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**Sudan University for Science and Technology**

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**Seroprevalence Of Brucellosis In Sheep In El-Gedarif  
State- Sudan**

الانتشار المصلي لمرض البروسيلا في الضأن بولاية القضارف – السودان

**Athesis sumitted in Partial for the Requirements  
of Degree of Master in Preventive Veterinary Medicine  
(MPVM)**

**BY**

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## **DEDICATION**

My final gratitude is reserved to my wife Rawda Abdalla Abdu, my sons Fadi, Faris and my lovely daughters Shorog and Shafag, the rest of all my beloved families for their invaluable material and moral support in all facet of life and their constant.

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## ملخص :

تمت هذا لدراسه فى الفترة ما بين 2016م و2018م حيث تم جمع عدد 558 عينه سيرم من الضان من خمس محليات[باسنده،الفشقة،القلابات الغربية،القلابات الشرقية وبلدية القصارف].

تم استخدام تقنية التراص [الروزبنقال] وتقنية الاليزا للكشف عن الاجسام المضاده لمرض البروسيللا وتحليل تلك النتائج بالتركيز على الجنس والعمر والمحليه . كشفت هذه الدراسة إصابة العينات المفحوصة نسبة 10.8% باستخدام الروزبنقال ونسبة 603% باستخدام تقنية الاليزا .

اظهرت نتيجة تحليل الروزبنقال وجود اهمية معنويه فى المكان (P=000) ولم تظهر اهمية معنوية فى الجنس والعمر وكانت نسبة المرض فى الذكور 1107% وفى الاناث 8.3% وكانت محلية الفشقة بها اعلى نسبة من المرض 55% . و اقل نسبة 22.9% وفى الذكور والاناث على التوالى وكانت اكبر نسبه للمرض هى من اعمار من 1-2 سنه وكانت 48.6% وكانت اعلى نسبة مرض فى محلية باسنده وهى 71.4%، كماظهرت نسبة صفر % فى فحص الروزبنقال سيما فى الاليزا كانت 11.4% فى محلية بلدية القصارف .

كان الغرض من هذه الدراسة هو الكشف عن الاجسام المضاده لمرض البروسيللا باستخدام تقنية الروزبنقال والاليزا وقياس العوامل المؤثرة

ولقد أستخلصت من الدراسة بأن مرض البروسيللا منتشرأ إنتشارأ واسعأ فى الضأن فى كل مناطق الولاية التي أجريت فيها الدراسة . وإذ تعتبر هذه مشكلة صحية للإنسان والحيوان بالولاية كما تؤثر على تجارة الضأن والذي يعتبر مورداً مهماً للإقتصاد ودعم خزينة الدولة

لذا لابد من إحاطة المسؤولين ،المربيين ،الرعاة والأطباء البيطريين بأهمية المرض

# Abstract

This study was done in Gedarif state during the period of 2016 – 2018 in which 558 sera samples were collected from sheep from 5 localities ( Basonda , Fashaga, GalabatGarbia, GalabatShrgia and Baladia ) Rose Bengal test was used for detection of Brucella Antibodies and for all 558 sample and Elisa test was done only for 184 sera samples all data was analyzed by SPSS programme version 16 .

This study revealed 10.8% by using Rose Bengal test and 6.3% by using Elisa test.

Significance was found ( $p: 0.000$ ) in location and no significant in age and sex Percentage of Brucella antibodies was found 11.7% in Males and 8.3% in females, Phasga locality it was the highest percentage which is 55% , on the other hand Baldia locality is lower percentage with 0%, in Elisa analysis it was found 22.9% and 77.1% in male and female respectively and in ages of 1-2 year is the highest percentage which 48.6%, and highest percent of the disease in Basonda locality 71.4% , as appeared 0% in Rose Bengal but in Elisa was 11.4% in Baladiat Algardarif.

The aim of this study is to estimate prevalence of sheep brucellosis and risk factor of age / sex and locality in El-Gadarif state using Rose Bengal and Elisa.

The study expressed that brucella disease is widely spread in sheep in hole regions of the state which is study done, that is mean health problem to human and animal in the state and its effect on sheep commercial which is important income to the economic.

Wherefore must surround officials, breeders, herdsmen and veterinarians.



## INTERODUCTION

Brucellosis is a zoonotic disease, widely distributed in both human and animal, especially in developing countries. The occurrence of the disease in human largely depends on the animal reservoir. High rate of brucellosis infection in sheep and goats usually causes the greatest incidence of human infection (WHO, 1997). The disease is highly contagious and economically important bacterial of animals (OIE, 2000). The causative agent of the disease was isolated from spleen of human cases that had died as a result of undulant or Mediterranean fever (culture *et al.*, 2005). Brucellosis still a major problem, mainly in developing world due to traditional feeding habits and failure to maintain standards of hygiene because of socio-economic conditions (Ozekicit *et al.*, 2003). The disease causes physical and psychological sufferings. Farmers suffer loss of income due to abortion, the consequent decrease of milk yield, death of infected animals and rejection of export consignments (Chukwu, 1987). Up to the present time, brucellosis remains major problem of public health in many countries which have promoted change from traditional to intensive method of dairy farming (Abu-Eisha, 2000).

Brucellosis is caused by species of the genus *Brucella*. These are facultative intracellular Gram-negative pathogens. The ability of *Brucella* to replicate and persist in host cells is directly linked with its capacity to cause persistent disease and to circumvent innate and adaptive immunity (Smith and Kadrimansor, 2005). There are ten species presently known in the genus *Brucella*. They are nearly similar morphologically, culturally, biochemically and serologically. With the acceptance of *B. ovis* and *B. neotomae*, all the accepted species are pathogenic for human (Blasco, 2010). Most species of *Brucella* are primarily associated with certain host; however infection can also occur in other species particularly when they are kept in close contact. *B. melitensis* mainly infects sheep, goats, cattle, camels and dogs, rarely in horses and pigs (OIE 2007). *B. abortus* is found in cattle, bison and water buffalo and occasionally in sheep, goats and dogs. *B. suis* infect feral pigs, reindeer, caribou, hares, mice, arctic, foxes, wolves, rodents and occasionally cattle and dogs. *B. ovis* infects sheep and deer. *B. canis* infect dogs and *B. neotomae* the American wood rats. *Brucella* species have also been found in deer, bison, elk, coyotes, camels, moose, horse and chickens (OIE, 2004). The significant of marine

mammal's brucellosis remains to be established, both as potential zoonotic agents and as source of infection for terrestrial mammals (Brew, 1999; Rhyan *et al.*, 2001; Sohn *et al.*, 2003). The species of the genus *Brucella* localises and produce infection in the reproductive organs, joints and other swellings on the body. In females, this infection extends to placenta and foetal tissues often producing abortion in pregnant animals (Musa *et al.*, 1990). *Bruclla* species are able to escape phagocytic killing through inhibiting the phagosome-lysosome fusion and reproducing inside macrophages (Young, 2005). Brucellosis in the Sudan is widely spread in different part of the country infecting domestic, wild animal species and man(Musa, 1995).

No enough information is available about the disease in El-Gedarif state. El-Gedarif state is located in the southern part of eastern sudan and has long international board with neighbouring Ethiopia. The soil of El-Gedarif is very fertile enabeling many agricultural activities in the rainy season part of agriculture. The state has about 1,454,222 head of cattle,3,688,218 of sheep,884,087 of goats and 880,162 of camels(Anon,2012). Temperature ranges from 17 c to 40 c (Suleiman and Buchroithner, 2006).

**Aims of the study:**

1. To study seroprevalence of brucellosis in sheep in El-Gedarif state.
2. To compare the results of the Rose Bengal Plate Test (RBPT) and Indirect Enzyme Linked Immunosorbent Assay (I-ELISA).

# CHAPTER ONE

## LITERATURE REVIEW

### 1.1 Definition:

Brucellosis is essentially a disease of animals, especially domesticated livestock, caused by bacteria of the *Brucella* group with humans as an accidental host. It is also known as “Undulant fever”, “Mediterranean fever” or “Malta fever” and transmitted by direct or indirect contact with infected animals or their products.. In other words it is a zoonotic disease. (Corbel, 2006). Because of the major economic impact on animal health and the risk of human disease, most countries have attempted to provide the resources to eradicate the disease from the domestic animal population (Radostits *et al.*, 2006).

### 1.2 Historical background:

In 1884, Captain David Bruce and several others working on Mediterranean fever isolated an agent they called *Micrococcus melitensis* from human spleens. Hospital patients were fed raw goat's milk for many illnesses and this was an early example of nosocomial infection. In 1895, Professor L.F. Benhard Bang, Danish veterinary pathologist and bacteriologist, described a different causative organism isolated from cattle, called *Bacillus abortus* (Radostits *et al.*, 2006). *Brucella melitensis*, the main aetiologic agent of brucellosis in small ruminants, was the first species in the genus *Brucella* described (Alton, 1990). The origin of the disease remained a mystery for a lot of years until it was discovered that goats were the source of infection for human populations (EC, 2001). Bennet (1943) was first reported the disease in Sudan in animals in a dairy farm in Khartoum, The prevalence of the disease was 160(80%) of 200 Friesian and 49(38%) of 130 local Zebu cattle.

### 1.3 Causative agents:

Six species were known in the genus *Brucella*: *B. melitensis*, *B. abortus*, *B. suis*, *B. neotomae*, *B. ovis*, and *B. canis*. The first three species (called “classic brucella”) have been subdivided into biovars that are distinguished by their different biochemical characteristics and/or reactions to the monospecific A. (abortus) and M. (melitensis) sera (PAHO, 2001).A four new species of *Brucella* have recently been described. Recently, following analysis of a small number of strains, two new species names, *Brucella. Ceti* and *Brucella. Pinnipedialis* were validly published (Foster *et al.*, 2007).*B. ceti* is associated with isolates from cetaceans (predominantly porpoises and dolphins) while *B. pinnipedialis*

predominantly associated with pinnipeds (seals) but host specificity of the species does not appear absolute (Groussaud *et al.*, 2007; Dawson., 2008). Recently, an additional novel species, *Brucella. microti*, isolated initially from voles and soil (Scholz *et al.*, 2008). While only a single isolate has been described this will shortly be formally published as the tenth *Brucella* species, *Brucella. inopinata*(Scholz *et al.*, in press) (Table 1).

**Table 1: Animals affected by *Brucella* species (Corbel, 2006)**

<b>Host</b>	<b>B. abortus</b>	<b>B. melitensis</b>	<b>B. suis</b>	<b>B. canis</b>
<b>B. ovis</b>				
Cattle	+	+	+ (rare)	-
-				
Buffaloes	+	+	-	-
-				
Bison	+	-	-	-
-				
Sheep	+ (rare)	+	+ (possible)	-
+				
Goats	+ (rare)	+	-	-
-				
Swine	+ (rare)	+ (rare)	+	-
-				
<b>c</b>				
Dogs	+	+	+ (rare)	+
-				
Camels	+ (rare)	+	-	-
-				
Caribou/Reindeer	-	-	+ (biovar 4)	-
-				
Elk	+	-	-	-
-				
Horses	+	+ (rare)	+ (rare)	-
-				
rodents	+ (rare)	+ (rare)	+ (biovar 5)	-
-				

#### **1.4 Epidemiology:**

##### **1.4.1 Geographic Distribution:**

##### **1.4.1.1 The global distribution of the disease:**

Worldwide, the distribution of the different species of *Brucella* and their biovars varies with geographic areas. *B. abortus* is the most widespread; *B. melitensis* and *B. suis* are irregularly distributed; *B. neotomae* was isolated from desert rats (*Neotomaelepida*) in Utah (USA), and its distribution is limited to natural foci, as the infection has never been confirmed in man or domestic animals. Infection by *B. canis* has been confirmed in many countries on several continents, and its worldwide distribution can be asserted. *B. ovis* seems to be found in all countries where sheep raising is an important activity (PAHO, 2001). It is well established in the Middle-East and that it affects both cattle (*B. abortus*) and small ruminants (*B. melitensis*) (WHO/MZCP, 1998). The disease vary widely from country to country, bovine brucellosis caused mainly by *B. abortus* is still the most widespread form. The disease remains amajor problem in the Mediterranean region, western Asia, and parts of Africa and Latin America. Sheep and goats and their products remain the main source ofinfection, but *B. melitensis* in cattle has emerged as an important problem in somesouthern European countries, Israel, Kuwait, and Saudi Arabia. *B. melitensis* infection is particularly problematic because *B. abortus* vaccines do not protect effectively against *B. melitensis* infection; the *B. melitensis*Rev.1. Vaccine has not been fully evaluated for use in cattle. Thus, bovine *B. melitensis* infection is emerging as an increasingly serious public health problem in some countries. Arelated problem has been noted in some South American countries, particularly Brazil and Colombia, where *B. suis* biovar 1 has become established in cattle (Garcia, 1990).

In some areas, cattle are now more important than pigs as a source of human infection (Corbel, 1997).The spread of the disease was investigated by Thimm and Wundt (1976)and found it higher or lesser over the whole Africa, and that 40(82%) of its 49 countries at that time (now 55) were infected or suspected with the disease. They had found that all domestic animals, 21 of 26 herbivorous and five of 12 carnivorous wild animals screened were serologically positive for the disease. The author reported that 18.8 to 61.5 of individual animals were positive for Milk Ring test (MRT), 3 to 23.5% to Serum Agglutination test (SAT) and Complement Fixation test (CFT), 44.4 to 88% of the herds were positive for MRT and 42.2 to 100% of villagers cattle were positive to the disease. Chukwu, (1987) stated thatthere was high prevalence rate of the disease in cattle in Africa, and that it is prevalent in man, sheep, goats, camels, dogs and wildlife, caused by *B. abortus* biovars 1, 2, 3, 4, 5, 6, 7, 8 and 9 (notice the previous identification of the species to 9 biovars and presently to 7), *B. melitensis* biovars 1, 2 and 3, *B. suis* and *B. ovis* and *B. canis* and this shows the complexity of the situation of the disease in the continent.

#### **1.4.1.2 The distribution of the disease in the Sudan:**

Brucellosis caused by *B. abortus* was first reported in the Sudan a dairy farm in Khartoum. 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. Musa, (1990) reviewed its situation from 1943 – 1990 and found its prevalence in individual animals varying from low (0 – 5%), moderate (6 – 15%), high (16 – 25%) and very high (above 25%), according to the criteria of (Thimm and Wundit (1976). Most of the herds examined in East, West, Central and South (previously) of the Sudan were infected with 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).

#### **1.4.2. Transmission of the disease and Source of Infection:**

##### **1.4.2.1 Infection in animals:**

The natural reservoirs of *B. abortus*, *B. suis*, and *B. melitensis* are, respectively, cattle, swine, goats and sheep. The natural host of *B.canis* is the dog and that of *B. ovis* the sheep (PAHO, 2001).

##### **1.4.2.1.1 Infection in cattle:**

The main pathogen is *B. abortus* Biovar 1 is universal and predominant among the ten species that occur in the world. Cattle can also become infected by *B. suis* and *B. melitensis* when they share pasture or facilities with infected pigs, goats, or sheep. The infection in cattle caused by heterologous species of Brucellosis usually more transient than that caused by *B. abortus*. However, such crossinfections are a serious public health threat, since these *brucellae*, which are highly pathogenic for man, can pass into cow's milk. Infection caused by *B. suis* is not very common. By contrast, infections caused by *B. melitensis* have been seen in several countries, with a course similar to those caused by *B. abortus*.

The main sources of infection for cattle are fetuses, afterbirth, and vaginal discharges containing large numbers of *brucellae*. To a lesser extent, farm areas can be contaminated by fecal matter of calves fed on contaminated milk, since not all the organisms are destroyed in the digestive tract. The most common route of transmission is the gastrointestinal tract following ingestion of contaminated pasture, feed, fodder, or water. Moreover, cows customarily lick afterbirth, fetuses, and newborn calves, all of which may contain a large number of the organisms and

constitute a very important source of infection. Cows' habit of licking the genital organs of other cows also contributes to transmission of the infection. It has been shown experimentally that the organism may penetrate broken and even intact skin. The extent to which this mode of transmission is involved in natural infection is unknown. Bang and others experimentally reproduced infection and disease via the vaginal route. The results of those experiments indicate that a large number of *brucellae* are necessary to infect a cow by this means. However, there is no doubt that the intrauterine route used in artificial insemination is very important in transmitting the infection since the infection can thus be spread to many herds. In closed environments, it is likely that infection is spread by aerosols; airborne infection has been demonstrated experimentally (PAHO, 2001).

Congenital infection and the so-called latency phenomenon have also been described, in six experiments Fensterbank, (1980) admitted that under natural range conditions the frequency of the latency phenomenon could be much lower. Conjunctival inoculation, skin contamination and udder inoculation from infected milking cups are other possibilities. The use of pooled colostrums for feeding newborn calves may also transmit infection. Sexual transmission usually plays little role in the epidemiology of bovine brucellosis (Corbel, 2006).

#### **1.4.2.1.2 Infection in goats and sheep:**

In sheep and goats, *B. melitensis* is nearly always the infecting species. *B. ovis* can also infect sheep but is of little significance in relation to human disease. The mode of transmission of *B. melitensis* in sheep and goats is similar to that in cattle but sexual transmission probably plays a greater role. The transmission of disease is facilitated by commingling of flocks and herds belonging to different townships and by purchasing animals from unscreened sources. The sharing of malebreeding 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 or fairs. In cold climates, it can be the custom to house animals in close space and this also facilitates transmission of infection (Corbel, 2006). Infection of goats *in utero* is not unusual, and kids can also become infected during the suckling period; such infection may persist in some animals. In ram epididymitis caused by *B. ovis*, semen is the main and possibly the only source of infection. The infection is commonly transmitted from one ram to another by rectal or preputial contact.

Transmission may also occur through the ewe when an infected ram deposits his semen and another ram breeds her shortly thereafter. *B. ovis* does not persist very long in ewes and is generally eliminated before the next lambing period (PAHO, 2001). After delivery or abortion, the excretion of brucellae in the vaginal discharge continues for about 3 weeks but may last up to 2 months. Therefore the soil where deliveries take place becomes massively contaminated. The number of



brucellae excreted in milk is generally not relevant for sheep-to-sheep transmission, but is important for the transmission of the infection to humans (Aitken, 2007). Persistent infection of mammary glands is associated with constant or intermittent shedding of the organisms in the milk in succeeding lactations (Philippon *et al.*, 1971). The number of *brucellae* excreted in milk is relatively low but is sufficient to allow transmission to lambs and kids, and indirectly through the milker's hands (EC, 2001). In sheep the organism appears to be transmitted orally from ram to ram or ram to ewe, but not from ewe to ewe (Pugh, 2002).

**Table 2: Diseases and principle hosts of the Brucella species. (Quinn *et al.*, 1994).**

Species	Host	Disease	Geographical Distribution
<i>B.abortus</i>	CATTLE *	Abortion and orchitis	Bio type: 1.worldwide (common) 2.worldwide (not common) 3. India, Egypt, East Africa 4.Britain and Germany Other bioypes are frequently isolated
	Sheep, goat and pigs	sporadic abortion	
	Horses	Associated with bursitis(poll evil and fistulous withers)	
	Human	Undulant fever	
<i>B.melitensis</i>	GOAT, Sheep	Abortion	Many sheep- and goat- raising regions except New Zealand , Australia and North America
	Cattle	Occasional abortion and excretion in milk	
	Human	Malta fever	
<i>B.suis</i>	PIGS	Abortion, orchitis, spondylitis and herd infertility	Biotype: !. World wide 2. western and central Europe 3. USA , Argentina and Singapore 4. Arctic circle
	Human	Undulant fever	

			(Canada, Alaska and Siberia )
<i>B.ovis</i>	SHEEP	Epididymitis in rams and sporadic abortion in ewes	New Zealand, Australia and some other raising countries: USA, Romania, Czechoslovakia, South Africa and South America.
<i>B.canis</i>	DOGS	Abortion, epididymitis, discospondylitis and permanent infertility in males	North America and part of Europe becoming worldwide but not common
	Human	Undulant fever	
<i>B.neotomae</i>	Desert wood rat ( <i>Neotomalepida</i> ).	Non-pathogenic for the wood rat and has not been recovered from any other animal species	USA (Utah)

\*Natural hosts given in capital letters

### 1.4.3 Survival of *Brucella* on the environment:

The ability of *Brucella* to persist outside mammalian hosts is relatively high compared with most other non-spore forming pathogenic bacteria, under suitable conditions. Numerous studies have assessed the persistence of *Brucella* under various environmental conditions. Thus, when pH, temperature and light conditions are favorable, *i.e.* pH>4, high humidity, low temperature and absence of direct sunlight, *Brucella* may retain infectivity for several months in water, aborted fetuses and foetal membranes, faeces and liquid manure, wool, hay, on buildings, equipment and clothes. *Brucellae* are able to withstand drying particularly in the presence of extraneous organic material and will remain viable in dust and soil. Survival is prolonged at low temperatures, especially below 0° C (Alton,1985;WHO, 1986 and Nicoletti, 1980).

Contaminated equipment can be sterilized by autoclaving (121° C). Chemical treatment is recommended to destroy *Brucella* in contaminated premises. Xylene (1 ml/liter) and calcium cyanamide (20 kg/m<sup>3</sup>) have been found to be

effective in liquid manure after 2–4 weeks. A 1 hour treatment with 2.5% sodium hypochlorite, 2-3% caustic soda, 20 % freshly slaked lime suspension, or 2% formaldehyde solution will suffice to destroy *Brucella* on contaminated surfaces. The survival of *Brucella* in milk and dairy products is related to a variety of factors including the type and age of product, humidity level, temperature, changes in pH, moisture content, biological action of other bacteria present and conditions of storage. At low concentration in liquid media, *Brucellae* are fairly heat sensitive. Thus, dilute suspensions in milk are readily inactivated by pasteurization (high-temperature short-time or flash methods) or by prolonged boiling (10 min) (Davies and Casey, 1973). *Brucella* do not persist for a long time in ripened fermented cheese. The optimal fermentation time to ensure safety is not known, but is estimated at 3 months (Nicoletti, 1989). However, in normally acidified soft cheese, the strictly lactic and short-time fermentation and drying increase the survival time of *Brucella*. Previous pasteurization of milk or cream is the only means to ensure safety of these products. *Brucella* are fairly sensitive to ionizing radiation and are readily killed by normal sterilizing doses of gamma-rays under conditions which ensure complete exposure, especially in colostrum (Garin-Bastuji *et al.*, 1990). In contrast to dairy products, the survival time of *Brucella* in meat seems extremely short, except in frozen carcasses where the organism can survive for years. The number of organisms per gram of muscle is small and rapidly decreases with the pH drop of the meat.

Direct contamination of abattoir workers is prevented by a proper and hygienic removal and disposal of mammary glands, reproductive organs and lymph nodes which are the most heavily contaminated. These precautions also prevent the contamination of the carcass by utero-vaginal secretions. Most commonly available disinfectants readily kill *Brucella* in aqueous suspensions at normally recommended concentrations (Phenol 10g/l, Formaldehyde and Xylene 1ml/l), except in the presence of organic matter or at low temperature, which drastically reduces the efficacy. Decontamination should be carried out by heat treatment, especially on surfaces. Diluted hypochlorite solutions, ethanol, isopropanol or iodophores and better, substituted phenols are effective for decontamination of the exposed skin. In contrast, the alkyl quaternary ammonium compounds are not recommended (WHO, 1986).

## **1.5 Economical impact of the disease:**

### **1.5.1 Economical impact in cattle:**

Losses in animal production due to this disease can be of major importance, primarily because of decreased milk production in aborting cows. The common sequel of infertility increases the period between lactations, and in an infected herd the average intercalving period may be prolonged by several months. In addition to the loss of milk production, there is the loss of calves and interference with the

breeding program. This is of greatest importance in beef herds, where the calves represent the sole source of income. A high incidence of temporary and permanent infertility results in heavy culling of valuable cows, and some deaths occur as a result of acute metritis following retention of the placenta (Radostits, 2006). The bovine infection presents a particularly serious problem because of the extensive environmental contamination that even single abortions or infected births can produce (Corbel, 2006).

In study in Mexico, Munozdel *et al.*, (2007) estimated the economic impact of the governmental eradication programme for a Brucellosis outbreak in a dairy herd by comparing the economic costs and losses. The results indicated that the use of the government recommended programme had excessively high costs which made the programme economically unfeasible. Zinsstage *et al.*, (2005) wrote about the disease in Brazil and USA. In Brazil the disease causes a 50% decrease in calf production, thereby decreasing the fertility indexes. Infected animals experience a decrease of 20 to 25% in milk production and of 15% in meat production.

In The USA the yearly cost of Brucellosis in the United States is \$30 million. In Sudan the cost of brucellosis In Kuku dairy Scheme was estimated Based on the results on prevalence rates together with the burden of the disease on at risk population. The total losses of the dairy sector due to brucellosis in the year 2004 accounted to SD 66,910,503.8 equivalents to US\$ 267,642. (92.4%) of the losses were attributed to reduction of milk yield and 7.6% to reduced fertility. Cost to the health accounted to SD 216,450 equivalent to US\$ 865.8. (Angara, 2009).

### **1.5.2 Economical impact of sheep and goats Brucellosis:**

The economic effects of the disease are subtle but significant. The effect of the disease on ram fertility can influence the number of rams that are required in a flock: the required ram to ewe ratio is significantly reduced in *B. ovis*-free flocks. The percentage of lambs born early and within the first 3 weeks of the lamb in period is also markedly increased. Lambing percentage may be reduced by 30% in flocks recently infected and by 15-20% in those where the infection is endemic. Additional costs are the loss of rams of high genetic potential and the cost of repeat serological testing. In the USA, an additional return of 12 US\$ per ewe mated has been calculated as the advantage in a control program. Brucellosis has major veterinary and human importance in affected countries(Radostits, 2006).

Costs include production loss associated with infection in animals, the considerable cost of preventive programs, and human disease. There is further loss from restriction in international trade in animals and their products. The occurrence of *B. melitensis* in the sheep and goat population of countries that have eradicated *B. abortus* poses a threat for the continuing problem of brucellosis in cattle herds (Radostits, 2006). In Sudan, from 1999-2005, a total of 17 ships carrying 40428 sheep, 5334 goats and 2298 camels were rejected by Saudi authorities because of

detection of some cases positive for brucellosis and that had resulted in heavy financial losses and bankruptcy of some animal traders (Musa *et al.*, 2008). The total of animals exported and rejected in the year 2006 and 2007 are shown by Shigidi( 2010) in this table:

**Table 3: Shipments of animals rejected because of brucellosis during the years 2006 and 2007 (Shigidi( 2010)).**

Year		No of animals			Total
		Sheep	Goats	Camels	
2006	Total exported	1,419,270	102,128	6,874	1,528,272
	Rejected	28,061(2%)	6,717(6.6%)	2,792(40.6%)	37,570(2,5%)
2007	Total exported	61,4447	29,995	9,832	654,274
	Rejected	6,537(1.1%)	0(0%)	4,232(43%)	10,769(1.7%)

## 1.6 Clinical signs:

### 1.6.1 Clinical signs in cattle:

The clinical signs in cattle are limited to abortion of fetuses - usually during the last half of gestation. Abortion storms may occur when the disease has recently been introduced in a herd, whereas abortion in first-calf heifers or new additions typifies endemic infection (Divers and Peek, 2008). In some areas, abortion is relatively uncommon. In some parts of Africa, hygromas and abscesses are the major clinical signs in nomadic or semi-nomadic cattle herds infected with *B.abortus* biovar 3. There is lowered milk production due to premature births. Interference with fertility is usually temporary and most infected animals will abort only once and some are unaffected. The udder is often permanently infected (Corbel, 2006).

### 1.6.2 Clinical signs in sheep and goats:

The main clinical manifestations of brucellosis in sheep and goats are, as in all female ruminants, reproductive failure, *i.e.* abortion and birth of weak offspring. Abortion generally occurs during the last 2 months of pregnancy and is followed in some cases by retention of foetal membranes. In the male, localization in the testis, epididymis and accessory sex organs is common, and bacteria may be shed in the

semen. This may result in acute orchitis and epididymitis and later in infertility. Arthritis is also observed occasionally in both sexes (EC, 2001).

The udder is often permanently infected, especially in the case of goats. Shedding of organisms in milk is frequent (Corbel, 2006). In pregnant ewes, the bacterium enters the uterus, where it reproduces in the placenta and fetal tissues, inducing an infective state not necessarily followed by abortion. The percentage of aborting animals varies according to circumstances. In non-pregnant animals, *Brucella* can cause a chronic infection, which is of epidemiological importance because, after an initial serological reaction in the animal, the infection becomes non-apparent thus creating problems in diagnosis (Aitken, 2007).

### **1.7 Diagnosis:**

Diagnosis and control of the disease in animals must be carried out on a herd basis. There may be a very long incubation period in some infected animals and individuals may remain serologically negative for a considerable period following infection. The identification of one or more infected animals is sufficient evidence that infection is present in the herd, and that other serologically negative animals may be incubating the disease and present a risk. Diagnostic tests fall into two categories: those that demonstrate the presence of the organisms and those that detect an immune response to its antigens.

The isolation of *Brucella* is definitive proof that the animal is infected, but not all infected animals give a positive culture and the methods and facilities that must be employed are not always readily available. The detection of antibody or a hypersensitivity reaction provides only a provisional diagnosis, but in practice is the most feasible and economic means of diagnosis. False positive reactions to serological tests can occur through a number of factors, including vaccination, and this must be borne in mind when interpreting results. Similarly, dermal hypersensitivity only indicates previous exposure to the organism, not necessarily active infection, and may also result from vaccination (Corbel, 2006).

#### **1.7.1 Bacteriological methods:**

Microscopic examination can be used for materials in which large numbers of brucellae are suspected, such as, for example, the placenta, stomach content of the fetus as well as its lungs and liver and the vaginal discharge in the case of abortion (Aitken, 2007). 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. It should be noted that all infected materials present a serious hazard, and they must be handled with adequate precautions during collection, transport and processing (Corbel, 2006).

##### **1.7.1.1 Stained smears:**

Smears of placental cotyledon, vaginal discharges or fetal stomach contents may be stained using modified Ziehl-Neelsen (Stamp) or Kusters' methods. This is

the usual procedure for the examination of smears of organs or biological fluids that have been previously fixed with heat or ethanol. The presence of large aggregates of intracellular, weakly acid-fast organisms with *Brucella* morphology is presumptive evidence of brucellosis. Care must be taken as other infectious agents such as *Coxiella burnetii* or *Chlamydia psittaci* may superficially resemble *Brucella* (Corbel, 2006 and OIE, 2009b).

#### **1.7.1.2 Culture:**

*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 most importance that fecal 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 homogenized 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 (Corbel, 2006). There are two *Brucella* cultures usually used:

##### **1.7.1.2.1 Basal media:**

Direct isolation and culture of *Brucella* are usually performed on solid media. This is generally the most satisfactory method as it enables the developing colonies to be isolated and recognised clearly. Such media also limit the establishment of non-smooth mutants and excessive development of contaminants. However, the use of liquid media may be recommended for voluminous samples or for enrichment purpose. A wide range of commercial dehydrated basal media is available, e.g. *Brucella* medium base, tryptose (or trypticase)–soy agar (TSA). The

addition of 2–5% bovine or equine serum is necessary for the growth of strains such as *B. abortus* biovar 2, and many laboratories systematically add serum to basal media, such as blood agar base (Oxoid) or Columbia agar (BioMérieux), with excellent results. Other satisfactory media, such as serum–dextrose agar (SDA) or glycerol dextrose agar, can be used. (SDA) is usually preferred for observation of colonial morphology (OIE, 2009a).

#### **1.7.1.2.2 Selective media:**

All the basal media mentioned above can be used for the preparation of selective media. Appropriate antibiotics are added to suppress the growth of organisms other than *Brucella*. The most widely used selective medium is the Farrell’s medium, which is prepared by the addition of six antibiotics to a basal medium (OIE, 2009b). However, nalidixic acid and bacitracin, at the concentration used in Farrell’s medium, have inhibitory effects on some *B. abortus* and *B. melitensis* strains. Therefore the sensitivity of culture increases significantly by the simultaneous use of both Farrell’s and the modified Thayer–Martin medium (Marin *et al.*, 1996). Contrary to several biovars of *B. abortus*, growth of *B. melitensis* is not dependent on an atmosphere of 5–10% CO<sub>2</sub> (OIE, 2009b). Bacterial colonies may be provisionally identified as *Brucella* on the basis of their cultural properties and appearance. Definitive identification of suspect colonies can only be made using techniques available at *Brucella* Reference Centers (Corbel, 2006).

#### **1.7.2 Serological methods:**

Serological tests can be divided broadly into two groups: Screening tests: used in the field clinics or in regional laboratories, such as the Rose Bengal or buffered plate agglutination. The Rose Bengal test has a very high sensitivity to ensure that infected animals are not missed. The milk ring test is also an excellent screening test for dairy cattle. Indirect ELISA tests are also being used to screen milk and serum. Confirmatory tests used in a central or regional laboratory, such as competitive ELISA, immunodiffusion or complement fixation tests. They are very useful in distinguishing vicinal antibody responses from those induced by field infections (FAO, 2003). Serological results must be interpreted against the background of disease incidence, use of vaccination and the occurrence of false positive reactions due to infection with other organisms (Corbel, 2006).

##### **1.7.2.1 Rose Bengal plate test (RBPT) (buffered plate antigen or card test):**

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 and other tests such as the buffered plate agglutination tests and the card test play 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



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. Positive samples should be checked by the CFT or by an IgG specific procedure such as ELISA. False-negative reactions occur especially in the early stages of acute infection. The RBPT can be used in all animal species but positive results should be confirmed by a quantitative test. False negative results are common in sheep, goats and pigs. The procedure can be automated but this requires custom-made equipment (Corbel, 2006). On the other hand, it has been proved that an increase in the volume of sera to be tested also improves significantly the sensitivity of the test (Aitken, 2007).

#### **1.7.2.2 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 mean that it should only be used in the absence of alternative techniques. In each set of tests, a positive control serum calibrated against the International Standard for *B. abortus* antiserum (ISABS) must be included. This enables the results to be expressed in IUs. And permits tests that have been performed in different laboratories to be compared. For cattle, titers equivalent to 50 IU or more for unvaccinated animals and 100 IU or more for vaccinates are regarded as indicative of infection. Micro agglutination methods using a stained antigen may be performed in microtiter plates instead of tubes (Corbel, 2006).

The SAT is widely employed for sheep and goats but limited by the possibility of negative or suspicious results in chronic brucellosis. The SAT may be influenced by Rev-1 and other antigens, and the response can be variable even in the same animal. For these reasons, the SAT must be used only as a screening test and, in cases in which a low titer is found, additional methods are necessary (Aitken, 2007). SAT is widely used in case of *B.abortus* infection, but its limitations include the following, the test detects nonspecific antibodies as well as specific antibodies from *B.abortus* infection and vaccination, during the incubation stage of the disease the test is often the last to reach diagnostically significant levels, after abortion due to *B. abortus* it is often the last test to reach diagnostically significant levels. In the chronic stage of the disease, the serum agglutinins tend to wane, often becoming negative when the results of some other tests may be positive (Radostits, 2006). It should be stressed that the serum agglutination test SAT is generally regarded as being unsatisfactory for the purposes of international trade (OIE, 2009b).

#### **1.7.2.3 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 isotypes 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 (Corbel, 2006). CFT is diagnostically more specific than the SAT, and also has a standardized system of unit age(OIE,2009a). CFT remains the prescribed test for international trade because of the lack of standardized methods recognized at the international level for I-ELISA and Agar Gel immune Diffusion (AGID) (OIE, 2009c).

#### **1.7.2.4 ELISA tests:**

Two main types of immune sorbent assay have been used: the indirect and competitive formats:

1. **Indirect ELISA (IELISA)** has been a useful test during an eradication program, after vaccination has ceased, for screening or as a supplementary test to CFT. It has gained wide acceptance for serological diagnosis of bovine brucellosis because of its ability to detect antibody of all isotopes, unlike the conventional tests. The sensitivity and specificity of indirect ELISA has been excellent but it could not distinguish between the antibody response induced by vaccination with *B. abortus* strain 19 and natural infection with the organism.

2. **Competitive ELISA (CELISA)** can differentiate between the induced antibody responses. An improved competitive enzyme immunoassay (CELISA) has a sensitivity of 100% and specificity of 99.7% and is considered a reasonable alternative as a single assay for serological diagnosis of brucellosis. The indirect ELISA kits produced by the Joint Food and Agriculture Organization and the International Atomic Energy Authority (FAO/IAEA) were able to detect residual anti-*B. abortus* strain 19 antibodies in adult cows vaccinated with strain 19 vaccine between 3 and 8 months of age but which were negative to the Rose Bengal and Rivanol tests. A 'dipstick' enzyme immunoassay is also available and being evaluated (Radostits, 2006). 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 RBPT or CFT (Corbel, 2006).

#### **1.7.2.4 Supplementary tests:**

Many other serological tests have been employed. Some, such as the Rivanol or 2-ME test, are variations of the SAT and, although more specific, share many of its disadvantages. At present, the use of such procedures in the place of the standard test is not advised (Corbel, 2006).

##### **1.7.2.4.1 Milk ring test:**

The milk ring test (MRT) is used widely for surveillance of *B. abortus* infection in dairy cattle. Bulk tank milk samples from each producer are tested at regular intervals by milk plants. A haematoxylin-stained suspension of killed *B. abortus* is added to fresh milk and incubated in a water bath at 37.0° C. Agglutinating antibodies in the milk will be detected by a color change (blue ring) in the cream layer because fat globules cause clumps of agglutinated organisms to rise in the tube, leaving decolorized milk below. A negative test result is confirmed when the milk in the tube remains colored (Divers and Peek 2008). The milk ring tests are unreliable with sheep milk (Quinn *et al.*, 1994). MRT is reasonably sensitive but may fail to detect a small number of infected animals within a large herd. Non-specific reactions are common with this test, especially in brucellosis free areas. The milk ELISA is far more specific than the MRT (Corbel, 2006).

##### **1.7.2.4.2 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 (Corbel, 2006). On the other hand EC(2001) thinks that the tests lacks the sensitivity compared with tests performed on serum, this due to the low rate and frequency of *Brucella* antibodies in milk.

##### **1.7.2.4.3 Fluorescence polarization assay (FPA):**

This test can be done outside the diagnostic laboratory, allowing for rapid and accurate diagnosis. FPA can be done almost anywhere using a portable analyzer, which receives power from a laptop computer, using serum, milk, or EDTA anticoagulated blood. The FPA technology has been developed and validated for the serological diagnosis of brucellosis in cattle, pigs, sheep, goats, and bison. The FPA was initially developed for testing serum; however, the technology has been extended to testing whole blood and milk from individual animals or bulk tank samples pooled from 2000 or fewer animals. The accuracy results of the FPA equals or exceeds those obtained using other serological tests

such as the rose Bengal plate test, the milk ring test, the CFT, the IELISA, and the CELISA. Validation of studies of the FPA and the CELISA for the detection of antibodies to *B. abortus* in cattle sera and comparison to the standard agglutination test, the complement fixation test and the indirect ELISA, found that the FPA is highly superior. The FPA offers clear advantage due to its ease of use. Full implementation and acceptance of FPA methods for the diagnosis of brucellosis will necessitate the use of an International Standard Serum panel containing at least a low titer positive sample and a negative (Radostits, 2006). However, Corbel (2006) thinks that the FPA technique requires special reagents and reading equipment and it is claimed to have advantages in sensitivity and specificity over other methods.

#### **1.7.2.4.4 Intradermal test:**

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.

#### **1.7.2.4.5 Antiglobulin test (Coombs') test:**

The antiglobulin test or Coombs' test (Coombs *et al.*, 1945) was developed to detect antibodies which, although they combine with cellular antigens of *Brucella* do not give rise to agglutination. The presence of these so-called "incomplete agglutinins" can be detected by using an antibody directed against the IgG fraction of the animal species being tested (MacMillan, 1990). The classical time-consuming methodology of the test in man has been considerably improved by its adaptation to a microtiter plate format (Otero *et al.*, 1982). Farina (1985) reported that the Coombs' test could be useful to check sera from animals that give negative, suspicious or non-conclusive responses to SAT, due to the presence of incomplete antibodies in these sera. There is evidence (Alton, 1990 and Unel *et al.*, 1969) suggested that the antiglobulin test is effective in diagnosing brucellosis in sheep and also in goats, but because of the complexity of the technique its use may be restricted to special situations, *e.g.* for the detection of antibodies in anti-complementary sera. Its use is not recommended in bovines vaccinated with strain S19 (MacMillan, 1990) or in small ruminants immunized with Rev.1 vaccine (Farina, 1985), because of its low specificity as compared to the CFT.

### **1.7.3 Molecular methods:**

#### **1.7.3.1 Polymerase Chain Reaction (PCR):**

The PCR-based assays for *Brucella* have been developed and are simple. The PCR has been applied to tissues such as aborted fetuses and associated maternal tissues, blood nasal secretions, semen, and food products such as milk and soft cheeses. The detection of *Brucella* DNA from aborted bovine fetuses by PCR has been compared with microbiological techniques and the estimated

concordance calculated by Kappa index was 0.73 which is considered satisfactory. *Brucella spp.* can be detected in the milk of naturally infected cattle, sheep, goats, and camels using a PCR assay which is more sensitive than the culture method. (Radostits, 2006).

#### **1.7.4 Diagnosis of sheep and goats brucellosis:**

Abortion of an infective nature may be suspected on the basis of history and clinical examination, especially when several ewes are involved. However, only bacteriological and serological tests may confirm the presence of *B. melitensis* (Aitken, 2007). The existence of clinical lesions (unilateral or, occasionally, bilateral epididymitis) in rams may be indicative of the existence of infection (OIE, 2009c). Positive blood culture soon after the infection occurs, or isolation of the organism from the aborted fetus, vaginal mucus, or milk, is the common laboratory procedures used in diagnosis. The organism is moderately acid-fast and staining smears from the placenta and fetus with a modified Ziehl Neelsen method may give a tentative diagnosis; however this does not distinguish this infection from *B. ovis* or the agent of enzootic abortion and culture is required. The rose Bengal test has excellent specificity and high sensitivity, is easy to perform, and is suitable for herd and flock testing (Radostits, 2006). CFT and agar gel immune diffusion (AGID) test can be used (OIE, 2009c). The organism can be detected by PCR in the abomasal fluid of aborted fetuses and, compared with culture; PCR has a sensitivity and specificity of 97.4% and 100%, respectively. PCR can also be used to detect the organism in semen (Radostits, 2006). The SAT must be used only as a screening test and, in cases in which a low titer is found, additional methods are necessary (Aitken, 2007). Other tests that have been developed include ELISA tests, radial immune diffusion, and counter immune electrophoresis; the sensitivity and specificity of these appears to vary between laboratories. An ELISA test using purified antigen is described as being able to differentiate the seropositivity of *B. melitensis* from that of *B. ovis* (Radostits, 2006).

#### **1.8 Prevention, Control and Eradication of Brucellosis:**

Justifications for prevention of the introduction of brucellosis in populations of animals are the same as those for the control of the disease in populations which are already infected: economic benefits and the protection of public health (Corbel, 2006).

##### **1.8.1 Prevention:**

It is nearly always more economical and practical to prevent diseases than to attempt to control or eliminate them. For Brucellosis, the measures of prevention include:

1. Careful selection of replacement animals. These, whether purchased or produced from existing stock, should originate from *Brucella*-free herds or flocks. Pre-

purchase tests are necessary unless the replacements are from populations in geographically circumscribed areas that are known to be free of the disease.

2. Isolation of purchased replacements for at least 30 days. In addition a Serological test prior to commingling is necessary.
3. Prevention of contacts and commingling with herds of flocks of unknown status or those with brucellosis.
4. If possible, laboratory assistance should be utilized to diagnose causation of abortions, premature births, or other clinical signs, suspected animals should be isolated until a diagnosis can be made.
5. Herds and flocks should be included in surveillance measures such as periodic milk ring tests in cattle (at least four times per year), and testing of slaughtered animals with simple screening serological procedures such as the RBPT.
6. Proper disposal (burial or burning) of placentas and non-viable fetuses. Disinfection of contaminated areas should be performed thoroughly.
7. Cooperation with public health authorities to investigate human cases.

Animal brucellosis, especially when caused by *B. melitensis*, can often be identified through investigations of cases in humans (Corbel, 2006).

## **1.8.2 Control:**

### **1.8.2.1 Treatment of Animal Brucellosis:**

Treatment is unsuccessful because of the intracellular sequestration of the organisms in lymph nodes, the mammary gland, and reproductive organs. *Brucella* spp. are facultative intracellular bacteria that can survive and multiply within the cells of the macrophage system. Treatment failures are considered to be due not to the development of antimicrobial resistance but rather to the inability of the drug to penetrate the cell membrane barrier. So that treatment is unlikely to be economically or therapeutically effective (Radostits, 2006).

The aim of an animal control programme is to reduce the impact of a disease on human health and the economic consequences. The elimination of the disease from the population is not the objective of a control programme, and it is implicit that some “acceptable level” of infection will remain in the population. Control programmes 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. In many countries, methods for the control of brucellosis are backed by governmental regulation/legislation. In others, no authorities exist. Therefore, the procedures for management of infected herds and flocks may vary widely. Nevertheless, certain principles apply, namely: 1) the reduction of exposure to *Brucella* spp. and 2) the increase of the resistance to infection of animals in the populations. These procedures may be further classified under the general categories of test and isolation/slaughter, hygiene, control of animal movement, vaccination (Corbel, 2006).

### **1.8.2.2 Test and isolation/slaughter:**

There are no pathogenomonic signs of brucellosis in animals at individual level; the occurrence of abortion storms in naive herds/flocks is usually a strong indicator of infection. Therefore, serological (and sometimes allergic) tests are the usual method of identifying possible infected animals. Bacteriological procedures are useful for confirming test results and for epidemiological studies. The decision about slaughter of test-positive animals is made after regulatory, economic and prevalence factors are considered. 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%). Retention of positive animals is less hazardous if the remaining animals have been vaccinated but should only be considered as a last resort. The isolation of test-positive animals is essential, especially during and after parturition. 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 (Corbel, 2006). For the implementation of such a program it is essential that the flocks are under strict surveillance and movement control. Animals must be individually identified and an efficient and well organized veterinary service for surveillance and laboratory testing must be in place (Alton, 1989; Nicoletta, 1993).

The flock size and the prevalence of brucellosis are the most important factors of this strategy which has been shown to be ineffective and unreliable when attempted in large flocks with a high prevalence of brucellosis (Kolar, 1995). The limited reliability of the diagnostic tests used which are unable to reveal all infected animals and which may give false negative results due to incubation period, latency or due to criteria used to interpret the results must also be considered. These drawbacks apply more to sheep and goat flocks than to cattle herds where a test and slaughter strategy has been more effective (Kolar, 1984; Nicoletta, 1993). Before embarking on the implementation of such a strategy it is necessary to ensure that the epidemiological situation is favorable, the necessary facilities and financial resources are available, a pool of healthy replacement animals is available and that the resources exist for continuing surveillance for a considerable period. It is also essential that full co-operation of farmers is available as slaughter of seropositive animals can be resisted by owners because of lack of clinical signs, inadequate compensation or lack of replacement animals (Nicoletti, 1993). A brucellosis control and eradication plan based on test and slaughter

strategy can be either voluntary or compulsory. Voluntary schemes, which apply to individual flocks, may be useful in the early stages of the campaign but may need to be supported by adequate incentives such as a bonus on the sale of milk from brucellosis-free herds or per capita payments. Compulsory eradication is required in the final stages but is often advisable from the start (WHO, 1986).

#### **1.8.2.3 Hygiene:**

The goal in the application of hygiene methods to the control of brucellosis is reduction of exposure of susceptible animals to those that are infected, or to their discharges and tissues. This is a classical procedure in disease control. Factors such as the methods of animal husbandry (e.g. commingling of herds or flocks), patterns of commerce, prevalence of clinical signs, type of facilities, and degree of dedication of the owners of animals, will also determine success. Owners are often poorly informed about disease transmission and recommendations, such as separation of parturient animals, can be difficult or impossible to implement. 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. A limited number of studies have shown rapid reductions in the incidence of brucellosis when the herd or flock was treated but this procedure is considered to be restricted in practice. Treatment has been used in animals of special breeding value, but because of the uncertain outcome it is not generally recommended (Corbel, 2006). In case of infection by *B. melitensis* hygiene measures must include at kidding or lambing and the disposal of infected or reactor animals. Separate pens for kidding does that can be cleaned and disinfected, early weaning of kids from their does and their environment, and vaccination are recommended. In endemic areas all placentas and dead fetuses should be buried as a routine practice (Radostits, 2006).

#### **1.8.2.4 Control of animal movement (Quarantine):**

This is a period of time during which cattle movement is restricted and the cattle are tested. This will prevent interherd transmission by infected cattle, especially those that are test-negative and incubating the disease. The quarantine period should be sufficiently long that all cattle have had sufficient time to develop brucellosis and insure that the remaining cattle will not be a source for interherd transmission. The time will usually range from 120 days to 1 year, or until all breeding animals have completed a gestation without test evidence of infection (Radostits, 2006). Control of animal movement may be regarded as an aspect of hygiene. However, it is essential in any programme to limit the spread of brucellosis. Animals should be individually identified by brand, tattoo or ear tag. Unauthorized sale or movement of animals from an infected area to other areas should be forbidden. Similarly, 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. In practice, it is much more difficult to control the movement of camel's 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 (Corbel, 2006).

#### **1.8.2.5 Vaccination:**

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. 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. 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 (Corbel, 2006).

##### **1.8.2.5.1 Brucella abortus strain 19 vaccine:**

*B. abortus* strain 19 vaccine has been most widely used to prevent bovine brucellosis. The vaccine protects uninfected animals living in a contaminated environment, enabling infected animals to be disposed of gradually. This overcomes the main disadvantage of the test and disposal method of eradication, in which infected animals must be discarded immediately to avoid spread of infection. Strain 19 *B. abortus* has a low virulence and is incapable of causing abortion except in a proportion of cows vaccinated in late pregnancy, although it can cause undulant fever in humans. Its two other weaknesses are its failure to completely prevent infection, especially infection of the udder, and the persistence of vaccinal titers in some animals. The optimum age for vaccination is between 4 and 8 months and there is no significant difference between the immunity conferred at 4 and 8 months of age. In calves vaccinated between these ages the serum agglutination test returns to negative by the time the animals are of breeding age, except in a small (6%) of cases. The lipopolysaccharide with an O-chain on

*B. abortus* strain 19 explains the appearance and persistence of antibodies in serum following vaccination. These antibodies are detectable in the serological assays used for the diagnosis of brucellosis and are the major problem with strain 19 vaccination, since they prevent easy differentiation of vaccinated from infected cattle. The appearance and persistence of these antibodies depends on age, dose, and route of vaccination. This situation makes the continued use of the vaccine incompatible with simultaneous application of test and slaughter procedures for the control of brucellosis. In most control programs, vaccination is usually permitted up to 12 months of age, but the proportion of persistent post vaccinal serum and whey reactions increases with increasing age of the vaccinates. Such persistent reactors may have to be culled in an eradication program unless the reaction can be proved to be the result of vaccination and not due to virulent infection.

Vaccination of adult cattle is usually not permitted if an eradication program is contemplated but it may be of value in reducing the effects of an abortion 'storm'. Vaccination of bulls is of no value in protecting them against infection and has resulted in the development of orchitis and the presence of *B. abortus* strain 19 in the semen. For these reasons the vaccination of bulls is discouraged (Radostits, 2006). The main objective of systematic and mandatory vaccination of calves in a given area or country is to reduce the infection rate and obtain herds resistant to brucellosis, so that eradication of the disease may then begin. It is estimated that 7 to 10 years of systematic vaccination are necessary to achieve this objective. The recommended dose is one to three billion cells of strain 19 *Brucella* administered subcutaneously (PAHO, 2001).

#### **1.8.2.5.2 *Brucella abortus* strain RB 51 vaccine:**

*Brucella abortus* strain RB51 (SRB51) is a live, stable, rough mutant of *B. abortus* strain 2308 that lacks much of the lipopolysaccharide O-side chain. The O side chains are responsible for the development of the diagnostic antibody responses of an animal to brucellosis infection. Heifer calves vaccinated at 3, 5 and 7 months of age with the SRB51 vaccine were protected when challenged against infection and abortion during their first pregnancy. None of the heifers developed antibodies that reacted in the standard agglutination test, but did react in a dot blot assay using RB51 antigen. In pregnant cattle, SRB51 vaccine has a tropism for the bovine placental trophoblast but when given subcutaneously does not cause placentitis or abortion and the induced humoral and cell-mediated immune response does not interfere with the serological diagnosis of field infections. Vaccination of mature sexually intact bulls and pregnant heifers with a standard calfhood dose of SRB51 is not associated with shedding or colonization in tissues, and does not appear to cause any reproductive problems when administered to sexually mature cattle. One study found that *B. abortus* RB51 isolated from the milk of a cow was no different from the RB51 vaccine strain and it is possible that

shedding of vaccine strains may be associated with the vaccine. Use of the vaccine in cattle already vaccinated with strain 19 vaccine will not cause positive responses on confirmation tests and does not interfere with brucellosis surveillance. Vaccination with a reduced dosage of SRB51 (reduced dose vaccination) protects adult cattle against abortion or infection caused by exposure to virulent *B. abortus* during the subsequent pregnancy. Revaccination of cows with a reduced dose of SRB51 in endemic zones does not cause abortion and protects 94 % of animals against field infection but may cause an atypical response to conventional serological tests. The summary of studies with strain RB51 vaccine indicate that it is as efficacious as *B. abortus* strain 19 vaccine but is much less abortigenic in cattle. It does not produce any clinical signs of disease after vaccination, nor does it produce a local vaccination reaction at the injection site. The organism is cleared from the blood stream within 3 days and is not present in nasal secretions, saliva, or urine. Immunosuppression does not cause recrudescence and the organism is not spread from vaccinated to non-vaccinated cattle. The vaccine is safe in all cattle over 3 months of age. In case of human exposure, strain RB51 is sensitive to a range of antibiotics used in the treatment of human brucellosis but is resistant to Rifampin and Penicillin. Strain RB51 vaccine must be administered by an accredited veterinarian or by a state or federal animal health official. Calves must be vaccinated with the calf dose (10-34 billion organisms) between 4 and 12 months of age. Only animals in high-risk areas should be vaccinated over 12 months of age. Vaccinates must be identified with the standard metal vaccination ear tag and a vaccination tattoo (Radostits, 2006). Preliminary experiments suggest that SRB51 vaccine can be effective for the prophylaxis of *B. melitensis* infection in goats (Suarez *et al.*, 1998). In contrast, it has been demonstrated that this vaccine does not confer protection against *B. ovis* in controlled experiments in rams (Jiménez de Bague *et al.*, 1995).

#### **1.8.2.5.3 Brucella abortus strain K45/20A vaccine:**

Strain K45/20A vaccine may be used and must be given in two doses at 6-month intervals. It is preferable to retest the herd before the second vaccination and to cull cows with a threefold rise in agglutination titer (Radostits, 2006).

#### **1.8.2.5.4 Brucella melitensis Rev. 1 vaccine:**

The live attenuated *B. melitensis* Rev.1 strain is presently recognised as the best available vaccine for the prophylaxis of brucellosis in sheep and goats. Numerous independent field and controlled experiments confirm its value for this purpose. Moreover, “correctly standardized Elberg 101 strain Rev.1 vaccine should continue to be considered as the basis of brucellosis control in small ruminants where vaccination is applied, until new safer and effective versions of *B. abortus* and *B. melitensis* vaccines, based on rough strains, are tested under controlled experimental and field conditions and shown to be at least equivalent to the Rev.1

vaccine.” (WHO, 1997). The ability of the vaccine (Rev.1 strain) to produce a high level of immunity against both artificial and natural challenge has been convincingly demonstrated both for sheep and goats (Alton, 1990). It has been well established that a large proportion of vaccinated animals is protected against infection (Elberg, 1959, and Garrido, 1992), and in those vaccinated animals where infection occurred, it is often transitory. Hence, the period of *Brucella* excretion from the udder or vagina is shorter, the degree of microbial contamination of the surroundings is reduced and, consequently, disease transmission within and between herds is significantly reduced (Garrido, 1992). As with all highly-contagious diseases, the effect of vaccination increases the greater the coverage of the animal population. Erratic administration of vaccines or their use without adequate quality control is not effective. Adequate protection is only possible if the vaccine quality is good and if the vaccines are administered to at least 80 % of the animals at risk (Garrido, 1992). The duration of immunity conferred by vaccination with Rev.1 was investigated by vaccinating Maltese goats when they were 4 to 12 months of age and challenging some at 2 ½ years (Alton,1966) and others at 4 ½ years (Alton, 1968) after vaccination. Those challenged at 4 ½ years were as resistant as those challenged at shorter intervals after vaccination, and it was concluded that immunity could be considered lifelong. Similar results were observed in sheep in Iran challenged 2 ½ years after vaccination (Biggi, 1956 and Alton, 1990). More recent work has demonstrated the efficacy of Rev.1 vaccine in sheep either vaccinated as lambs (CJ or SC route) and challenged 9-10 months (Fensterbank *et al.*, 1985) or 7.5- 15.5 months later, respectively (Verger *et al.*, 1995), or vaccinated as adults (CJ route) and challenged 2 ½ years after (Durán-Ferrer, 1998). Likewise, good results of protection were obtained when young goats were vaccinated at 4 months of age (CJ or SC route) and challenged 8.5-12.5 months after (Fensterbank *et al.*, 1987), and used exhaustively in whole flock vaccination programmes, the live *B.melitensis* Rev.1 vaccine greatly decreases the prevalence of brucellosis in both sheep and human population (Elberg, 1981, 1996). Once the prevalence has been diminished, a more efficient control of the disease may be achieved through the implementation of a programme based on Rev.1 vaccination of lambs combined with the test-and-slaughter of adults. Finally, it may be possible to use a test-and slaughter programme only (Garin-Bastuji *et al.*, 1998).

#### **1.8.2.5.5 Other recently developed vaccines of sheep:**

##### **1.8.2.5.5.1 *Brucella melitensis* H-38 vaccine:**

H-38 vaccine is produced by a virulent strain of *B. melitensis* biotype 1, inactivated with formaldehyde and suspended in adjuvant oil (Arlacel A). One dose containing 3 x10<sup>11</sup> bacteria induces good protection which lasts 15 months. As it is an inactivated vaccine, it may be used in pregnant and lactating animals.

Unfortunately, two disadvantages have been reported: the antibody response develops more slowly compared with Rev-1, and H-38 frequently causes a local reaction at the inoculation site, which can be severe. Moreover, as the characteristics of the vaccine may change from one batch to the other, the use of H-38 has been limited (Aitken, 2007).

**1.8.2.5.5.2 *B. suis* S2:**

*B. suis*S2, a classically obtained Brucella attenuated strain with smoothLPS, was apparently successfully used in controlling brucellosis following oral administration to small ruminants in field conditions in China (Xin, 1986)and Libya (Mustafa and Abusowa, 1993). However, this vaccine showed no protective effect against *B. melitensis* in sheep in fully controlled experimental conditions (Verger *et al.*, 1995).

**1.8.2.5.5.3 VTRM1:**

VTRM1 is a live rough strain of *B. melitensis*. It does not induce antibodies against the S-LPS and does not interfere with classical serological tests. However, the VTRM1 vaccine does not confer adequate protection against *B. melitensis* in goats (Elzer *et al.*, 1998).

**1.8.2.5.5.4 RfbK strain:**

The mutant rfbK is a live rough strain obtained by transposing mutagenesis from smooth *B. abortus*2308 (Adams *et al.*, 1998). Preliminary experiments suggest that this mutant strain can be effective for the prophylaxis of *B. melitensis* infection in goats (Suarez *et al.*, 1998). Table 4 summarizes the advantage and disadvantage of various strategies to control Brucellosis:

**Table 4: Summary of the advantages and disadvantages of various strategies (EC, 2001).**

<b>STRATEGY</b>	<b>ADVANTAGES</b>	<b>DISADVANTAGES</b>
Mass vaccination	Reduces zoonetic impact. Herd immunity quickly established. Effective disease control and reduction in losses due to disease. Well accepted by owners. Easy to manage and economical. Flock immunity can be maintained by	Vaccine induced abortions in pregnant animals. Distinguishing infected from vaccinated animals is not feasible in the short term. Infected animals remain on farms for some time.

	vaccinating young animals.	
<b>Vaccination of young animals and test and slaughter of older infected animals</b>	Minimizes vaccine induced abortions. Serological response reduced in vaccinated non-infected animals allowing test to differentiate infected and vaccinated animals.	Herd immunity slowly established (unless moving from mass vaccination strategy). Serological tests to differentiate infected and vaccinated animals are not optimal and cannot be relied upon for accurate diagnosis of an individual animal.
<b>No vaccination test and slaughter</b>	If successful will result in elimination of the infection in the region. Diagnostic tests are more accurate in non-vaccinated animals but still not optimum.	Risk of epidemics and subsequent human infection. Higher cost. Need efficient veterinary services (animal identification, laboratory support, movement control). Suitable for low disease prevalence areas only. Removal of protective cover of vaccination may allow disease prevalence to increase. May require whole herd slaughter to be effective.

### 1.8.3 Eradication:

Eradication means the elimination of a pathogenic agent from a country or a zone (*i.e.* part of the territory of a country with a distinct animal health status). 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 is neither a casual nor an automatic consequence of a control programme, no matter how well

planned and implemented the control programme is. It is based on sanitary measures and on an organization of activities completely different from those implemented for a control programme. 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. The strategies described above for prevention and control can be applied for eradication; however, they are not mutually exclusive, on the contrary they can be arranged in a cascade as shown in diagram 1. On a long-term basis, eradication programmes in general are more economically advantageous compared to control programmes. This advantage, however, cannot always be translated into practice. In fact, an eradication programme involves the mobilization of an amount of resources (financial and human) that may not be available or whose returns for the investment may require a time span longer than any decision-making authority can afford. Cost-benefit and cost-effectiveness analysis can be used to support decisions on control strategies. However, no in-depth analysis is possible in absence of epidemiological surveillance. 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 (Corbel,2006).

## **Chapter Two**

### **Materials and methods**

#### **2.1. Area of sample collection:**

Samples were collected from the following localities of El-Gedarif state :

1. Basundalocality:Gellabat city, Otrub, Am-Khraeet, Kiseiba, Allam, Saraf Saeed and Kuneina.
2. El-Gellabat El-Sharqia locality: Rashid, Assaraf El-Ahmer, Mahala, and Alhamraa.
3. El-Gellabat El-Gharbiya locality: Sabony
4. El-Baladiya : El-Gedarif city
5. El-Fashaqa locality: El-Shuwak city

#### **2.2collection of samples:**

After shaving and disinfecting the site of jagular vein, 3–5 ml of blood samples were collected in sterile plain vacutainer tubes from each study animals. Atotal 558 blood samples were kept in a slanting position overnight at room temperature to separate the serum and the clotted red blood cells ( OIE manual, 2009a).The blood tubes placed in racks and left to stand at ambient temperature for 1–2 hours in slanting position until the clot begins to contract. The bottles placed in a refrigerator at 4°C. After overnight, sera decanted with a pipette in eppendorf tubes, labeled and preserved in an ice box which is transported to the. All sera samples kept at -200° C before serological tests, labeled and transported in cold chain to the animal research laborotary in El-Gedarif city.

#### **2.3 Serological diagnosis of brucellosis:**

##### **2. 3. 1: Rose Bangal Plate Test:**

All serum samples from sheep were examined by the RBPT. The RBPT antigen was removed from a refrigerator, placed at room temperature and brought to 37° c in an incubator, then about 30UL of each serum sample was mixed with an equal volume of the RBPT antigen on an enamel plate. Both were mixed with wooden applicator, rocked gently by hand for 4 minutes and the test was read with unaided eye for agglutination (OIE, 2004).

##### **2. 3. 2: Competitive ELISA (c ELISA):**

The 184 sample of sheep serum were retested by c ELISA. The test was used for examination of the serum samples use in COMP ELISA test kits and procedures supplied by the Veterinary Laboratories Agency (VLA) UK. The c ELISA kit comprised the following:



- Microtiter plates pre-coated with *B.melitensis* lipopolysaccharide antigen.
- Diluting buffer: Tablets of phosphate buffered saline (PBS) + Phenol Red Indicator + Tween 20.
- Wash solution: Na<sub>2</sub>HPO<sub>4</sub> + Tween 20.
- Conjugate: *B.abortus* monoclonal antibody IgG1 conjugated to horseradish peroxidase.
- Chromogen: OPD tablets (Toxic).
- Substrate: Urea hydrogen peroxidase tablets.
- Stopping solution: citric acid.
- Controls: positive and negative serum samples.

#### **Equipment Required:**

- Microtitre plate reader with 450 nm filter (Lab systems Multiskan Ms, Finland)
- Single and multichannel variable volume pipettes.
- Disposable tip for the above.
- Reagent troughs for multichannel pipetting.
- 10 litre container for wash solution.
- Refrigerator.
- Rotary shaker, (Henry Troemner L.LC, USA) capable for 160 Revs/Min.
- Microtitre plate shaker (Stuart scientific CO.LTD, Great Britain).
- Sterile distilled or deionized water.
- Bottles, tubes and beakers for storage of sera and reagents.
- Absorbent paper.
- Freezer for storage of the conjugate.

#### **Method:**

1. The conjugate solution was prepared immediately before use and diluted to the working strength with diluting buffer according to the instructions of the supplier.
2. Of each test serum was added to each well of each microtitre plate, (precoated with *B.melitensis* lipopolysaccharide antigen) using columns 1-10 and wells of columns 11 and 12 were left for control.
3. Twenty microliter of the negative control serum was added to wells A11, A12, B11, B12, C11 and C12.
4. Twenty microliter of the positive control serum was added to wells F11, F12, G11, G12, H11 and H12.
5. The remaining wells (had no serum) were acted as the conjugate controls.
6. Immediately, 100 ul of the prepared conjugate solution was dispensed to all wells. This gave a final serum dilution of 1/6.

7. Each plate was then vigorously shaken on the microtitre plate shaker for 2 minutes in order to mix the serum and conjugate solution. The plate was covered with the lid and incubated at room temperature (21+\_ 6 c) for 30 minutes on arotary shaker set at 160 revs/min.
8. The contents of the plate were shaken out and the plate was rinsed 5 times with washing solution and then thoroughly dried by tapping on absorbent paper towel.
9. The microplate reader was switched on, set at 450 nm and allowed to stabilizer for 10 minutes.
10. The substrate chromogen solutions were prepared by dissolving one tablet of urea H<sub>2</sub>O<sub>2</sub> in 12ml of distilled water, mixed thoroughly. And then 100 ul amounts of this solution were added to all wells. The solution was not stored after preparation.
11. The plate was left at room temperature for 10 to 15 minutes for reaction.
12. The reaction was slowed by addition of 100ul of stopping solution to all wells.
13. Condensation from the bottom of the plate was removed with absorbent paper towel before the plate was read.

**Analysis of the result:**

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

**Evaluation of the test results:**

- The test results of each plate were evaluated by checking the following values.
- Binding ratio =  $\frac{\text{Mean of 6 negative control wells}}{\text{Mean of 6 positive control wells}}$
- The Binding ratio must be greater than 10.
- The mean OD of six negative control wells must be greater than 0.7.
- The mean OD of six positive control wells must be than less0.1
- The OD of the four conjugate control wells must be greater than 0.7.
- Any results which did not comply with the above values were rejected and samples-examined.

## **Data Analysis**

The Statistical Package for Social Sciences (SPSS) for Windows version 18.0 (SPSS Inc., Chicago, Illinois) was used for all appropriate statistical analyses. Descriptive statistics of the variables were obtained. For each variable (age, sex, breed, body condition, parity and locations), frequencies (number of observations within variable) and prevalence rates by crosstabbing (number of positive valid samples/number of individuals sampled in the variable) were obtained. Hypotheses of differences of age group, breed, sex, and locations between test-positive and test negative animals were first tested by univariate analysis by means of the 2-tailed chisquare test. In a second step, a logistic regression model was used to assess the association between the potential individual and management risk factors and the outcome variable brucellosis serological status. Associations in the Chisquare test and the logistic regression model were deemed significant when  $p \leq 0.05$

## Chapter three

### RESULTS

#### Results

- Frequency of tested sheep sera was shown in table (1)
- Over all percentage of sheep Brucellosis determining 1 igG antibodies using Rose Bengal and Enzyme linked Immunsorbant assay (Elisa) shown in table (2)
- Estimated percentage of igG antibodies of sheep Brucellosis in sex , age and locality notice only significant with location (p : 0.000) using Rose Bengal test and also with location using Elisa lest (table (3) and (5).
- Confidence internal 95% cc of all sera samples which were collected in this study . (table (4) and (6)) .
- Situation of sheep Brucellosis among male and female and association with location and age using Rose Bengal test and Elisa test figure No (1) (2) (3) (4) (5) and (6).

**Table 1:** The characteristic features of the sample size

<b>Risk factors</b>	<b>Frequency</b>	<b>Frequency %</b>	<b>Cumulative Frequency %</b>
Sex	male	73	13.1
	female	485	86.9
Age	-1	85	15.2

1-2	131	23.5	38.7
2-3	181	32.4	71.1
3-4	139	24.9	96.1
4-5	18	3.2	99.3
5 above	4	.7	100
location			
Albaladia	100	17.9	17.9
Basonda	202	36.2	54.1
Alfashaga	110	19.7	73.8
Alglabat Algrbia	46	8.2	82.1
Alglabat Alshargia	100	17.9	100

**Table 2 : the result of sera using Rose Bengal and Elisa test**

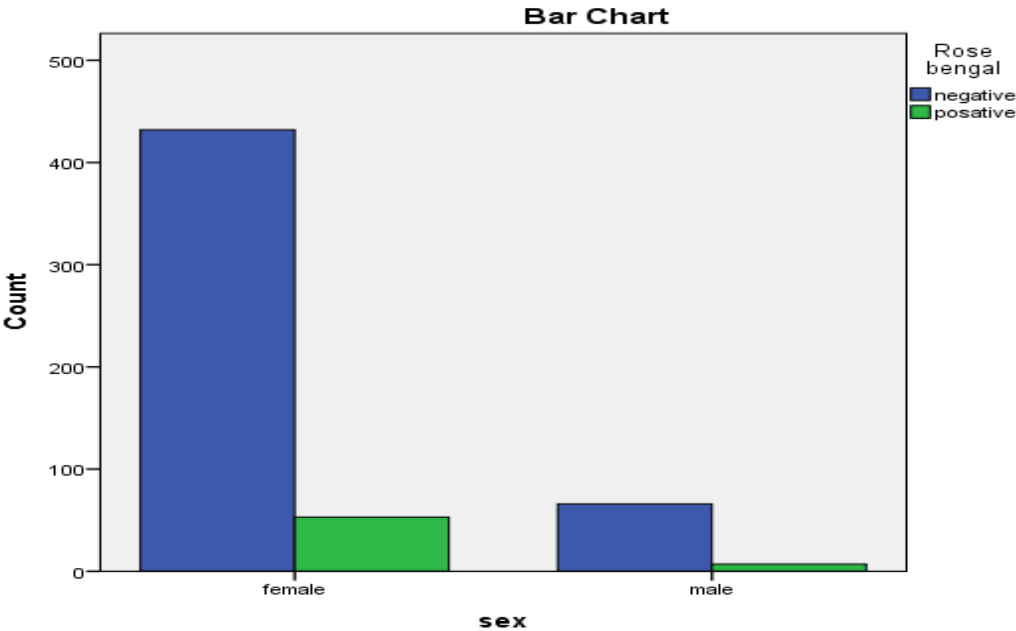
Rose Bengal		Elisa	
No. inspected	No. affected(%)	No. inspected	No. affected(%)
558	60(10.8)	185	35(6.3)

The result shown the number of affected % is less when using Elisa test

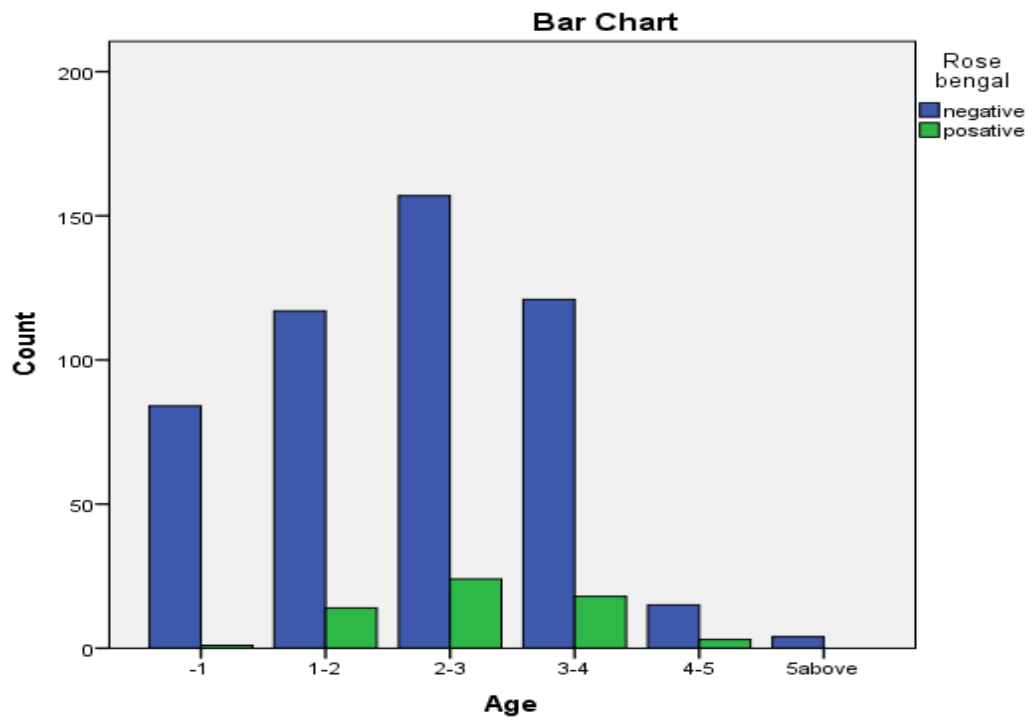
**Table 3:**

Risk factors	No. inspected	No. affected(%)	Odds ratio	95% Confidence Interval

				Lower	Upper
Sex					
female	485	53(8.3)	.864	.377	1.982
male	73	7(11.7)			



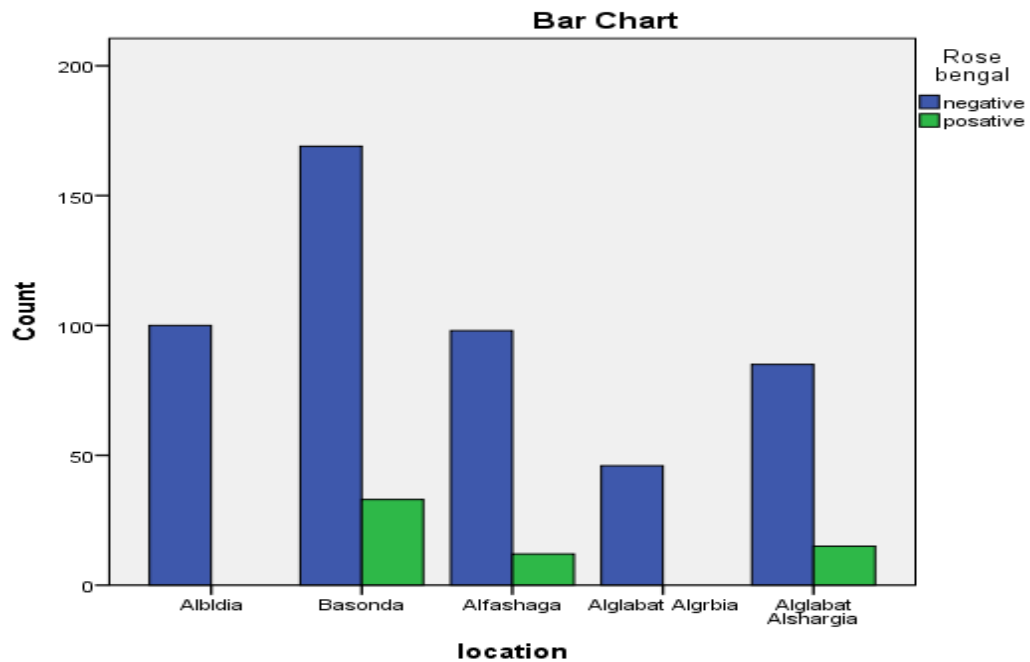
**Rose Bengal Test association with factor sex :**



**Rose Bengal Test association with factor Age :**

Risk factors	No. inspected	No. affected(%)	Df	Chi-square	p-value
Sex					
Male	23	8(22.9)	1	4.309 <sup>a</sup>	.048
Female	161	27(77.1)			

**Rose Bengal Test association with factor location :**



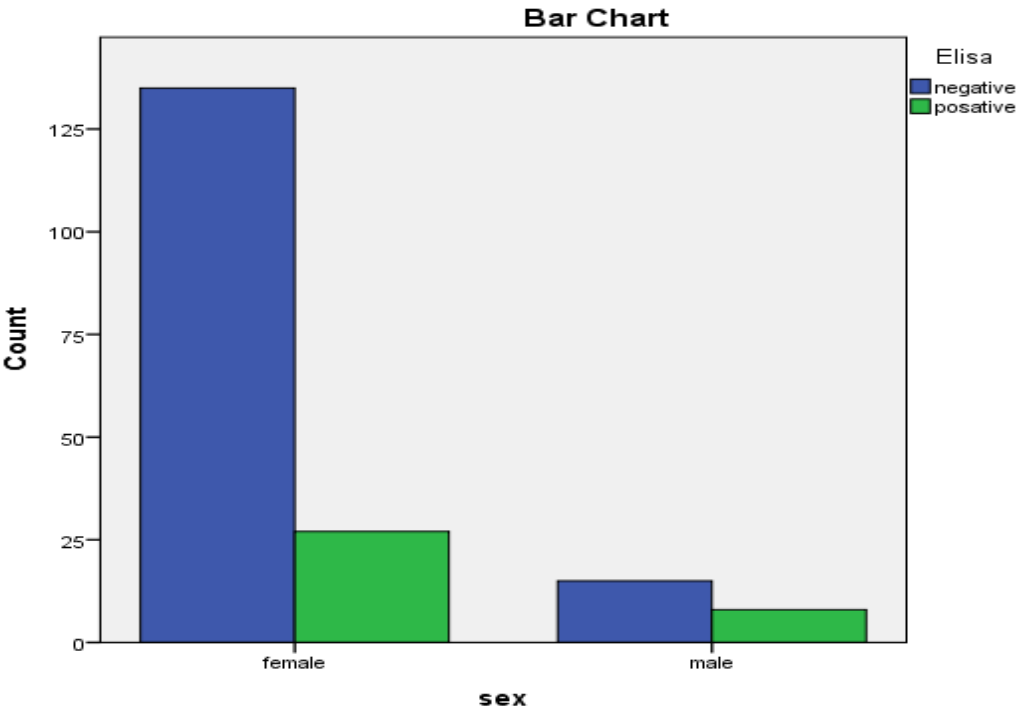


Age					
-1	29	2(5.7)			
1-2	47	8(22.9)			
2-3	57	17(48.6)			
3-4	44	8(22.9)	5	9.144 <sup>a</sup>	.103
4-5	5	0(0)			
5 above	3	0(0)			
Location					
Albaladia	41	4(11.4)			
Basonda	66	25(71.4)			
Alfashaga	43	1(2.9)	3	25.919 <sup>a</sup>	.000
Alglabat	35	5(14.3)			
Algrbia					

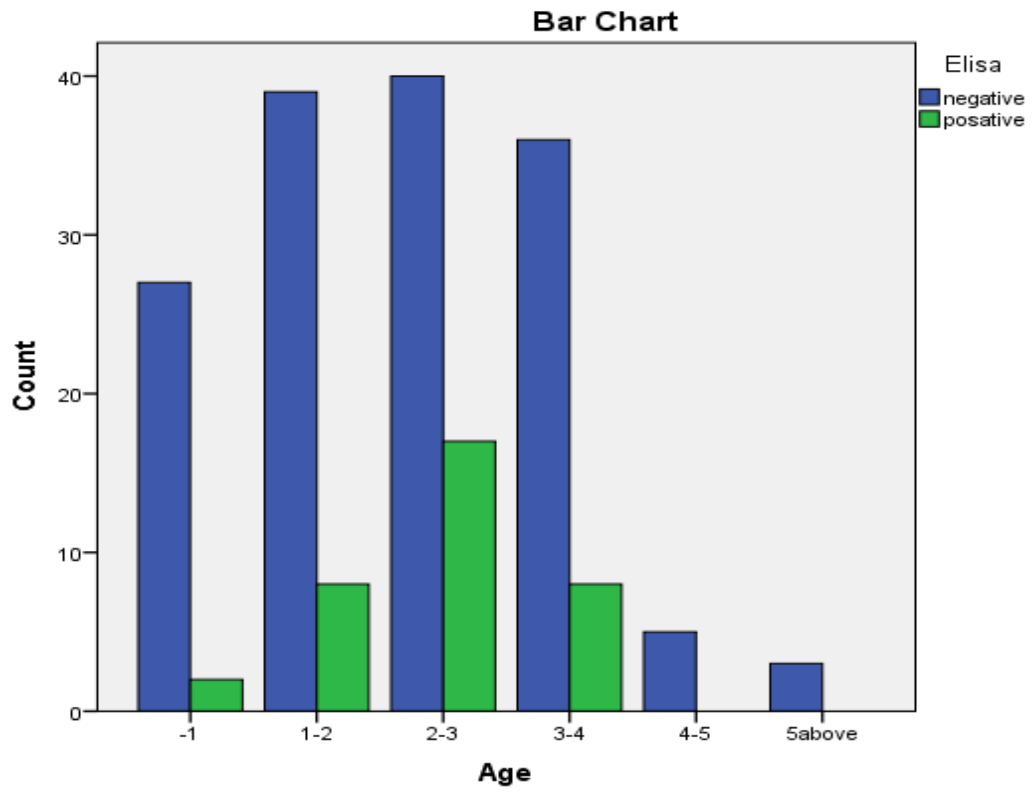
### Elisa

Risk factors	No. inspected	No. affected(%)	Odds ratio	95% Confidence Interval	
				Lower	Upper

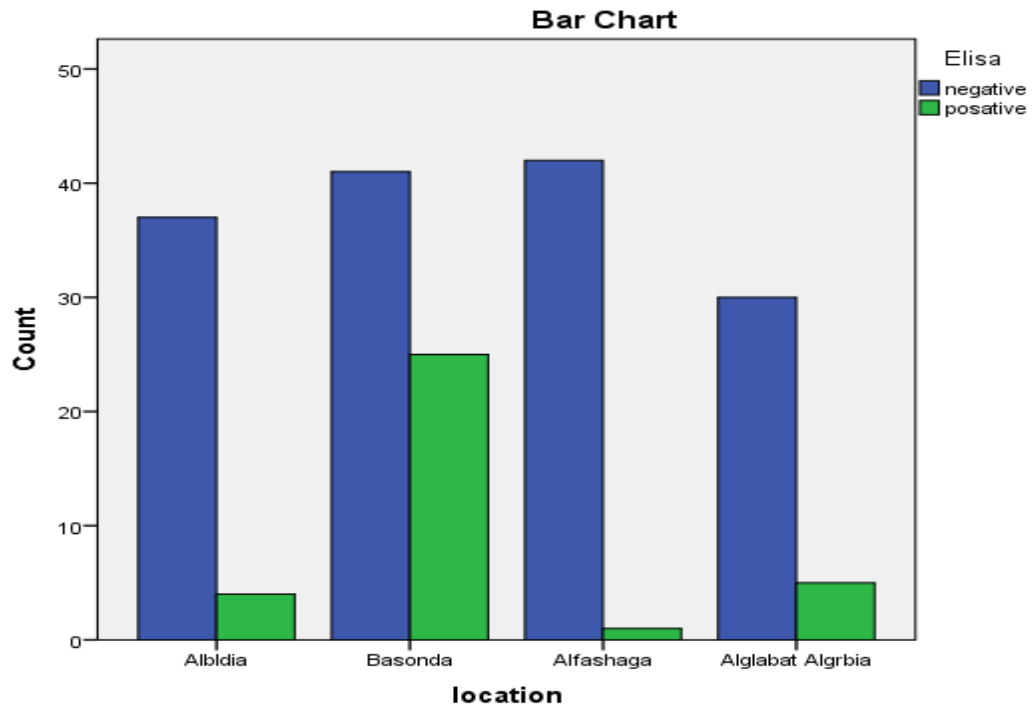
Sex					
female	161	27(77.1)	2.667	1.029	6.912
male	23	8(22.9)			



**Elisa Test association with factor sex :**



**Elisa Test association with factor age :**



**Elisa Test association with factor location :**

## **Chapter four**

### **Discussion and Recommendation**

Brucellosis in animals is considered a great problem in most countries of the world. Thus, early detection of *Brucella* infection in a herd is a pre-requisite for successful control.

In this study the overall prevalence rate in sheep is 10.8% using rose Bengal test and 6.3% using Elisa test , these finding go with seroprevalance study of sheep Brucellosis in Kassala which is 2.1% by using Rose Bengal. (Guma *et al.*, 2014).

However In kordofan(Ahmed *et al.*,2015) found prevalence of sheep Brucellosis is 2.2%.

In this study sheep seroprevalance of Brucellosis is 11.7% in male and 8.3% in females and these findings are In line with studies done by (Musa ,1990) .

In our study it is found that no significance in age association as well as location.

Low seroprevalance rate of sheep Brucellosis could be due to export or consumption of sheep in early ages

Assessment of the economic effect of disease on an individual farm is relatively straight forward, economic analysis becomes more and more complex as one moves from the individual farm to a larger sector. For proper economic assessment more quantitative information has to be collected in order to carry out a sound economic evaluation of the disease at the national or even regional level. Data must include disease awareness, disease occurrence, effect of disease on production process as well as potential of control measures and cost of control (Otte and Chilonda), these information were not available for this study.

Brucellosis is consistently ranked among the most economically important zoonoses. The economic losses due to disease have been divided in direct loss include the milk reduction, abortion and infertility while the indirect include the cost of disease control. In current investigation 80.6% of respondents, mentioned that reduction of milk yield accounted to approximately 100% while the reduction in infertility accounted to 22.8% .This indicates that milk yield reduction causes significant losses more than infertility and loss of calves due to abortion where may occurs once in a life time in she camel.

Competitive ELISA was initially developed to improve the diagnostic specificity of immunoassays for brucellosis while maintaining a high sensitivity (23, 24). The present study confirms that the mRBPT shows an enhanced sensitivity compared to the RBPT given that only 1/400 samples classified negative by the mRBPT was additionally detected by the cELISA

When the all possible economic losses due to disease are considered together, it was evident that the total losses due to the disease in camels were quite considerable. This emphasizes that control of the disease by vaccination and other method of control will have its impact camel production in the area.

Since the state export goats, sheep and camels directly from its quarantines to the Gulf countries, it is always important to confirm the RBPT results with cELISA or in direct ELISA, and likewise for control purpose of the disease in farm animal in the state and the country as a whole. Immediate control of the disease is deeming essential in the state and in the country for food security, public health and export purposes.

### **Recommendations:**

1. More surveys are required to investigate the sheep brucellosis in the states.
2. There is need to plan, implement and monitor sudan eradication strategy for brucellosis in the state based on epidemiological reality.
3. Follow up on capacity building of laboratory technical staff as well as of strengthening of national laboratories should be favourably considered in all

programs of eradication and can be achieved by collaborating international organizations including OIE, FAO, WHO and Codex Alimentarius, where possible.

4. Educational programmes to other stakeholders such as farmers, grazers and animal production consumers

5. Effective enforcement of legislation in conjunction with animal disease control

6. Public awareness about the disease transmissible to human.

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