4.2 Epidemiology of Brucellosis

The most important factors affecting the epidemiology of brucellosis are the Geographic distribution, transmission and risck factor.

1.4.2.1 Geographical distribution

The geographical distribution of brucellosis is constantly changing, with new foci emerging or reemerging. The epidemiology of human brucellosis has drastically changed over the past few years because of various sanitary, socioeconomic, and political reasons, together with increased international travel. New foci of human brucellosis have emerged, particularly in central Asia, while the situation in certain countries of the Middle East is rapidly worsening (Pappas *et al*., 2006).

Brucellosis is a disease of worldwide distribution occurring in domestic as well as wild animals. It has been reported wherever animals are raised all over the world (Seifert, 1996). Although some of the industrialized countries in Europe and America have achieved eradication of brucellosis in domestic animals through Intensive control and eradication schemes, the disease is still a serious problem in developing countries (Warner, 2001; Ragan, 2002). *Brucella melitensis* is the most virulent species of the

Brucella genus and has three biovars, with biovars 1 and 3 being the ones isolated most frequently in small ruminants in the Mediterranean, the Middle East and Latin America (Lucero *et al*., 2008; Blasco and MolinaFlores, 2011). Brucellosis is a barrier to trade in animals and animal products and causes significant losses from abortion, as well as being a serious zoonosis (Benkirane, 2006; Banai, 2007; Seleem *et al.*, 2010).

1.4.2.2 Transmission of the disease

Animal brucellosis can be transmitted by both vertical and horizontal transmission. Horizontal transmission occurs through ingestion of contaminated feed, skin penetration, via conjunctiva, inhalation and udder contamination during milking. Congenital infection during parturition is frequently cleared and only few animals remained infected as adult (Radostits *et al.,* 2007). Spread of the disease is due to movement of infected animals to disease free herds. Proximity of infected herds to non-infected herds occurs at water where animals come together. The important epidemiological risk factors are large herd size, poor management, abortion, milking more animals by a single person and herding with other ruminants. Survival of the organisms in the environment may also play a role in epidemiology of the disease (Abbas *et al*., 1987; Radwan *et al.,* 1992; Abuo -Eisha, 2000).

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

1.4.3. Brucellosis in livestock

Brucellosis affects camel and small ruminants

1.4.3.1 Brucellosis in Camels

The camel (*Camelus dromedarius*, one-humped camel) plays an important socio-economic role within the pastoral and agricultural system in dry and semi dry zones of Asia and Africa (Gwida *et al.*, 2011). Brucellosis is considered by the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO) and Office International des Epizootics (OIE) as one of the most.

During the last few years, camel brucellosis has been a subject for many researches in many countries of the world ally cause significant loss of productivity through late first calving age, long calving interval time, low herd fertility and comparatively low milk production in camels (Gessese *et al.*, 2014). Moreover, brucellosis causes a serious illness. In man especially that contact with infected animals and those consume infected animal products and are considered as one of the great public health problem all over the world (Marei *et al.*, 2011; Shimol *et al.*, 2012; Sayour *et al.*, 2015).

During investigations conducted by Radwan *et al.* (1995) it was found that brucellosis was diagnosed in 30% of the camel handlers and milkers and the same *Brucella melitensis* biovars were cultured from aborted sheep and goats sharing the same premises. Therefore the intense alertness which is directed nowadays toward brucellosis all over the world can be justified by the economic impact of the disease, the ease with which the disease transmits among animal population, the substantial difficulties associated with its control and finally the public health significance of the disease. Cross transmission can occur between cattle, sheep, goats, camels and other species (Ghanem *et al.*, 2009). Camels are not known to be primary hosts of Brucella organisms, but they are susceptible to both *Brucella abortus* and *Brucella melitensis*

(Cooper, 1991; Seifert, 1996; Abbas and Agab, 2002; Gwida *et al*., 2012).

1.4.3.2 Brucellosis in small ruminants

Brucellosis is a disease of many animal species including humans but especially of those that produce food: cattle, sheep, goats, camels and other species (Corbel, 2006; Ghanem *et al*., 2009). 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. So the disease causes significant losses in reproduction and productivity of sexually mature animals through high morbidity (Pappas *et al*., 2006; Radostits *et al*., 2007). *B. melitensis*, *B. abortus* and *B. suis* are zoonotic pathogens which can infect humans. *Brucella canis* may cause infections in immune-suppressed individuals Brucellosis in sheep and goats (excluding *Brucella ovis* infection) is primarily caused by one of the three biovars of *B. melitensis*. Sporadic infections caused by *B. abortus* or *B. suis* have been observed in sheep and goats, but such cases are rare. In most circumstances, the primary route of transmission of *Brucella* is the placenta, fetal fluids and vaginal discharges expelled by infected ewes and does when they abort or full-term parturition. Shedding of *Brucella* is also common in udder secretions and semen and *Brucella* may be isolated from various tissues, such as lymph nodes from the head, spleen and organs associated with reproduction (uterus, epididymides andtestes), and from arthritic lesions (OIE, 2009). Goats are the classic and natural host of *B. melitensis* and together with sheep are its preferred hosts. In pathological and epidemiological terms, *B. melitensis* infection in small ruminants

is similar to *B. abortus* infection in cattle: the main clinical manifestations of brucellosis in ruminants are abortion and stillbirths, which usually occur in the last third of the pregnancy following infection and usually only once in the animal's lifetime (Elzer *et al*., 2002; Blasco and Molina, 2011).

1.4.4 Pathogenesis

The major route of infection is through mucous membranes of the oropharynx and upper respiratory tract or conjunctiva (Tabak *et al*., 2008). Another route is through the mucous membrane of the male and female genital tract. On entering into the body of the host, the organism encounters the cellular defences of the host but generally succeed in arriving via the lymph vessels at the nearest lymph node after escaping the cellular defenses (Ko and Splitter, 2003). The fate of the invading bacteria is mainly determined by cellular defences of the host chiefly macrophage and T-lymphocytes though specific antibody also plays apart (Radostits *et al*., 2007). The outcome depends on the ruminant species infected, age, immune status of the host, pregnncy status, and the virulence and the number of invading *Brucella* (Seifert, 1996).

When the bacteria prevail over the host's defences, a bacteraemia is generally established. The bacteraemia is always detected after 10 to 20 days and persists from 30 days to more than two months. If the animal is pregnant, bacteraemia often leads to the invasion of the uterus (Olsen, 2010). At the same time, infection becomes established in various lymph nodes and organs, often in the udder and sometimes in the spleen (WHO, 2006). The main lesions which appear in the male animals are orchitis and epididymitis, as well as inflammation of the joints and bursa. Abortion may also occur in the females presenting the typical yellowish sticky layers on the placenta. The consequences of brucellosis in small

ruminants are infertility, high mortality rate in calves, mastitis and reduced milk production (Oyedipe *et al*., 1981; Radostits *et al*., 2007).

1.4.5 Immune Responses

Infection with *Brucella* usually results in the induction of both humoral and cell-mediated immune responses, but the magnitude and duration of these responses is affected by various factors including the virulence of the infecting strain, the size of infecting inoculum, pregnancy, sexual and immune status of the host _Joint FAO/WHO Expert Committee on Brucellosis, 1986).

1.4.6 Cellular Immunity

As the *Brucellae* are facultative intracellular organisms characteristic chronic granulomatous lesions develop in infected tissue where macrophage, neutrophils and lymphocytes respond to *Brucella* antigens. Phagocytes play a key role in initiating T-cells by processing and presenting antigens. Sensitized T-cells release cytokines that activate macrophages which in turn combat *Brucella* by reactive oxygen intermediates. Both CD4 and CD8 subsets are involved in cell-mediated protection. Cytokines also play a role in controlling *Brucella* infections (WHO, 1997). Neutrophils effectively utilize the myeloperoxidase H2O2 halide system in killing *Brucella*. However, the organisms inhibit degranulation and the respiratory oxidative burst and they are able to survive with in the cell (Riley and Robertson, 1984). Macrophages readily ingest *Brucella* when opsonized with either complement orspecific antibodies. The survival of the organisms in macrophages may result from a failure of phagosome-lysosome fusion and resistance to oxidative killing by producing superoxide dismutase and catalase (Frenchick *et al.*, 1985; Harmon *et al.,* 1988; Quinn *et al.,* 2002). Tatum *et al.* (1992) suggested that anti-oxidant Cu-Zn superoxide dismutase plays a role in the survival of *Brucella* in phagocytic cells.

1.4.7 Diagnosis of Brucellosis

Many workers used serological tests for diagnosis of the disease. A Definitive diagnosis of brucellosis requires the isolation and identification of the etiological agent (Davis, 1980; Volk, 1982).

Several methods are used for diagnosis of brucellosis these include:-

1.4.7. 1 Direct smear

Smears of placental cotyledon, vaginal discharge or fetal stomach contents may be stained using modified Ziehl-Neelsen (Stamp) method. The presence of large aggregates of intracellular, cocco bacillus 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 also stained red (Alton *et al*., 1988; FAO, 2006).

1.4.7.2. Cultural isolation

The only 'gold standard' method for the diagnosis of brucellosis is the cultural isolation or detection of *Brucella* organisms from the infected host (Alton *et al.*, 1988; OIE, 2009; Smirnova, *et al*., 2013). This can be made by means of microscopic examination of smears stained with the modified Ziehl-Neelsen method from vaginal swabs, placenta, or aborted foetuses (Stamp,1950). However, morphologically related micro organismis such as *Chlamydia psittaci* and *C. burnetii* can mislead one in the diagnosis (Garin, 2006; Radostits *et al.,* 2007). So bacterial culture plays an important role in confirming the presence of 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). Important clinical samples include aborted fetuses (stomach, spleen, and lung), fetal membranes, vaginal secretions, colostrum, milk, sperm, 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. Care should be taken to minimize the fecal and environmental contamination of the material to give the greatest chance of successfully isolating *Brucella.* However vaginal swabs and milk from aborted animals are the best materials/samples for the isolation of *Brucella* species, while spleen and lymph nodes (iliac, mammary and prefemoral) are the most reliable samples for isolation purposes in necropsied animals (Marin *et al*., 1996). For the isolation of *Brucella* species the most commonly used medium is Brucella Selective Medium (HiMedia) with sterile inactivated horse serum, which contains antibiotics Able to inhibit the growth of other bacteria present in clinical samples.

1.4.7.3 Guinea pig inoculation

More successful than direct culture especially contaminated material and small number of organisms. Guinea pigs are injected intramuscular or intra peritoneal if the emulsified material is free from contamination, and killed after 4-5 weeks of inoculation. Typical lesions include nerotic foci in liver, spleen, lymph nodes and orchitis in male guinea pigs. The spleens are cultured on SDA and the sera are subjected to SAT. Recovery of the organism from the spleen or positive serum agglutination test is a justifying diagnosis of brucellosis (Alton, 1975)

1.4.7.4 Serological Diagnosis

In the Sudan, detection of immunoglobulins is based on the Rose Bengal Plate Test (RBPT), complement fixation test (CFT) and enzyme- linked immunosorbent assay (ELISA) on serum, and the milk ring test (MRT) on milk. Two ELISAs are mentioned in the OIE (2008): an indirect ELISA specific for IgG1; and a competitive (inhibition) ELISA using monoclonal antibodies. The USA Department of Agriculture, animal and

Plant health Inspection Service have also reported anew serologic test for detection of *Brucella* antibodies, termed the rapid automatic presumptive (RAP) test. It uses acomputer reader and recorder device to assess and report test results. This minimises subjectivity and has enhanced laboratory- to-laboratory uniformity. Cross –reactions to other organisms may cause some diagnostic problems. Several authors have reported serological reactions to the presence of *Yersinia enterocolitica*. This reaction was later considered to have been caused by previous exposure to yersinia enterocolitica (Hilbink *et al*., 1995).

1.4.7.4. 1 Rose Bengal plate test (RBPT)

This test was developed by Rose and Roekpe (1957) for the diagnosis of bovine brucellosis to differentiate specific *Brucella* agglutinins from non-specific factors. When the antigen was buffered at pH 4.0 they observed that agglutination of *B. abortus* cells by non-specific agglutinins of bovine serum was inhibited whereas the activity of specific *Brucella* antibodies was not affected. Despite the scanty and sometimes conflicting information available (Alton, 1990), this test is internationally acknowledged as the test of choice for the screening of brucellosis in cattle as well as in small ruminants (Garin and Blasco, 2004; WHO, 2006). However, the standardization conditions suitable for diagnosing cattle infection (European Commission, 2001; Garin and Blasco, 2004) are not adequate in sheep and goats and account for the low sensitivity of RBPT in small runinants. If the antigen is standardized differently, to give a higher analytical sensitivity, the diagnostic sensitivity to *B. melitensis* infection will be improved. The RBPT is based on the detection of specific antibodies of the IgM and IgG types but more effective in detecting antibodies of the IgG1 type than the IgG2 and IgM types. Also the low pH (3.65) of the antigen enhances the specificity of the test by inhibiting non specific agglutinins. The temperature of the

antigen and the ambient temperature at which the reaction takes place may influence sensitivity and specificity (Macmillan, 1990).

The RBPT could be modified for testing of sera in endemic, low prevalence areas to increase the sensitivity of the test. This simple modification is achieved by increasing slightly the amount of sera for the test dose from 25 µl to 75 µl, at the same time maintaining the antigenvolume at 25 µl. This results in significantly increase in the sensitivity of the test without affecting the specificity (Blasco *et al*., 1994; Ferreira *et al*., 2003).

1.4.7.4. 2. 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 means that it should only be used in the absence of alternative techniques. (OIE, 2018)

1.4.7.4. 3 Complement fixation test (CFT)

Complement fixation test is the most widely used confirmatory test and recommended by OIE (Garin., 2006)**.** As in cattle brucellosis, there is agreement that this test is effective for the serological diagnosis of brucellosis in sheep and goats despite the complexity and the heterogeneity of the techniques used in different countries. The CFT is based on the detection of specific antibodies of the IgM and IgG1 that fix complement. It is highly specific but laborious and requires highly trained personnel as well as suitable laboratory facilities. Its specificity is very important for the control and eradication of brucellosis but may test negative when antibodies of the IgG2 type hinder complement fixation (Farina, 1985; Alton, 1990; Macmillan, 1990).

1.4.7.4.4 Enzymed linked immune Sorbent Assay

ELISAs are divided into two categories, the indirect ELISA (IELISAs) and the competitive ELISA (c-ELISA). Most IELISAs use purified smooth LPS as antigen but a good deal of variation exists in the anti-bovine Ig conjugate used (Saegerman *et al*., 2004). Most IELISAs detect mainly IgGs or IgG sub-classes. Their main quality is their high sensitivity but they are also more vulnerable to non-specific reactions, notably those due to YO9 infection. These cross-reactions seen in IELISAs motivated the development of c-ELISAs. The O-chain of the smooth LPS of *Brucella* contains specific epitopes that are not shared with the LPS of YO9. Therefore, by using monoclonal antibodies directed against specific epitopes of the *Brucella* LPS, the development of more specific c-ELISAs has been possible. These tests are more specific, but less sensitive, than IELISAs (Nielsen *etal*., 1995, Weynants *etal*., 1996). The OIE considers these tests "prescribed tests for trade" (OIE 2009).

1.4.7.5 Tests for detecting anti bodies in milk

1.4.7.5.1 Milk Ring Test (MRT)

The MRT is widely used as a herd test to determine brucellosis in dairy cattle, but it is not sensitive enough to detect brucellosis in goats (Shimi and Tabatabayi, 1981). The MRT was proved to be sensitive and specific for screening dairy herds and for identifying infected ones with milk from individual animals or bulk milk samples (Morgan, 1967).

1.4.7.5. 2 Whey Agglutination Test (WAT)

The test is of value for detecting animals which are excreting *Br.abortus*. After preparation, whey is tested by the same method as the TAT (Buxton and Fraser, 1977).

1.4.7. 6 Serological Cross – reactions

In both the agglutination and CFT strong cross-reactions occur between smooth species of *Brucella* and *Yersinia enterocolitica* serotype 9. Cross– reactions with *Brucella* were also reported in cases of infection or vaccination with some strains of *Campylobacter*, *Pasteurella* and *Salmonella* (Alton *et al*., 1975).

1.4.8 Treatment, control and prevention of brucellosis

1.4.8.1. Treatment

Treatment of brucellosis in animals is usually futile and normally not undertaken due to the possibility of exposure to humans from handling infected animals and also due to less effectiveness of available drugs and the cost implications (Timoney *et al.*, 1988; Quinn *et al.*, 1999). Different drugs and agents such as trace elements, vitamin mixtures, and antimicrobial agents such as phenol, azo and flavine dyes, have been shown to be lethal to *Brucella* organisms *in vitro*, but all have yielded mixed results when used *in vivo* (Quinn *et al.*, 1999). Under *in vitro* conditions, *B. abortus* have been found to be sensitive to gentamicin, kanamycin, tetracyclines and rifampin (Timoney *et al.*, 1988; Wanke, 2004). A combination of oxytetracycline and streptomycin was found to successfully treat 71.4% of the infected rams, while sulphonamides and penicillin were found to be less effective (Wanke, 2004). A four week continuous treatment using a combination of tetracycline and streptomycin or dihydrostreptomycin, administered within the first three months of infection have been found to give successful therapy (Shin and Carmichael, 1999; Wanke, 2004). However, recrudescence of infection after the cessation of antibiotic treatment is not uncommon (Wanke, 2004).

1.4.8.2 Control and prevention

Control and prevention of brucellosis in farm animals depend on the animal species involved, *Brucella* species involved, management practices and availability and efficacy of vaccines (Radostits *et al.,* 1994). The options to control the disease include immunization, testing and culling of positive reactors and improving management practices and movement control (Hunter, 1994).

Brucellosis has been controlled and successfully eradicated in some countries through vaccination, coupled with test and slaughter policies. In many countries, the practice of purchasing animals to improve genetics and intensive management systems often makes the control of brucellosis difficult due to exposure to infection of many highly susceptible animals (Nicoletti, 1984). Similarly, in developing countries in the subtropics, control of the disease is complicated by such practices as communal grazing, pastoralism and non-controlled livestock trade (Timoney *et al.*, 1988; McDermott and Arimi, 2002). Under such management, hygienic measures as segregation of purchased animals or keeping parturition animals separated from the herd is difficult and mostly impractical.

1.4.8.3 Control by vaccination

Several vaccines have been developed and are licensed and available for use in some countries. In 1906, Bang observed that cattle could be protected from infection by immunising them with live virulent cultures of *Brucella* organisms (Bishop *et al.*, 1994); it is, however, safe to use live attenuated vaccine, *B. abortus*, S19 which is safe and effective in controlling bovine brucellosis (Nelson, 1977). Vaccination with *B. abortus* S19 by itself will not eradicate bovine brucellosis, but it raises the level of immunity for individual animals such that undesirable consequences of brucellosis are minimised following exposure to virulent strains of *B. abortus* (Nelson, 1977). The use of *B. abortus* S19 vaccine

should only be recommended where the prevalence of the disease is high and cessation of vaccination should be considered when the prevalence is reduced to 0.2% or less (Alton *et al.,* 1988). Nevertheless, *B. abortus* S19 vaccine has been the most widely used vaccine in the control of bovine brucellosis (Schurig *et al.,* 1991).

The normal practice of using a standard dose of 5x1010 viable organisms per dose (Bishop *et al.,* 1994), to vaccinate calves between 3 to 6 months of age has been reported to give long term immunity and benefits of re-vaccination has not been firmly demonstrated (Berman and Irwin, 1952), contrary to what has been reported (Nicoletti *et al.,* 1978). Moreover, antibody titres would decline to a point where 6-8 months after vaccination it is rare to find IgG in the accination it is rare to find IgG in the serum (Nelson, 1977). This will be an added advantage in countries where test and slaughter is practiced since occurrence of *B. abortus* S19 cross-reacting antibodies will be minimised. Although some studies have advocated for the use of a reduced dose (2x 108 to 3x 109 organisms/dose) (Bishop *et al.,* 1994) to vaccinate adult animals to control bovine brucellosis (Alton and Corner, 1981), the benefits of this practice are debatable (Nelson, 1977). A major set-back of using *B. abortus* S19 vaccine in adult cattle is that significantly more animals will have persistent antibody titres than those vaccinated as calves (Nelson, 1977; Beckett and MacDiarmid, 1985). This will interfere with serological tests in herds where test and slaughter is being practiced. In addition, the use of *B. abortus* S19 has been associated with abortions in cows vaccinated during pregnancy (Beckett and MacDiarmid, 1985), sterility problems in males, occasionally with low levels of protection (Nelson, 1977) and arthropathy (Corbel *et al.,* 1989).

A variety of vaccines prepared from killed cells of *Brucella* organisms have been tried and tested (Schurig *et al.,* 1991), but with the exception of

B. abortus strain 45/20 (McEwen and Priestley, 1938), the practical use of these preparations has been very limited. *Brucella abortus* 45/20 was found to offer protection comparable to that of *B. abortus* S19 if administered as double doses in adjuvant (McEwen and Priestley, 1940). The need for a booster and the irritant nature of the adjuvant might make this vaccine more expensive to use and less desirable than *B. abortus* S19. Moreover, like any other killed vaccine, the use of *B. abortus* 45/20 may be associated with low level of cell-mediated immunity which is critical in protection against infection with *Brucella* species (Oliveira *et al.*, 2002). A potential vaccine candidate, *B. abortus* M-strain, was discontinued from trials because the strain offered low protection (Huddleston, 1946).

A rough mutant *B. abortus* RB51 has been a promising vaccine candidate, lacking the antibody inducing antigens but still giving a similar cellular protection as *B. abortus* S19 (Schurig *et al.*, 1991). However, its efficiency over *B. abortus* S19 remains a subject of debate (OIE, 2004). Similar to *B. abortus* S19, the *B. abortus* RB51 vaccine has been reported to cause placental infection and placentitis, and abortion in vaccinated cattle (Palmer *et al.*, 1996; OIE, 2004) as well as infections in humans (OIE, 2004). The use of DNA vaccines in farm livestock is not commonly used (Schurig *et al.*, 1991; Davis and Elzer, 2002).

In small ruminants, vaccination is recommended using Elbeg‟s *B. melitensis*, Rev. 1, a live attenuated vaccine (Elberg, 1981; Banai *et al.*, 2002). Although *B. suis* strain 2 vaccine has been advocated for vaccinating sheep against *B. melitensis* infection, it has been demonstrated that *B. melitensis* Rev 1 gives a better protection (Verger *et al.*, 1995). The use of a killed vaccine, H38, prepared from *B. melitensis* biovar 1 has been reported, but this vaccine has been associated with protection failures (Alton, 1987).

1.4.9. Prevalence of the disease in Sudan

Brucellosis caused by *B. abortus* was first reported in Khartoum state. The prevalence of the disease was 160 (80%) of 200 Friesian and 49 (38%) of 130 local zebu cattle (Bennet, 1943). Subsequently the disease was reported by many investigators all over the country.

In eastern Sudan, camel brucellosis was firstly reported by Mustafa and Nur (1968) in Gash and Tocker where the prevalence was ranged from 0.1 to 5.5%. In Kassala and Butana, Mustafa and El Karim (1971) examined 310 camels and reported that the prevalence was 1.75 and 5.7%, respectively. Abu-Damir *et al*., (1984) stated that the prevalence of *B. abortus* antibodies was 4.9% in 740 camel serum samples tested by RBT, SAT and CFT. Bitter (1986) examined 948 camels from different herds in eastern Sudan and reported a prevalence of 16.5- 32.3% . Abbas *et al* (1987) investigated 238 camel serum samples by slide agglutination test. Antibodies against *B. abortus* were detected in 8 (3%). They attributed the low prevalence of brucellosis in Sudan to the fact that camels were raised on extensive ranges without overcrowding. Yagoub *et al.* (1990) collected 1,502 serum samples from one humped camels *(Camelus dromedaries).* The prevalence rate of *B. abortus* tested by RBT was 6.54, 5.79, 9.32, 5.03 and 8.06%, respectively from 1985 to 1989. Agab *et al.* (1994) examined 38 serum samples by RBT. They found that 32 (84.2%) were positive for *Brucella* and they isolated *B. abortus* biovar 3 from 3 samples. Musa (1995) examined 416 camels from seven herds in western Sudan. The prevalence was 7.9, 9.32, 5.03 and 8.06 %, respectively from 1985 to 1989. The author suggested that EL- camels are the second most affected animal species besides cattle Ansary *et al*., (2001) randomly collected 64 camel sera from 5 herds. All sera were screened for *Brucella* antibodies by the slide agglutination test.

Seroprevelance for brucellosis was 0%. Musa and Shigidi (2001) examined 3,303 camel sera in Nyala abattoir, Sudan. Of which 3,274 camels were examined by conventional serological tests as RBT, SAT and CFT. 256 (7.82%) were positive. The remaining 29 sera were examined by RBT and competitive ELISA (cELISA). Four (13.8%) out of the 29 sera 16 samples examined by cELISA were positive, while only 3 (10.3%) were positive by RBT. Yagoub (2005) examined 756 camel serum samples. Only 12 (1.6%) showed high agglutination titres**.** On the other hand *Brucella* was not isolate from the herd. Omer *et al.* (2007) estimated the prevalence of brucellosis in camels in Kassala area during 2004 to 2006. The serum samples were collected from 14,372 camels. All samples were investigated using 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.

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

The prevalence of the disease in cattle and camels was medium and high but low in sheep and goats. *B. abortus* biovars 1, 3, 6 and 7 and *B. melitensis* biovars 2 and 3 were isolated in the Sudan. *B. abortus* biovar 6 and *B. melitensis* biovar 3 are associated with infection in indigenous animals throughout the country, but the other biovars occurred in cross breed dairy cattle in Khartoum town only. Prevalence of *B. melitensis* in sheep and goats and its spread to the secondary hosts, specially cattle and camel poses health and control problems. Work is going on in South

Darfur, El Gazera, South Kordofan and Sennar to reveal the present situation of the disease and brucella species biovars associated with infections (Musa *et al*., 2008).

1.4.10 Isolation of *Brucella*

Br. abortus was isolated from aborted bovine foeti (Bennett, 1943; Dafaalla and Khan, 1958; Musa and Mitchell, 1985; Khalafalla *et al.*, 1987 and Musa and Jahans, 1990). The organism was also isolated from synovial fluid of cattle by Shigidi and Razig (1973), from bovine milk (Ibrahim, 1975; Khalafalla *et al.*, 1987; Suliman, 1987 and Musa, 1995) from camels in Butan area (Agab *et al.*, 1995). *Br. melitnsis* was isolated from the milk of cattle, sheep and goats (Dafaalla and Khan, 1958) and from a ram in an infected flock (Musa, 1995). According to Musa (1995) the strains of *Br. abortus* isolated in the Sudan were typed as *Br.abortus* biovar 6 and those of *Br.melitensis* as *Br.melitensis* biovar3.

Chapter two

Materials and methods

2.1 Study area

The Red Sea State occupies an area of 218,887 km2, in eastern Sudan, between longitudes 17-22ºN and latitudes 23-38ºE. And is bordering Egypt to the North, Kassala State to the South, and river Nile State to the West and the Red Sea to the East. The State is sub-divided into eight mahallias (localities or districts): Port Sudan, Suakin, Gunub/Aulib, Sinkat, Hayya, Halaib and Tokar/Agig. Figure (1)

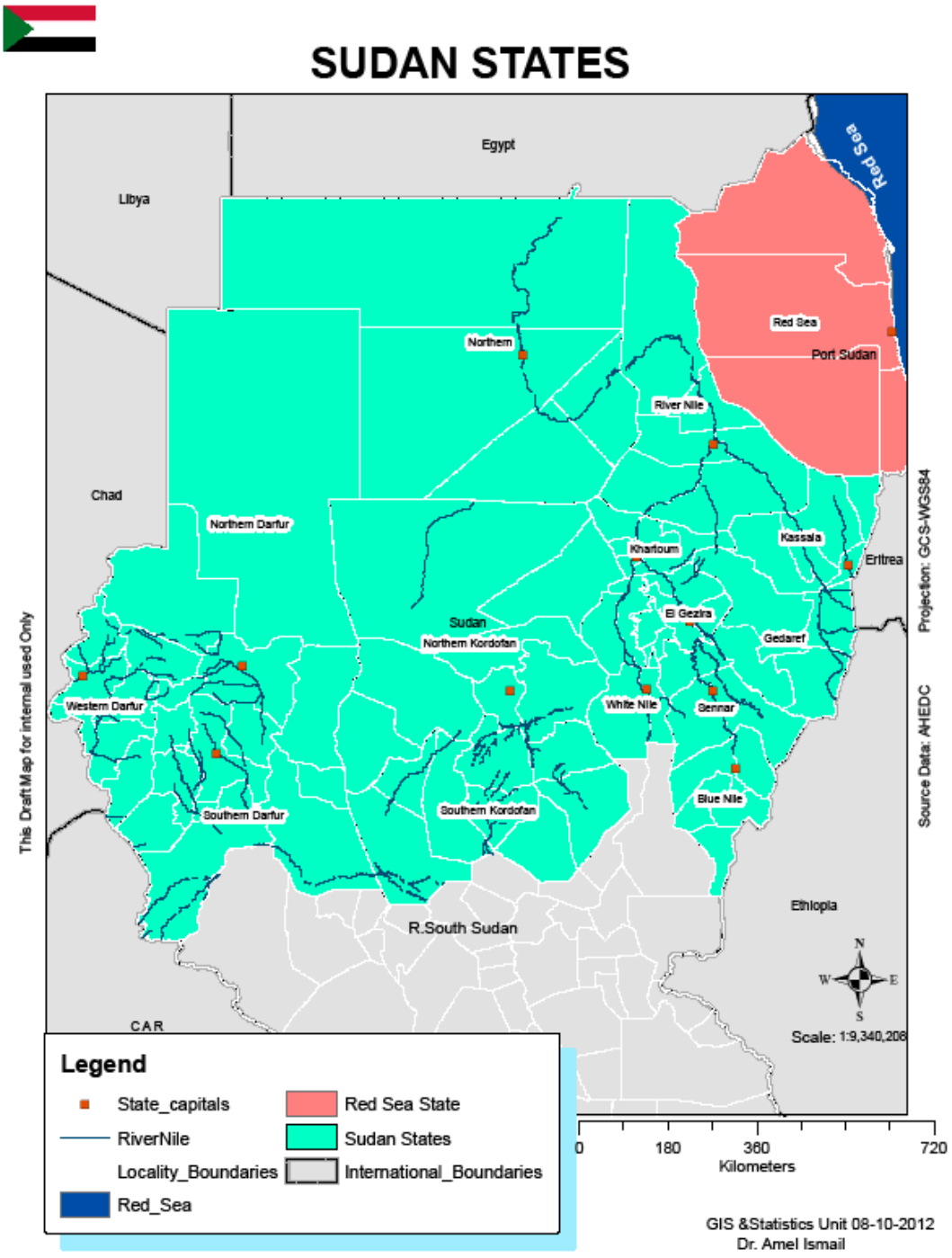

Figure 1: Map of SUDAN and Port Sudan showing the location of Suakin quarantine mager and its bordering countries.

2.2 Serum samples

A total of 1500 sera samples were collected from camels and 1500 sera sample were collected from goats intended for exportation in the period from (2019-2020).

2.2.1 Collection of blood

About 3ml to 5 ml of blood was collected from the jugular vein of animals in a plain tube with serum clot activator.

2.2.2 Separation of sera

Following the collection of blood samples, the vaccutainer tubes were put in rack then after clotting the samples were separated by centrifugation at 3000rpm x 15 second the sera were tested by Rose Bengal Plate test immediately for detection of brucella antibodies . Then separated sera were collected in a screw capped plastic vials and transported to the laboratory of the Centeral Veterinary Research laboratory (CVRL), Soba where they were stored at -20ºC till used.

2.3 Serological test

All sera samples were screened for antibodies against *Brucella* by RBPT as screening test. The test was done in quarantine meager lab in Suakin

2.3.1.1 Antigen for the test

The antigen used in the RBPT was obtained from Central Veterinary Research Laboratory, Soba (CVRL, Soba). The antigen was Prepared and standardized as described by OIE manual (2016).

2.3.1.2 Procedure of the test

The test was performed according to the OIE manual (2016), RBPT antigen was brought to room temperature then 75 µl of serum were transferred to a clean, dry and non greasy plate by micropipette. The antigen bottle was shaken to ensure homogenous suspension then 25 µl of the antigen was added. The antigen and serum were mixed thoroughly with a spreader and then the plate was agitated gently for 4 minutes by shaker. The result were noted immediately after 4 minutes. Any degree of agglutination was regarded as positive result, while no agglutination was Regarded as negative result.

2.3.2 Standard tube agglutination Test (SAT)

It is a quantitative test used to detect of immune globulins classes. The test was carried out according to the method used by OIE manual (2016)

2.3.2.1 Procedure of the test

To overcome the prozone phenomenon, if any to occur, a raw of seven Wasserman tubes was used per samples. An amount of 0.8 ml of phenol-saline was placed in first tube and 0.5 ml was placed in each of the remaining tube. To the first tube, 0.2 ml of the serum to be tested was added and thoroughly mixed, then 0.5 ml of the mixture was transferred to the second tube, from which, (after mixing), 0.5 ml of the diluted serum dilution was transferred to the next tube and so on then 0.5 ml of diluted serum from the last tube was discarded. This results in two fold dilutions of serum (1:5, 1:10, 1:20, and so on).By using an automatic pipette, 0.5 ml of diluted slandered antigen was added to each tube .(standard antigen was diluted 1 ml : 9 ml using phenol saline).

The Contents of the tube were thoroughly mixed by shaking the racks. This gives final dilution 1:10 to 1:640.The tubes were then incubated at 37 ^{o}c overnight. The Standard tubes were prepared at the time of the test and incubated with them. The antigen was diluted by mixing 2 ml of

antigen with 2ml of phenol- saline, the 5 standard tubes were prepared as following :In the first tube: 1 ml phenol saline as ++++, in the second tube 0.75 ml phenol saline with 0.25 ml diluted antigen (1:2) as +++, in the third tube 0.5 ml phenol saline with 0.5 ml diluted antigen as ++, in the fourth tube 0.25 phenol saline with 0.75 ml diluted antigen as + and in the last tube 1 ml of diluted antigen as -

2.3.2.2 Interpretation of the results

The degree of agglutination was assessed by the amount of clearing that had taken place in the tubes compared with the standard tubes. The tubes were examined, without being shaken, against ablack background. With a source of light coming from above and behind the tubes, complete agglutination and sedimentation with water-clear supernatant was recorded as ++++, nearly complete agglutination and 75% clearing as +++, marked agglutination and 50% clearing as ++, some sedimentation and 25% clearing as +, and no clearing as negative.

2.3.3 Buffered Acidified Plate Antigen (BAPA)

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

2.3.3.1 Materials

- Standard BAPA test antigen
- Control sear (negative, low positive and high positive).
- Adjustable pipette, with disposable tips
- Minnesota testing box with glass plate. (illuminator with indirect light source,black background, and lid to prevent evaporation of test materials)
- Stirrer/spreader
- Paper towel

2.3.3.2 Test procedure

The samples and antigen were allowed to come to room temperature About 20, 40 and 80 µl of sample were placed on the centre of glass plate of the Minnesota testing box. Known high positive, controls were included in each day's work. 30µl of BAPA antigen were added to each quantity of serum (Mix 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. (Spreaders should be rinsed in water and wiped dry between samples). The glass plates were tilted in a circular motion for 4 rotations and leave for 4 minutes in the Minnesota box with the lid covered. And Rotated 4 times again, incubated for another 4 minutes in the box and finally rotated 4 further rotations.

The reaction was read immediately against the illuminated background of the Minnesota box. Any visible agglutination within 8 minutes was considered positive. Complete agglutination with very clear fluid as ++++, nearly complete agglutination with clear fluid as +++, marked agglutination with less clear fluid as ++, slide agglutination with turbied fluid as + and no agglutination within 8 minutes is negative.

2.3.4 Enzyme-linked Immune Sorbent assay (ELISA)

Competitive Enzyme- Linked Immune Sorbent (c-ELISA) assay for camel and Indirect Enzyme- Linked Immune Sorbent assay (I-ELISA) for small ruminant used (Boehringer Ingelhein Svanova Kitts) were used in Brucella Department in Central Veterinary Research Centre, Soba.

2.3.4.1 Materials

1- Precision pipettes.

2- Disposable pipette tips

3- Distilled, deionised or any similar high quality water.

4- Wash bottle, multichannel pipette or plate washer.

5- Container 1 to 2 liters for PBs-tween.

6- Micro plate photometer, 450nm filter.

2.3.4.2 Test procedure for Competitive ElISA

1- All reagents were equilibrated to room temperature 18-25ºc before use.

2- Adding samples:

A- 45 µl of sample Dilution Buffer were added into each well to be used for serum samples, serum controls and conjugate controls.

B- 5µl of positive, weak positive and negative serum controls were added into each of the appropriate wells, respectively .For confirmation purposes it is recommended to run the control sera in duplicates.

C- 5 µl of Sample Dilution Buffer were added into two appropriate wells (designated as Conjugate Control, Cc).

D- 5 µl of test sample were added to each of the appropriate wells. The samples can be tested in singlicates. However for confirmation purposes it is recommended to run the samples in duplicates.

3- 50 µl of mAb-Solution were added into all wells used for controls and samples.

4- The plate was sealed and the reagents mixed thoroughly for 5 minutes, either by using a plate shaker or by tapping the sides of the plate.

5- The plate was incubated at room temperature 18-25ºc for 30 minutes.

6- The plates/strips were rinsed 4 times with PBS-Tween Buffer, filled up the wells at each rinse; the plate was empted and taped hard to remove all remains of fluid.

7-100 µl of Conjugate Solution were added into each well .The plate were sealed and incubated at room temperature 18-25ºc for 30 minutes.

8- The plates/strips were rinsed 4 times with PBS-Tween Buffer.

9- 100 µl Substrate Solution were added to each well and incubated for 10 minutes at room temperature 18-25ºc.

10- The reaction was stopped by adding 50 µl of Stop Solution to each well and mixed thoroughly.

11- The optical density (OD) was measured of the controls and samples at 450 nm in micro plate photometer (air was used as blank). The OD was measured within 15 minutes after the addition of stop solution to prevent fluctuation in OD values.

2.3.4.3 Test procedure for Indirect Elisa (I-Elisa) for small ruminant

1- All reagents were equilibrated to room temperature 18-25ºc before use. Each strip was labeled with a number.

2- Samples were added.

A- 100 µl of sample Dilution Buffer were added to each well that was used for serum samples and serum controls.

B- 4µl of positive control serum (Reagent A) and 4µl of negative control serum (Reagent B) respectively were added to selected wells coated with *Brucella* antigen. For confirmation purposes it is recommended to run the control sera in duplicates.

C- 4 µl of serum sample were added to selected well coated with Brucella antigen. The sample was tested singlicates.

3- The plate was shacked thoroughly. The plate were sealed and incubated at 37ºc for 1 hour.

4- The plate was rinsed 3 times with PBS-Tween Buffer: filled up the wells at each rinsed, the plate were emptied and taped hard to remove all remains of fluid.

5- 100 µl of HRP Conjugate were added to each well and incubated at 37ºc for 1 hour.

6- The plate was rinsed 3 times with PBS-Tween Buffer.

7- 100 µl Substrate Solution were added to each well and incubated for 10 minutes at room temperature 18-25ºc.

8- The reaction were stopped by adding 50 µl of Stop Solution to each well and mixed thoroughly. The Stop solution was added in the same order as the Substrate solution was added in Step 7.

9- The optical density (OD) was measured of the controls and samples at 450 nm in micro plate photometer. The OD was measured within 15 minutes after the addition of stop solution to prevent fluctuation in OD values.

2.3.4.4. Preparation of reagents

2.3.4.4.1 PBS-Tween Buffer

The PBS-Tween Solution was diluted 20xconcentrate 1/20 in distilled water, 500 ml per plated were prepared by added 25 ml PBS-Tween solution to 470 ml distilled water and mixed thoroughly.

2.3.4.4.2 Anti-ruminant IgG conjugate

The lyophilized HRP Conjugate was reconstituted with 11.5 ml PBS-tween Buffer, the buffers were added carefully to the bottle. The solution left for one minute and mixed thoroughly. Conjugate was prepared immediately before use. The remaining reconstituted conjugate was stored at -20ºc.

2.3.5 Statistical Analysis

Data were statistically analyzed by using statistical package for social science SPSS (2008).The agreement between serological tests was calculated using kappa analysis

Chapter 3

Results

3.1 Serological tests for Camels:

This study was planned to valuation of serological test used for diagnostic brucellosis in Camel and Goat intended for export in Sudan. In the period from (2019-2020).

A total of 1500 serum samples for Camels collected from Suakin Veterinary Quarantine were subjected to the four recommended serological tests.

3.1.1 Rose Bengal Plate Test (RBPT)

Out of the 1500 serum samples from Camels 185 (12.3%) were positive for brucellosis by the Rose Bengal Plate test (RBPT) Fig (2) and table (3)

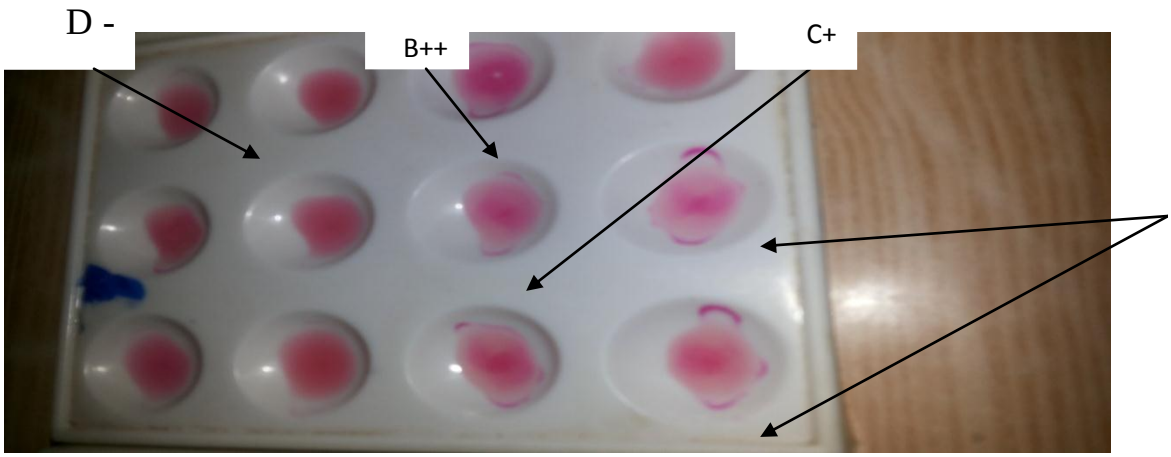

Fig (2): Rose Bengal Plate Test (RBPT) demonstrated the degree of agglutination from (+, ++, +++) of *Brucella* in Camel

Key:

Positive	
A	+++
B	++
C	+
Negative	
D	–

Table (3): Serological result according to diagnostic techniques of *Brucella* in Camel

Test	Total sample-No	Positive	Negative	Infection Rate%
RBPT	**1500**	**185**	**1315**	**12.3%**
BAPA	**185**	**143**	**42**	**77.3%**
SAT	**185**	**73**	**112**	**39.5 %**
c-ELISA	**185**	**102**	**83**	**55.1%**

3.1.2 Buffered Acidified Plate Antigen (BAPA)

All positive sera screened by RBPT 185/1500 (12.3%) were retested by Buffered Acidic Plate Antigen (BAPA) test , the result showed 143/185 (77.3%) were Positive and 42/185 (22.7%) were negative sera. The cross tabulation by statictical analysis comparing between RBPT and BAPA test were showed that 143/185 (77.3%) of the RBPT positive sera were found positive with BAPA and 42/185 (22.7%) of the positive sera by RBPT were found negative with BAPA. Show fig (3,4) and table (3,4)

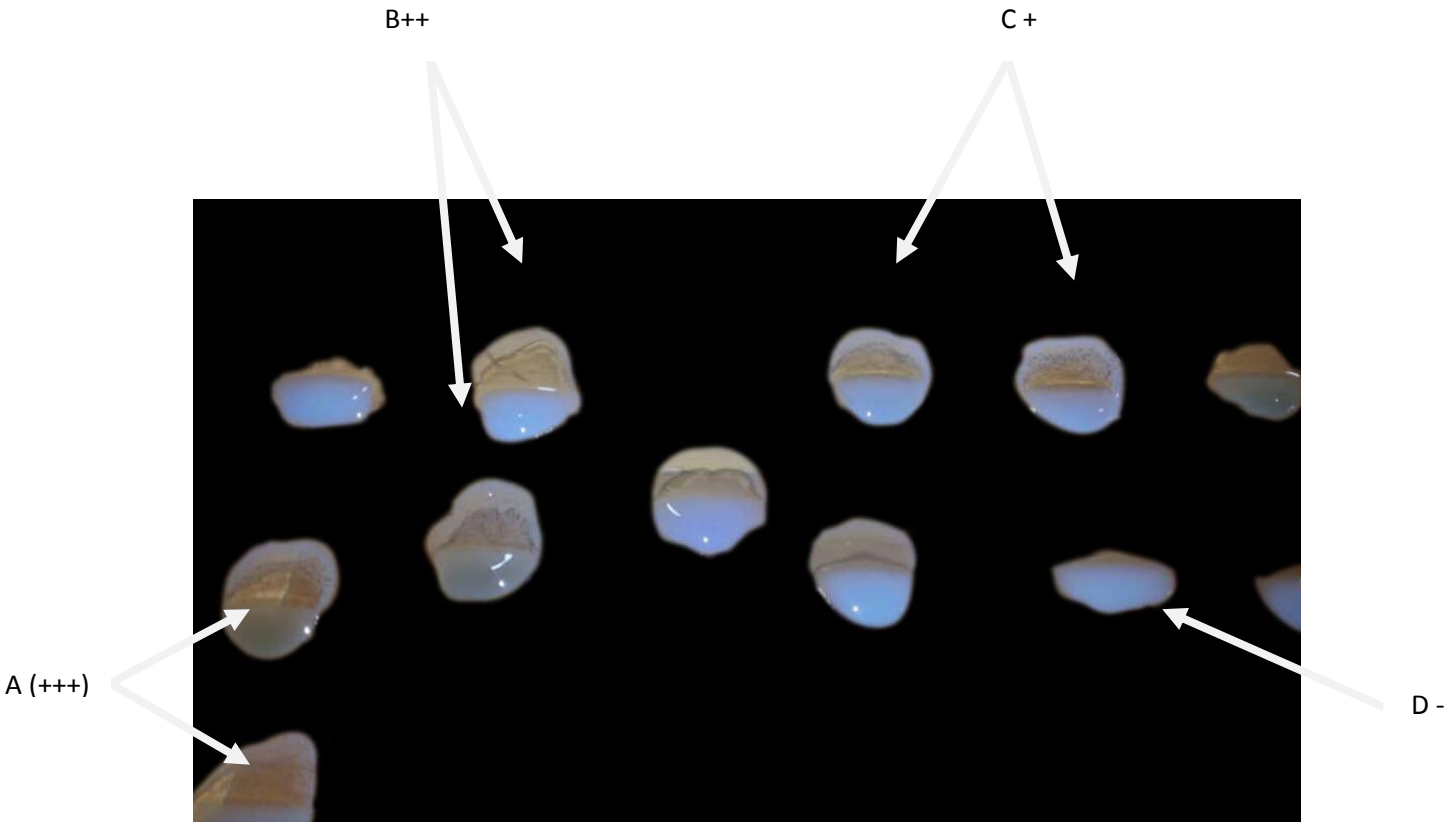

Fig (3): Buffered Acidified Plate Antigen (BAPA) demonstrated the degree agglutination (+, ++, +++) of *Brucella* in Camel

Key:
Positive
A +++
B ++
C +
Negative
D –

Table (4): cross tabulation between RBPT and BAPA test used for Brucellosis in Camel

			BAPA		Total
			+ve	- ve	
RBPT	**+ve**	**Count**	143	42	185
		% within RBPT	77.3%	22.7%	100.0%
		% within BABA	100.0%	100.0%	100.0%

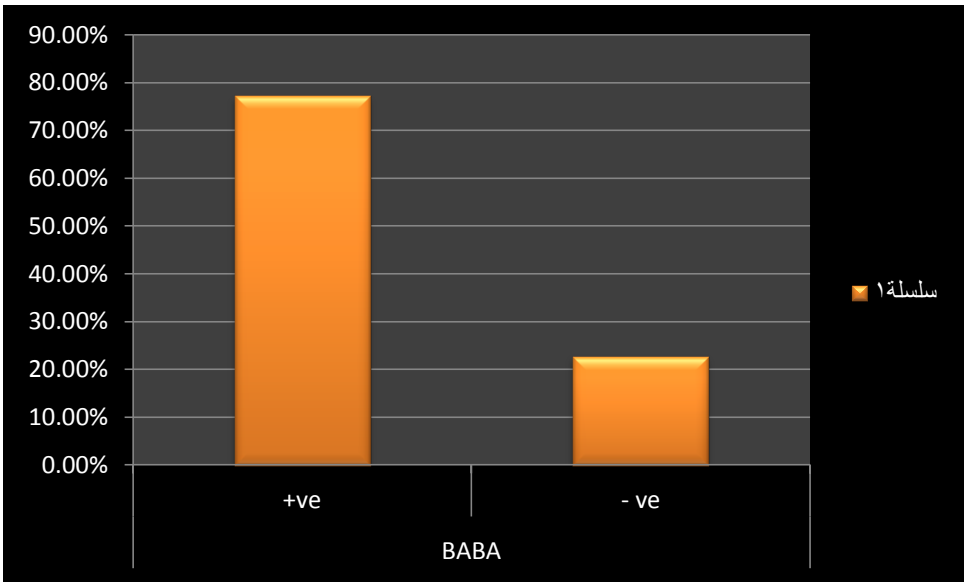

Fig (4): Cross tabulation between RBPT and BAPA test used for Brucellosis in Camel

3.1.3 Standard tube AgglutinationTest (SAT)

Positive sera tested by RBPT 185/1500 (12.3%) were retested by Standard tube Agglutination Test (SAT) , the result showed 73/185 (39.5%) were Positive and 112/185 (60.5%) negative sera. The cross tabulation by statictical analysis comparing between RBPT and SAT test were showed that 73/185 (39.5%) of the RBPT positive sera were found positive with SAT and 112/185 (60.5%) of the positive sera by RBPT were found negative with SAT. fig (5,6) and table (3,5)

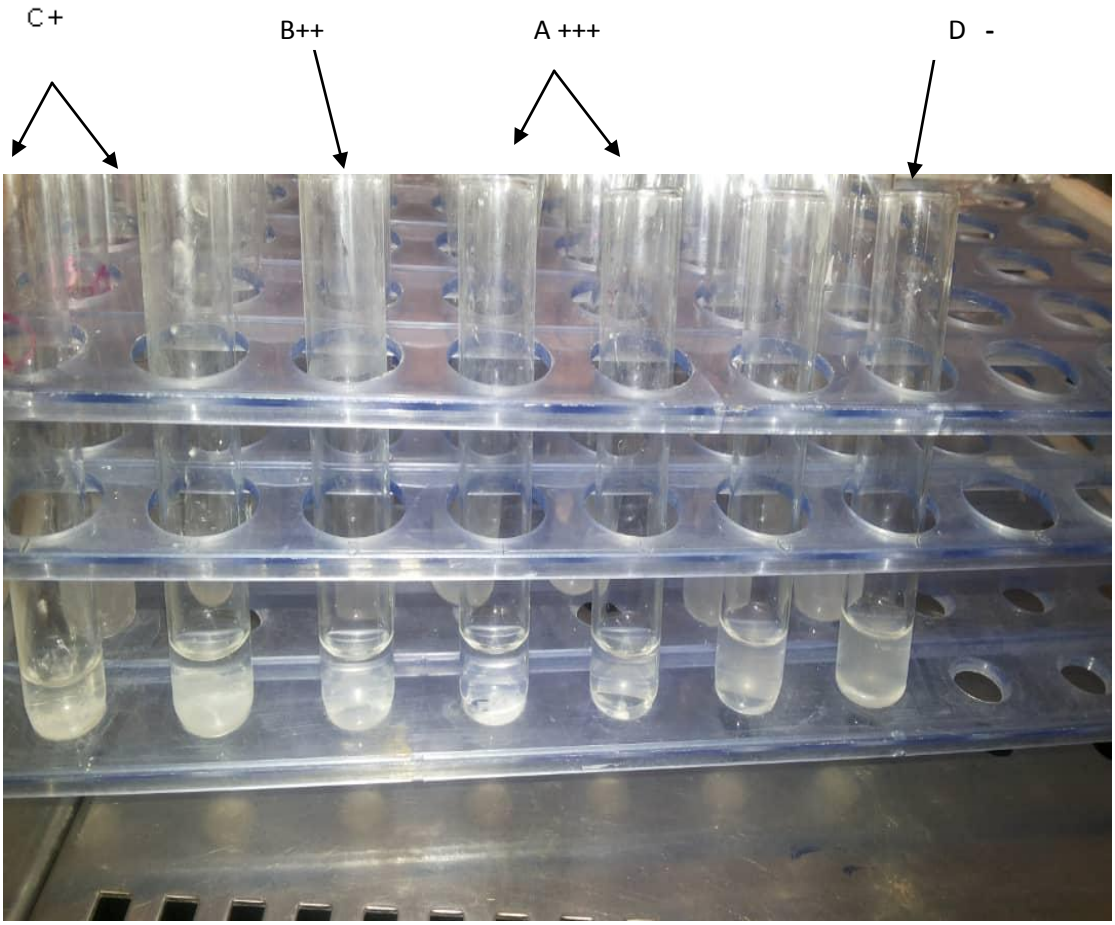

Fig (5): Standard tube agglutination Test (SAT) showing different degrees of Precipitation of *Brucella* antigen-antibody reaction in Camel

Key:

Positive	
A	+++
B	++
C	+
Negative	
D	–

Table (5): cross tabulation between RBPT and SAT test used for Brucellosis in Camel

			SAT		Total
			+ve	- ve	
RBPT	+ve	Count	73	112	185
		% within RBPT	39.5%	60.5%	100.0%
		% within SAT	100.0%	100.0%	100.0%

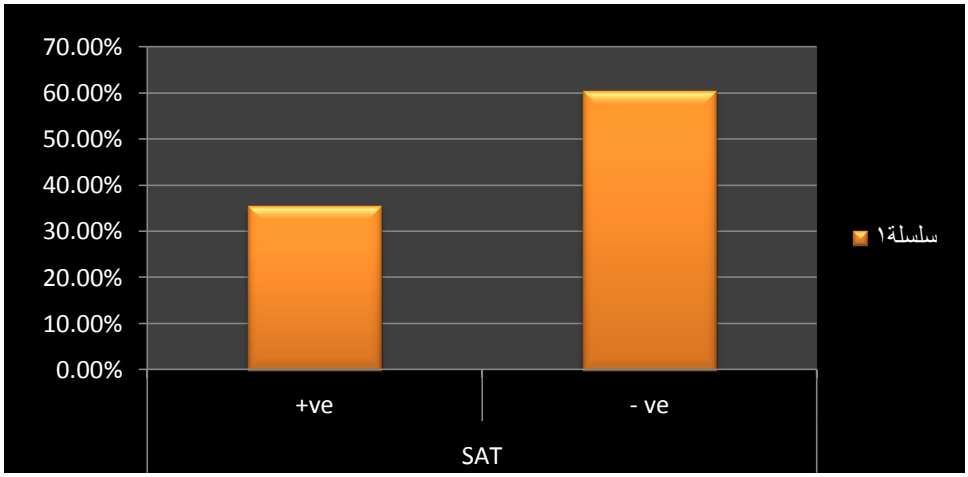

Fig (6): Cross tabulation between RBPT and SAT test used for Brucellosis in Camel

3.1.4 Competitive Enzyme-linked immune sorbent assay(c-ELISA)

Positive sera tested by RBPT 185/1500 (12.3%) were conformted by Competitive Enzyme-linked immune sorbent assay(c-ELISA**)** the result showed 102/185 (55.1%) Positive and 83/185 (44.9%) negative sera.

The cross tabulation by statictical analysis comparing between RBPT and c-ELISA test were showed that 102/185 (55.1%) of the RBPT

positive sera were found positive with c-ELISA and 83/185(44.9%) of the positive sera by RBPT were found negative with c-ELISA. fig (7,8)

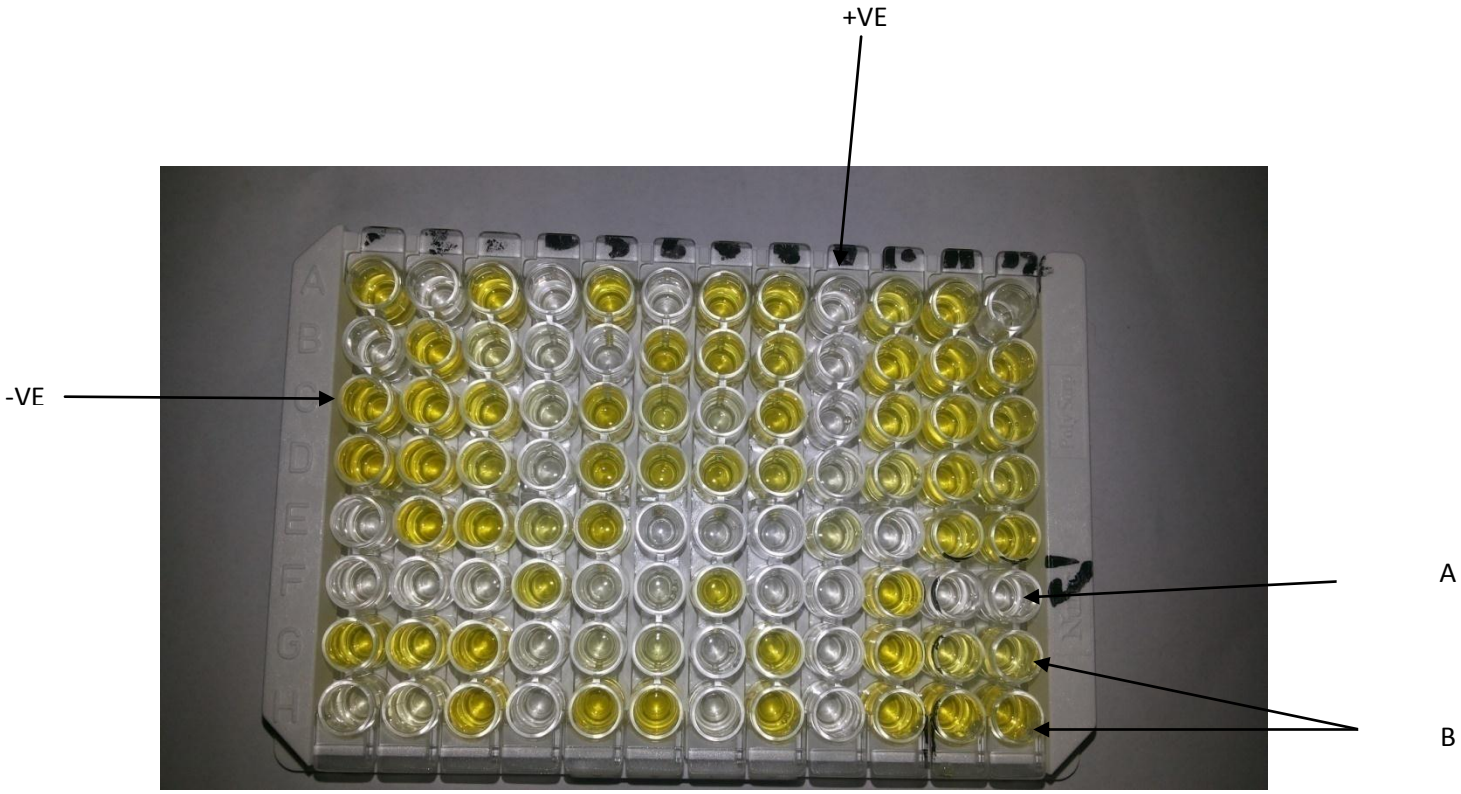

Fig (7): Competitive Enzyme-linked immune sorbent assay (c-ELISA): showing positive and negative results in the presence of standard serum controls of *Brucella* in camel

Key:

White colure is Positive result

Yellow colure is Negative result

Control:

A positive control

B negative control

Table (6): cross tabulation between RBPT and c-ELI test used for Brucellosis in Camel

			c-ELISA		Total
			+ve	- ve	
RBPT	+ve	Count	102	83	185
		% within RBPT	55.1%	44.9%	100.0%
		% within c-ELISA	100.0%	100.0%	100.0%

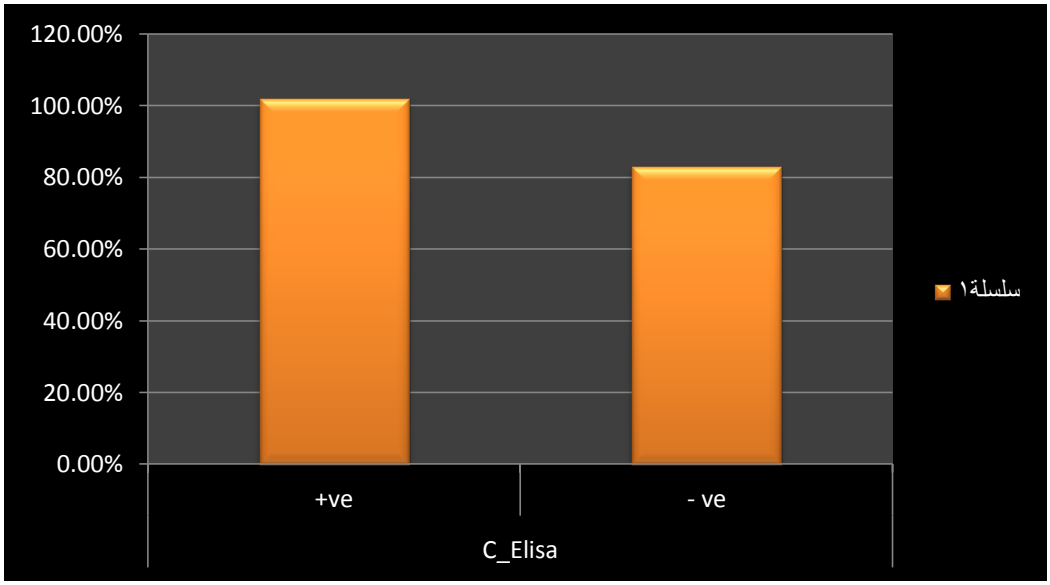

Fig (8): Cross tabulation between RBPT and c-ELISA test used for Brucellosis in Camel

3.2 Prevalence of *Brucella* in Camel:

The prevalence of Brucellosis in Camel was found 12.3 %, 77.3 %, 39.5 %, and 55.1% by RBPT, BAPA, SAT and c-ELISA respectively, Statistical analysis gave same result. Fig (9) and Table (7)

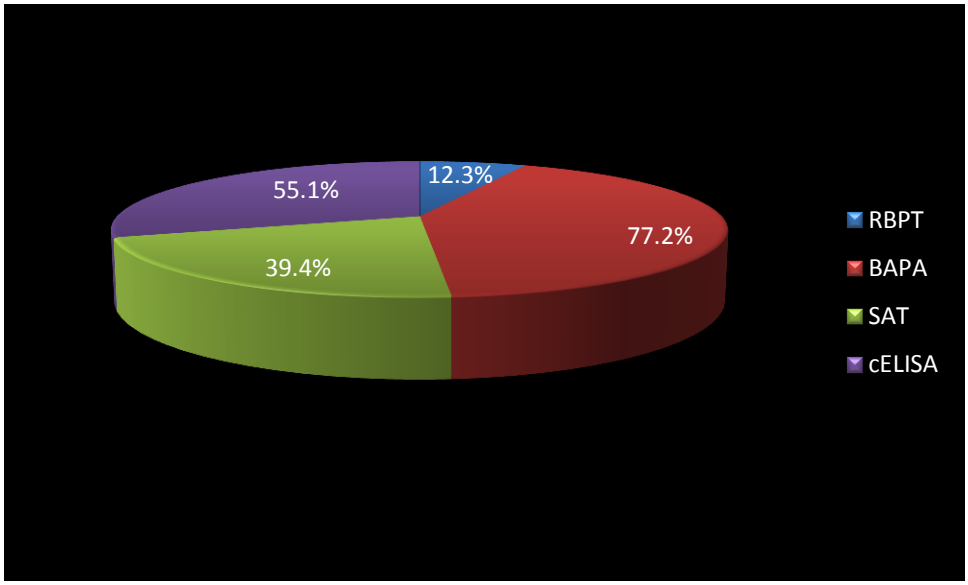

Fig (9): Occurrence of *Brucella* in Camel by using different Diagnostic tests

Table (7): Cross tabulation between RBPT, BAPA, SAT and c-ELISA tests used For Brucellosis in Camel

RBPT	BAPA		SAT		c-ELISA	
	+ve	- ve	+ve	- ve	+ve	- ve
COUNT	143	42	73	112	102	83
PRECENTAGE	77.3%	22.7%	39.5%	60.5%	55.1%	44.9%

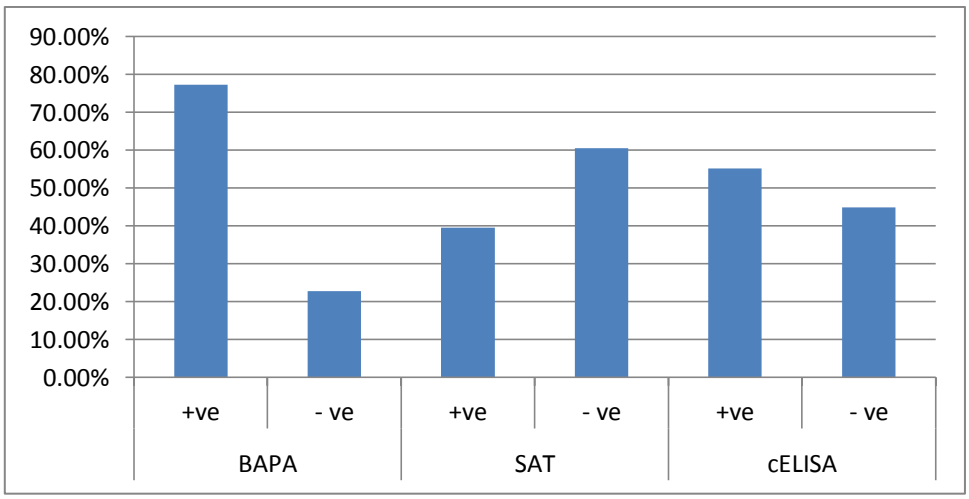

Fig (10): Positivity of Brucellosis used RBPT and the three tests in Camel

3.3. Serological tests in Goat:

A total of 1500 serum samples for Goat collected from Suakin Veterinary Quarantine were subjected to the four recommended serological tests.

3.3.1 Rose Bengal Plate Test (RBPT)

Out of the 1500 serum samples from Goats 180 (12%) were positive for brucellosis by the Rose Bengal Plate test (RBPT) Fig (2) and table (3)

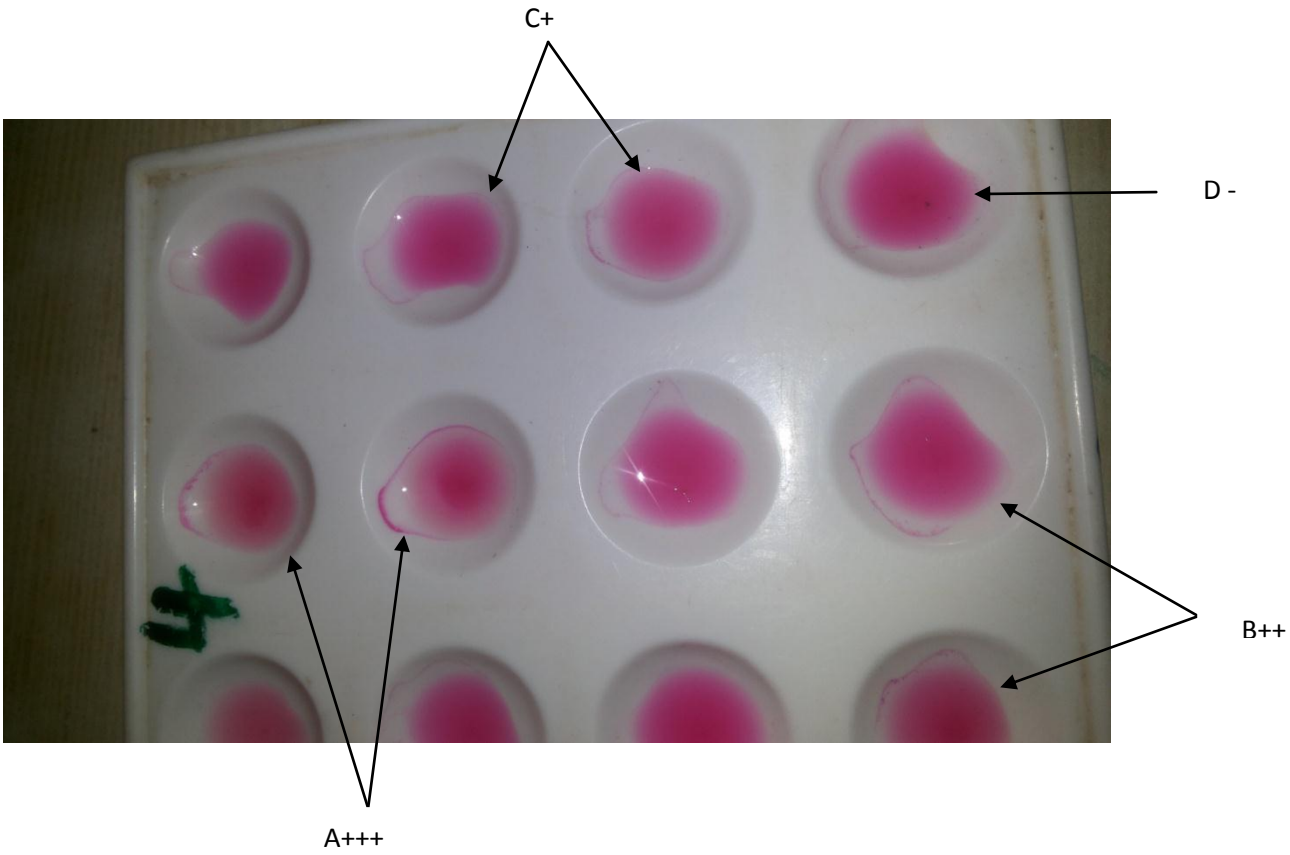

Fig (11): Rose Bengal Plate Test (RBPT) demonstrated the degree of Agglutination from (+, ++, +++) of Brucellosis in Goat

Key:

Positive	
A	+++
B	++
C	+
Negative	
D	–

Table (8): Serological result according to diagnostic techniques of *Brucella* in Goat

Test	Total sample-No	Positive	Negative	Infection Rate%
RBPT	**1500**	**180**	**1320**	**12 %**
BAPA	**180**	**135**	**45**	**75 %**
SAT	**180**	**36**	**144**	**20 %**
I-ELISA	**180**	**46**	**134**	**25.6 %**

3.3.2 Buffered Acidified Plate Antigen (BAPA)

All positive sera screened by RBPT 180/1500 (12%) were retested by Buffered Acidic Plate Antigen (BAPA) test , the result showed 135/180 (75%) were Positive and 45/180 (25%) were negative sera.

The cross tabulation by statictical analysis comparing between RBPT and BAPA test showed that 135/180 (75%) of the RBPT positive sera were found positive with BAPA and 45/180 (25%) of the positive sera by RBPT were found negative with BAPA. fig (3,4) and table (3,4)

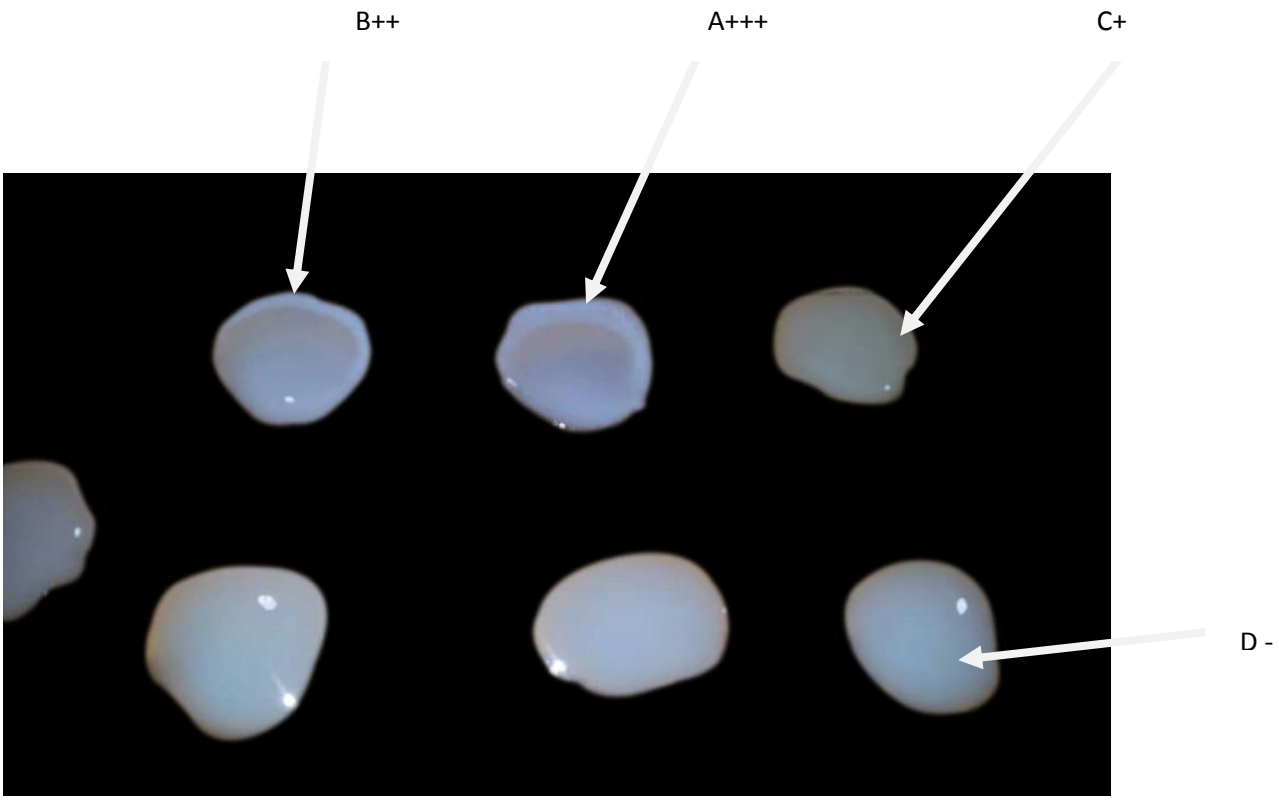

Fig (12): Buffered Acidified Plate Antigen (BAPA) demonstrated the degree agglutination (+, ++, +++) of *Brucella* in Goat

Key:
Positive
A +++
B ++
C +
Negative
D -

Table (9): Cross tabulation between RBPT and BAPA test used for Brucellosis in Goat

			BAPA		Total
			+ve	- ve	
RBPT	+ve	Count	135	45	180
		% within RBPT	75.0%	25.0%	100.0%

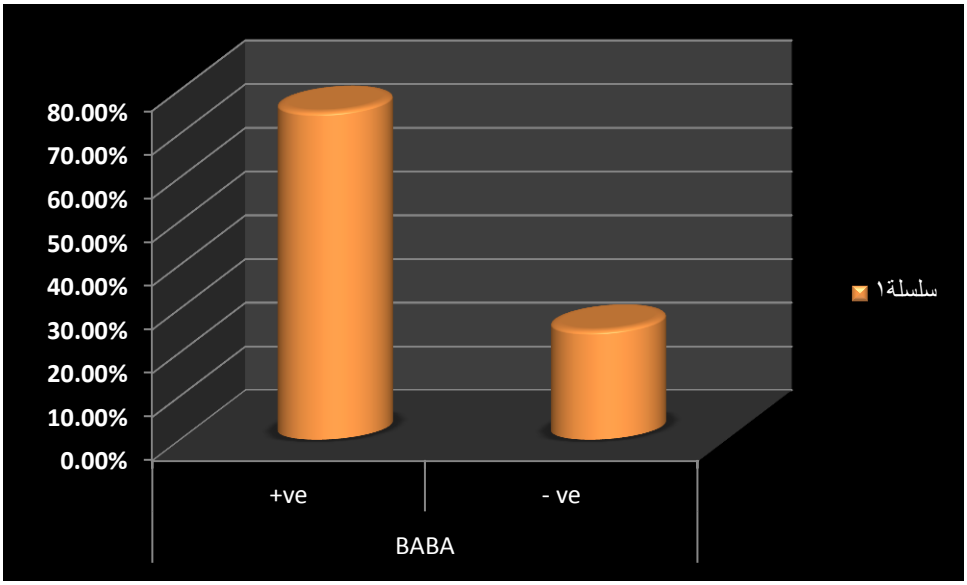

Fig (13): Cross tabulation between RBPT and BAPA test used for Brucellosis in Goat

3.3.3 Standard tube AgglutinationTest (SAT)

Positive sera tested by RBPT 180/1500 (12%) were retested by Standard tube Agglutination Test (SAT) , the result showed 36/180 (20%) were Positive and 144/180 (80%) negative sera .

The cross tabulation by statictical analysis comparing between RBPT and SAT test were found that 36/180 (20%) of the RBPT positive sera were found positive with SAT and 144/180 (80%) of the positive sera by RBPT were found negative with SAT . fig (5,6) and table (3,5)

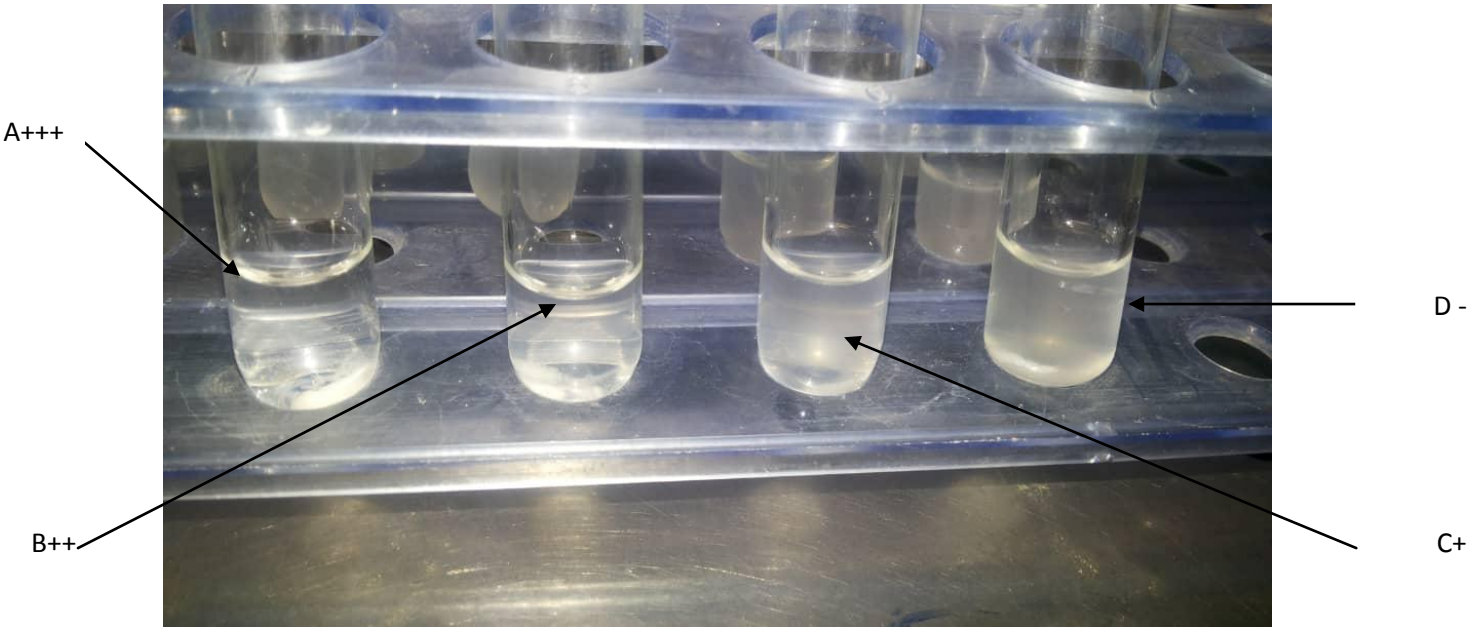

Fig (14): Standard tube agglutination Test (SAT) showing different degrees of Preseptation of *Brucella* antigen-antibody reaction in Goat

Table (10): cross tabulation between RBPT and SAT test used for Brucellosis in Goat

			SAT		Total
			+ve	- ve	
RBPT	+ve	Count	36	144	180
		% within RBPT	20.0%	80.0%	100.0%

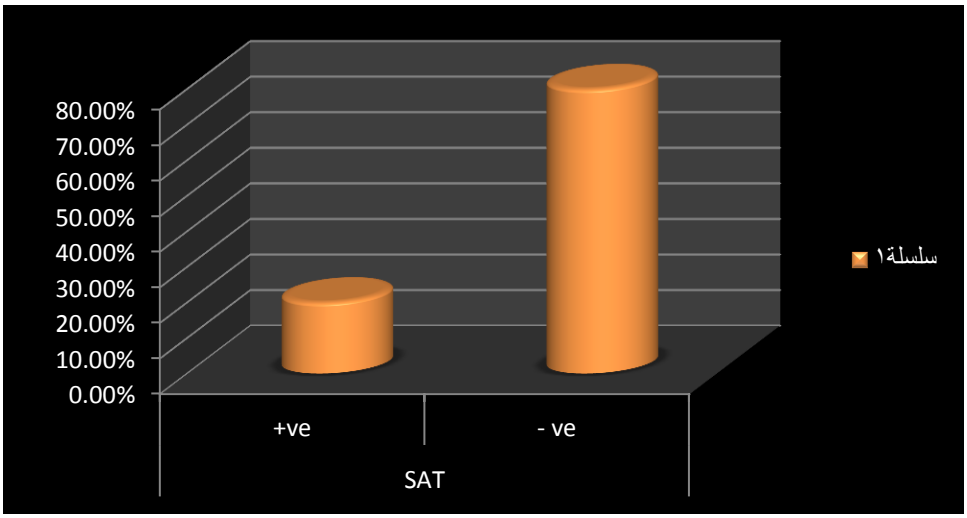

Fig (15): Cross tabulation between RBPT and SAT test used for Brucellosis in Goat

3.3.4 Indirect Enzyme-linked immune sorbent assay (I-ELISA)

Positive sera tested by RBPT 180/1500 (12%) were conformted by Indirect Enzyme-linked immune sorbent assay (I-ELISA**)** the result showed 46/180 (25.6%) were Positive sera and 134/180 (74.4%) were negative sera.

The cross tabulation by statictical analysis comparing between RBPT and I-ELISA test were showed that 46/180 (25.6%) of the RBPT positive sera were found positive with I-ELISA and 134/180 (74.4%) of the positive sera by RBPT were found negative with I-ELISA . fig (7,8) and table (3,6)

Fig (16): Indirect Enzyme-linked immune sorbent assay (I-ELISA): showing positive and negative results in the presence of standard serum controls of *Brucella* in Goat

Key :

Yellow colure is Positive result

White colure is negative result

Control:

A positive control

B negative control

Table (11): Cross tabulation between RBPT and I-ELISA test used For Brucellosis in Goat

			I- ELISA		Total
			+ve	- ve	
RBPT	+ve	Count	46	134	180
		% within RBPT	25.6%	74.4%	100.0%

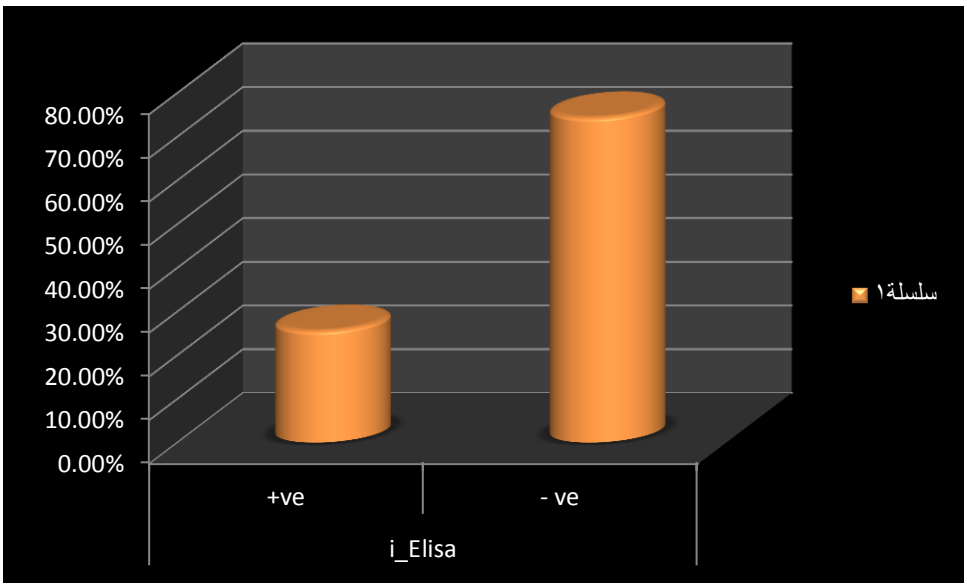

Fig (17): Cross tabulation between RBPT and I-ELISA test used for Brucellosis in Goat

3.4 Prevalence of Brucellosis in Goat:

The prevalence of Brucellosis in Goat was found 12 %, 75 %, 20 %, and 25.6% by RBPT, BAPA, SAT and c-ELISA respectively. Statistical analysis gave same result Fig (18) and Table (12) showed the result

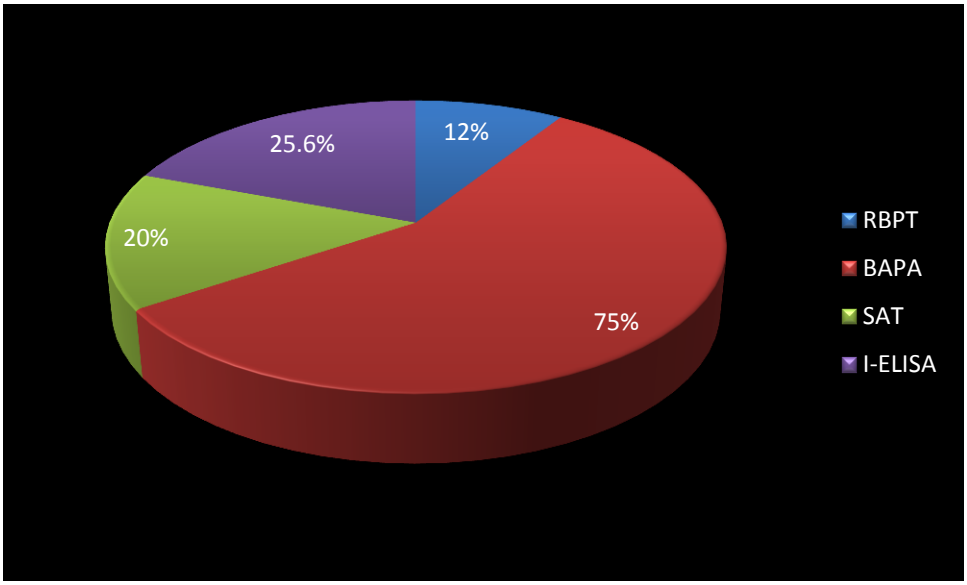

Fig (18) : Occurrence of *Brucella* in Goat by using different Diagnostic tests

Table (12): Cross tabulation between RBPT, BAPA, SAT and I-ELISA tests used for brucellosis in Goat

RBPT	BABA		SAT		I_ ELISA	
	+ve	- ve	+ve	- ve	+ve	- ve
COUNT	135	45	36	144	46	134
PRECENTAGE	75%	25 %	20%	80%	25.6%	74.4%

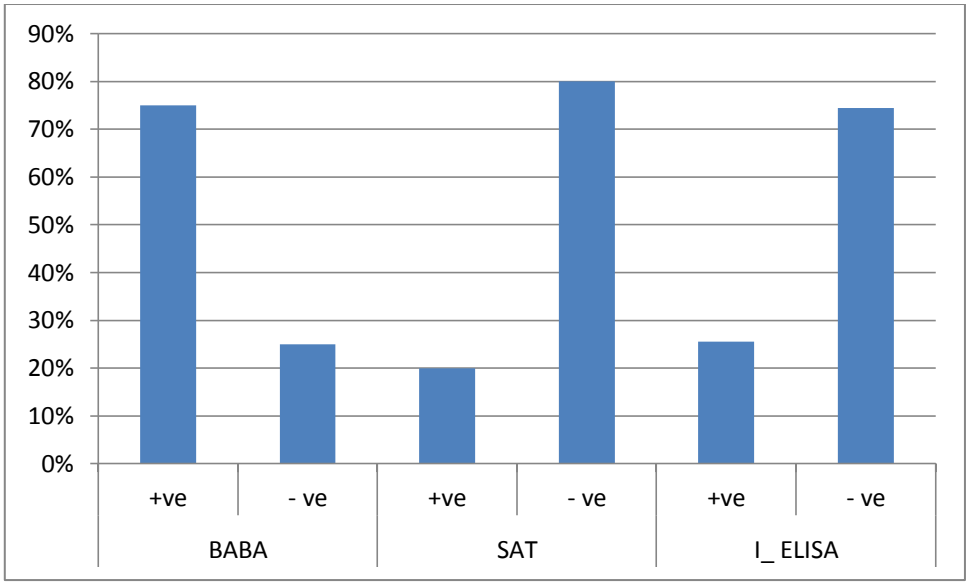

Fig (19): Positivity of Brucellosis used RBPT and the three tests in Goat

Chapter 4

Discussion

Brucellosis is a zoonotic infection caused by the bacterial of genus *Brucella* which was first recognized by David Bruce (Bruce, 1887). The disease is an old one that has been known by various names, including Mediterranean fever, Malta fever, gastric remittent fever, and undulant fever. Humans are accidental hosts, but brucellosis continues to be a major public health concern worldwide and is the most common zoonotic infection, (WHO, 2020).

Brucellosis is an important zoonotic bacterial disease widespread in the world, (Tian, *et al*, 2020). It has high public health significance and may poses threat to all human as diseases may transmit through consumption of raw and under cooked milk and milk products (Schelling *et al.*, 2003).

The signs of brucellosis in goats are similar to those in cattle. The disease is prevalent in most countries where goats are a significant part of the animal industry, and milk is a common source of human brucellosis in many countries. The causal agent is *Brucella melitensis.* Infection occurs primarily through ingestion of the organisms, (Nicoletti, 2013).

Sudan exports camels, goats, sheep and cattle to many countries especially to Saudi Arabia and other Arabic Gulf countries (Anon, 2011). These exported numbers influence with epidemics that emerge spontaneously. The number of tested animals in different species for Brucellosis depends on this exportation movement.

The present study was conducted to determine the occurrence of brucellosis by use of comparative serological tests, precisely, BAPA, SAT and ELISA for confirmation of Brucella detected with RBPT positive sera in Camels and Goat destined for export in the Sudan,. The

importing countries reexamine the animals with the same test upon arrival and reject the consignment if the total positive cases exceeded 3%. The RBPT is a sensitive test and is recommended by the OIE (2016) for screening animals for export and control measures.

This study also aimed to determine the evaluation the four serological tests used to diagnostic of brucellosis in exported Camels and Goat.

A total of 1500 camels and 1500 goats were studied.

All samples were screened for brucellosis with the RBPT. The positives with RBPT were confirmed by BAPA, SAT and ELISA.

In Camel the results showed that the prevalence of brucellosis by RBPT was (12.3) (185/1500). In another study the overall seroprevalence was found 11.4% (n=35), out of these 35 RBPT-positive samples, the positivity of 18 and 17 were confirmed by SAT and c-ELISA, respectively (Eman *et al*, 2018).

12.3% is in line with Omer M.M *etal* (2000) who reported prevalence rate of 12.1% in camel in Sudan however it is higher than Mokhtar (2007) and Ahmed who reported in camel in Somalia prevalence of 9.4% and 3.9% respectively while it is lower than Musa (2012) and Al- Maijali (2008) who reported prevalence rate of 22.2% and 13.9% respectively.

Bitter (1986) examined 948 camels from different herds in eastern Sudan and reported a prevalence of 16.5- 32.3%. Musa (1995), who examined 416 camels from seven herds owned by nomads of the same clan of brucellosis in camels and other species in the Darfur region.

The difference in seroprevelance might be due to different sample size, management condition or due difference of study area.

In this study, the seroprevalence estimated by RBPT (185/1500) was confirmed by BAPA which revealed prevalence of 77.2% (143/185). This result is higher than that reported by Mahmoud *et al* (2017) and Abdel

Rashed (2004) who found that seroprevelence of 39.83%, 9.42% respectivitaly In Egypt.

In present study all positive sera samples by RBPT (185/1500) was tested by SAT, the prevalence was 39.4% (73/185) this result is lower than that reported by Gwida (2011), 70.6 % and Ayman El Behiry (2014) 77.56%.

Competitive ELISA was initially developed to improve the diagnostic specificity of immunoassays for brucellosis. The present study confirms the positive samples of m RBPT (185) by c-ILISA the Prevalence rate was found 55.1% (102/185) this result is lower than that found by Elamer Gafar *etal* (2014) in Sudan and Gwida (2011) in Egypt 87.5%, 68.8% respectively dairy systems, where *brucella* can readily spread.

Another study with slight decrease in the prevalence rate of the disease was conducted in Red Sea State. The sero-prevalence of camel (*Camelus dromedaries*) brucellosis based on mRBPT and c-ELISA, with a total of 400 sera collected from dromedary camel from different localities. The overall sero-prevalence rate in the State using mRBPT was 10.8% (No .of positive cases = 43), (Atif, *et al*, 2016).

Multivariate analysis showed that BAPA test is more sensitive than SAT and ELISA. According to the findings, RBPT and BAPA had showed higher positive cases than the c-ELISA. Although the SAT is less specific than RBPT, BAPA and c-ELISA. It is concluded that a combination of the RBPT as a screening test and the BAPA, SAT and competitive ELISA as a confirmatory test would be an appropriate choice for those working on export and control measures of brucellosis in camels.

In Goat the results showed that the Prevalence of brucellosis by RBPT was (12%) (180/1500) this result is lower than Waffa A. *etal* (2016) and

higher than Eman. *etal* (2018) in Sudan and lower than Kallungo *etal* (2013) in Nigeria who reported 38.9%,11.4% and respectively 25.8.

In this study, the seroprevalence estimated by RBPT (180/1500) was confirmed by BAPA which revealed Prevalence of 75% (135/180) Ghobashy *etal* (2009) and Montasser (2011) who reported 100% using BAPA test in Goat in Egypt.

In present study all positive sera samples by RBPT (180/1500) were tested by SAT. The Prevalence was 20 % (36/180), this is higher than many reports from different parts of the country which were between 0.3% and 6.0% Ahmed (2004), Rayas (2004), Elnasry *etal* (2001), Samah, (2015) and Solafa *etal* (2014). On the other hand, it is higher than a report from the Northern part of Sudan which was 16.3% Zein, (2015).

In present study all positive sera samples by RBPT (180/1500) was tested by I-ELISA the Prevalence was 25.6 % (46/180) this result is higher than Sulafa *et al* (2014) used c-ELISA and reported 18% in Sudan And lower than Mukladerul Ahmed *etal* (2011) who reported 66.7% using I-ELISA in Bengladesh, statistical analysis showed that BAPA test more sensitive than SAT and I-ELISA in Goat. Another study was carried out to investigate the seroprevalence of brucellosis in dairy cattle in Port Sudan, Red Sea state, the Sudan. Two hundred and fifteen blood samples were collected from three locations in Port Sudan were used. Sera samples were tested by RBPT, mRBPT, SAT and c-ELISA. The seroprevalence were 12.6% by RBPT and 21.4% by mRBPT. Furthermore, SAT confirmed the positivity of 93.0% of the RBPT- and mRBPT-positive samples with titers ranging from 20 IU/ml to 1488 IU/ml. The c-ELISA confirmed the positivity of 27, 21, and 25 of the RBPT, mRBPT, and SAT positives, respectively (Miada, *et al*. 2016), the increased seroprevalence rates were attributed to the nature of intensive dairy systems, where *brucella* can readily spread.

Statistical analysis showed that BAPA test is more sensitive than SAT and I-ELISA. According to the findings, RBPT and BAPA had showed higher positive cases than the I-ELISA. Although the SAT is less specific than RBPT, BAPA and I-ELISA.

All differences observed in the prevalence rates reported between different areas and countries are likely to be associated with different environmental and management conditions, the type of study conducted and sample size.

CONCLUSION

It can be concluded that**:-**

- Camel and Goat brucellosis according to serological diagnosis is prevailing at a low rate in Swakin Qurantine, Red Sea State.
- BAPA test was performed in this study and was considered as cheap quick and effective tests for diagnosis and screening of brucellosis, BAPA test and Modified RBPT techniques are both recommended to be used for diagnosis of the disease in Sudan.
- It is concluded that a combination of the RBPT as a screening test and the BAPA, SAT and ELISAs as a confirmatory test would be an appropriate choice for those working on export and control measures of brucellosis in Camel and Goat.

RICOMONDATION

- Comparative serological diagnostic procedures should be implemented for diagnosis of brucellosis eg: RBPT and BAPA as rapid tests and SAT and ELISAs must be applied as confirmatory tests for definitive serological results.
- This study highlights the disease situation in animals intended for exportation, more Studies are needed to be done as different animal

species respond differently to serological evaluation especially Camels and goats.

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