Sudan University of Science and Technology

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Evaluation of Different Serological tests for diagnosis of Brucellosis in Camels and Male Goats Intended export in Sudan

تقويم اختبارات السيرولوجيه المختلفه في تشخيص مرض البروسيلا في الابل و ذكور الماعز المعده للصادر من السودان

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DEDICATION

إلى من كنا معهم واليوم نذكرهم ونحيا بهم

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الملخص العربي

تم جمع جملة 5700 عينة دم من حيوانات معدة للصادر بمظهر صحي وذلك بعدد ألفي عينة (2000) من الإبل الذكور، سبعمائة عينة (700) من الإبل الإناث وعدد ثلاثة آلاف عينة (3000) من الماعز الذكور. كل الحيوانات كانت غير محصنه .

في هذا البحث تم إجراء أربعة إختبارات علي مصل الدم وهي: فحص العينات لوجود الأجسام المضادة للبروسيلا بإختبار سريع وهو إختبار الروزبنغالRBPT. تم فحص العينات الموجبة بفحصين تأكيديين وهما إختبار التراص الأنبوبي SAT وإختبار اللوحة المحمضة المتعادل BAPA واخيراً باختبار المقايسة المناعية التنافسية بالإنزيم المرتبط بالإليزا cELISA.

كانت النتائج كالآتي : فحص RBPT إستبعد من الحيوانات المعدة للصادر نسبة 21% من الإبل الذكور ونسبة 26% من الإبل الاناث ومانسبته 14% من الماعز الذكور، وهي نسبة كبيرة جداً مقارنة بغيره من الفحوصات.

فحص SAT كان أقل إبعاداً للحيوانات الموجبة حيث كانت نسبة المبعد من الإبل الذكور12% والإناث 14.5% اما الماعز فكانت 3.5%.

اما بالنسبه لفحص BAPA فقد أبعد نسبة 13% من الإبل الذكور ونسبة 14.5%من الإناث ونسبة 3.5 % من الماعز .

أما فحص cELISA فقد أكد إيجابية نسبة 7.4% من الذكور الإبل ونسبة 7.4% من إناث الإبل ونسبة 1.5% من الماعز.

تم إجراء التحليلات الإحصائية باستخدام الحزمة الإحصائية للعلوم الاجتماعية تم حساب انتشار SPSS والجدولة المتقاطعة. اعتبرت الارتباطات في اختبار Chi-square ونموذج الانحدار اللوجستي مهمة عند $p\leq 0.05$.

تعتبر جميع الاختبارات ذات أهمية لأن قيمة P تتراوح من .000 إلى .003 وجميع النتائج أقل من .05.

ABSTRACT

A total of 5700 samples of blood were collected from animals intended for export with a healthy appearance, with two thousand samples (2000) from male camels, seven hundred samples (700) from female camels, and three thousand samples (3000) of male goats. All animals are not vaccinated.

In this research, four tests were performed on blood serum, namely examination of samples for the presence of antibodies to *Brucella* with a rapid test, which is the RosBengal RBPT test. The positive samples were examined by two confirmatory tests, namely the SAT and the BAPA test, and finally by the cELISA-bound enzyme competitive immunoassay.

The results were as follows: The RBPT examination excluded 21% of male camels, 26% of female camels, and 14% of male goats. It is a very large percentage compared to other tests. The SAT test was less remote for positive animals, where the percentage of deported males was 12% and females 14.5%, and goats were 3.5%.

As for the BAPA examination, it excluded 13% of male camels, 14.5% of females, and 3.5% of goats.

As for the cELISA test, it confirmed the positive rate of 7.4% of male camels, 7.4% of female camels, and 1.5% of goats.

Statistical analyses were carried out using the Statistical Package for Social Sciences version SPSS prevalence and cross-tabbing were computed. Associations in the Chi-square test and logistic regression model were deemed significant when $p \leq 0.05$.

All tests consider to be significant because the P value ranged from .000 to .003 and all the results less than .05.

CHAPTER ONE

Introduction

Brucellosis is an important zoonotic disease of domestic and wild animals including humans (Hamdy and Amin, 2002; Radostits *et al*., 2007).

Brucellosis is caused by the bacteria of the genus *Brucella* .

Brucella is named for the British army medical doctor, David Bruce, who isolated the organism from a dead soldier in 1887 on the island of Malta.

Brucellae are facultative intracellular, Gramnegative coccobacilli that lack capsules, flagellae, and endospores. The genus *Brucella* is composed of ten recognized species, six of which are the "classical" members *(B. abortus, B. suis, B. melitensis, B. canis, B. ovis, and B. neotomae)* (Cutler *et al.*, 2005).

B. melitensis, B. suis and B. abortus are of major impact by causing significant economic losses to animal owners and by provoking severe human disease.

Brucella spp. are also a focus of interest as they are categorized as biological agents due to their high contagiousness and their impact on human and animal health.

Brucella suis was among the earliest agents investigated and developed as a bioterrorism weapon in the United States offensive bioterrorism program in the 1950s. The zoonotic pathogens *B. abortus*, *B. melitensis*, and *B. suis* have been identified as category B bioterrorism agents (Rotz *et al*., 2002).

Camels can be infected by *B. abortus* and *B. melitensis* when they are pastured together with infected sheep, goats and cattle (Musa, 1995; Musa *et al*., 2008; Gwida *et al*., 2012). The main pathogenic species are *B. abortus*, responsible for bovine brucellosis, and *B. melitensis*, the main aetiologic agent of ovine and caprine brucellosis (Ali *et al*., 2013).

Most of the importing countries require that the exporting countries be free from communicable and infectious diseases, and there are agreements regarding immunization against some diseases.

Export animals are free of communicable and infectious diseases, one of the most important requirements of importing countries. Among the most important diseases that affect the export is *Brucella*. In the case of a positive test result, the animal is immediately excluded in the Sudanese veterinary quarantines.

In the case of animals exported to the Kingdom of Saudi Arabia, the Kingdom examines a random samples of animals for *Brucella*, and if the brucellosis has a positive percentage, it returns all the animals of the ship, which results in a great economic loss to the animal owners and the government in addition to losses in animals due to severe stress.

The approved examination for the export is Rose Bengal Plate Test, which is a screening test not specific for *brucella*, due to not specific test cross reaction may occur with other diseases leads to estimated percentage of false positives, which excludes an estimated number of exports animals .

B*rucella* disease is very important for animals exports in Sudan, and we should work hard for eliminate the country from it is bad effects.

In this research, we measure the importance of the diversity of laboratory tests for *Brucella*, its importance and its necessity for exports to prevent future economic losses.

It is important to have more than one test for *Brucella*, its ability to perform in the laboratory, and its availability, as there are general and other confirmatory tests for *Brucella*.

Objectives of the study

objectives to:

1\ Detect the prevalence of brucellosis in camels and goats intended for export.

2\Compare between different serological tests .

CHAPTER TWO

Literature Reviews

2.1 Animal Brucellosis

The world population of camels is about 20 million mainly in arid zones. Of which, 15 million camels live in Africa and 5 million in Asia (Glipha, 2006).

In 2001, the total camel population was 19 million. Of which, 17 million were dromedaries and 2 million were Bactrian (Farah and Fischer 2004).

Brucellosis is a disease of high economic and public health importance and has a worldwide distribution (AlMajali *et al*., 2008; Saegerman *et al*., 2008).

Brucella infection is still endemic in countries of the Mediterranean basin, the Middle East and Central Asia (Radostits *et al.*, 2007 Saegerman *et al*., 2008).

Brucellosis is a widely spread disease in camel producing horn of African countries such as Ethiopia, Eritera, Somalia and Sudan ,

Sudan has 43.8 million goats (AOAD, 1998) .These animals are of great economic importance and are kept for meat, milk, hair and skin. Goats are usually kept in small numbers for milk supply in pens or yards near human dwellings Therefore, there is close association between these animals and man in villages and towns, (Nisreen2003).

Caprine and ovine brucellosis is endemic in countries around the Mediterranean Sea, Iran, India, Kenya, and Southern part of Russia, Mexico, Latin America and the southern part of the United States (Nisreen2003).

table 1:Animals affected by *Brucella* spp.

HOST	*B. abortus*	*B. melitensis*	*B. suis*	*B. canis*	*B. ovis*
Cattle	**+**	**+**	**+(rare)**	**–**	**–**
Buffaloes	**+**	**+**	**–**	**–**	**–**
Bison	**+**	**–**	**–**	**–**	**–**
Sheep	**+(rare)**	**+**	**+(possible)**	**–**	**+**
Goats	**+(rare)**	**+**	**–**	**–**	**–**
Swine	**+(rare)**	**+(rare)**	**+**	**–**	**–**
Dogs	**+**	**+**	**+(rare)**	**+**	**–**
Camels	**+(rare)**	**+**	**–**	**–**	**–**
Caribou/Reindeer	**–**	**–**	**+(biovar 4)**	**–**	**–**
Elk	**+**	**–**	**–**	**–**	**–**
Horses	**+**	**+(rare)**	**+(rare)**	**–**	**–**
Rodents	**+(rare)**	**+(rare)**	**+(biovar 5)**	**–**	**–**

(2006WHO, FAOand OIE)

(Jennifer2015) describe the species of *Brucella* and the primary host and the zoonotic importance.

table2: *Brucella* species, preferred hosts, and zoonotic potential.

Species	**Primary host(s)**	**Zoonotic Potential**
Classical species		
Brucella abortus	Cattle	High
Brucella melitensis	Sheep, goats	High
Brucella suis	Swine, hare, reindeer, rodents	High
Brucella ovis	Sheep	None
Brucella canis	Dogs	Moderate
Brucella neotomae	Desert wood rat	None

Jennifer2015

2.2. The epidemiology of brucellosis

Risk factors for brucellosis can be categorized into those determinants necessary for the transmission and maintenance of the disease within herds Factors related to the host, the agent, the environment and management practices determine the extent of exposure, spread and maintenance of brucellosis in a geographical area (Godfroid 2002). The major routes of entrance of *Brucella* to the body are mucus membranes of the alimentary tract, conjunctiva and respiratory tract, damaged skin and male and female genital tracts (SANCO 2001; Neta *et al*. 2010).

contaminated pastures, feed, water, equipment, clothing and udder inoculation from infected milk cups (CFSPH 2007). The penning of sheep and goats at night is known to provide an ideal crowded environment for the spread of brucellosis within the flock. The bacteria do not survive for long periods in hot dry weather. Wet conditions prolong survival and increase the probability of transmission to the next host. In farm

slurry, *Brucella* bacteria can survive for up to seven weeks at ambient temperatures (SANCO 2001).

table3: *Brucella* survival times in the environment

Environment	Conditions	Survival time
Water	20°C	2.5 months
Water (lake)	37°C, pH= 7.2	< 24 hours
Water (lake)	8°C, pH =6.5	2 months
Soil	Dried at 18°C	69-72 days
Soil	Dried in laboratory	< 4 days
Urine	37°C, pH =8.5	16 hours
Manure/dung	Summer	3 months
Manure/dung	Winter	6 months
Pasture	Sunlight	< 5 days
Wool	In warehouse	4 months
Hay		Several days to months
Street dust		3-44 days

(SANCO 2001)

2.3. Camel Brucellosis

Camels are very susceptible to brucellosis and under extensive farming conditions high prevalence rates of the disease have been reported in this species.

Camels are highly susceptible to *Brucella abortus* (*B. abortus*), and *Brucella melitensis* (*B. melitensis*), but camels are not known to be primary hosts of *Brucella*. Thus, camel brucellosis depends on the *Brucella* species prevalent in other animal species sharing the same habitats, and on husbandry methods (Gwida *et al*.,2011).

Brucellosis, particularly due to *B. abortus*, is considered to be one of the most important zoonotic diseases of camels and other domestic animals in some countries of northern Africa. Camel brucellosis was recorded to be caused by *B. abortus* and *B. melitensis* (Abbas and Agab 2002).

Camels are very important for exporting and most of them goes to Saudi Arabia, so there is a routine test of Brucella in quarantine area and it is Rose Bengal test. The table below explain more clear .

table4: Brucella test of exported animals (Camels)

	Total number of animals	**Negative results**	**Positive results**	**The ratio percent**
2010	28368	26117	2251	7.93%
2011	10852	9878	974	8.97%
2012	26537	24688	1849	6.9%
2013	29,578	28,506	1,057	3.6%
2014	25,896	24,982	914	3.5 %
2015	34,225	31,823	2,402	7%
2016	35,518	33,868	1,650	4.6%
2017	53,571	51,492	2,079	3.9%
2018	11,196	10,507	689	6.2 %

(Ministry of animal 2018)

2.4 (Caprine) Brucellosis:

Brucellosis in small ruminants is caused mainly by *Brucella melitensis*, which was the first species in the genus *Brucella* described. It is the most virulent one and most widely encountered of all the species . Brucella melitensis infection may cause abortion in pregnant animals or orchitis and epididymitis in adult males of sheep, goat and cow which may result in infertility (Motamedi H,et.al 2010)

In goats, brucellosis is mainly caused by *Brucella melitensis* , Sheep and goats are the natural hosts but may infect other species such as cattle, pigs and man. (Nisreen 2003).

The transmission of disease is facilitated by commingling of flocks and herds belonging to different owners and by purchasing animals from unscreened sources. The sharing of male breeding stock also promotes transfer of infection between farms. Transhumance of summer grazing is a significant promoting factor in some areas as is the mingling of animals at markets or fairs. In cold climates, it can be the custom to house animals in close space and this also facilitates transmission of infection.(WHO and FAO2006). Caprine brucellosis is an endemic in most countries at the Mediterranean basin, Middle East and Central Asia (Seleem *et al*., 2010) **.** B. melitensis is particularly common in the Mediterranean. It also occurs in the Middle East, Central Asia, around the Persian Gulf (also known as the Arabian Gulf), and in some countries of Central America. This organism has been reported from Africa and India, but it does not seem to be endemic in northern Europe, North America (except Mexico), Southeast Asia, Australia, or New Zealand. Biovar 3 is the predominant biovar in the Mediterranean countries and the Middle East, and biovar 1 predominates in Central America (Ovine and Caprine Brucellosis2009).

In Sudan, interest in goat brucella has begun in recent years, given the importance of goats for exports and pastoralists, and there is an examination of *brucella* for export according to the statistics of the Ministry of Animal Resources.

The RBPT it is a routine test in quarantine department in animal ministry of Sudan as the table 5 explain.

table 5: Brucella test of exported animals (Goats)

	Total number of animals	**Negative results**	**Positive results**	**The ratio percent**
2010	102715	101428	1287	1.25%
2011	160669	160257	412	0.26%
2012	153520	152600	920	0.05%
2013	17061	178436	625	0.4%
2014	322945	321933	1012	0.3%
2015	422472	421378	1094	0.3%
2016	280885	280580	305	0.1%
2017	286165	285867	298	0.1%
2018	248,409	247,842	567	0.2 %

(ministry of animal 2018)

2.5. The Importance of Camels and Goats:

We find that camels and goats have a great and important opportunity to develop exports according to the latest census of the Ministry of Animal. in addition to their economic importance for pastoral communities.

table 6:Estimate of Livestock Population In Sudan 2019

CATTLE	SHEEP	GOATS	CAMELS	TOTAL
31489000	40896000	32032000	4895000	109321000

Camels are primarily the domestic animals of pastoral communities that ensure food security. They produce milk, meat, hair and hides, and also serve as a draught animal for agriculture and transport people as well as goods (Schwartz and Dioli, 1992, Bekele 2004).

Until the arrival of motorized transport in the arid and semi-arid zones, camels have been the sole means of transport in the areas where they are adapted. They are also used for wheel transport, water lifting and source of power for oil mill. Camel racing and other leisure activities such as camel safaris and trekking have recently become a tourist attraction and luxurious in some parts of the world (Schwartz and Dioli, 1992; Wilson, 1998).

Goats are the major source of livelihood for pastoral communities there is a good demand for its meat and milk. Approximately 90% of goats are located in the developing world, where they are considered one of the most important sources of protein for human (PLOS 2017, Tosser *et al.,* 2014).

Small, highly mobile animals that are easy to care for little boys and ladies they are suitable for cultivation on newly reclaimed lands in addition to non-reclaimed lands, and fertilizing those increases the fertility of these lands.

The price of one is cheap and a herd can be formed at a reasonable cost low cost of raising and food, they are sweeping animals for all field waste and have high food conversion efficiency compared to other animals.

You do not need sheds, and simple umbrellas are sufficient to accommodate them, and they can be raised indoors. Adaptability and different food

With high reproductive efficiency, as the rate of production of twins is high, and females will remain in the herd to increase the number of goats

Its small size makes it suitable for family consumption, especially on occasions

Goat meat tastes excellent and the percentage of fat in it is low, so its meat is preferred to reduce the incidence of arterial disease.

The production of milk is so abundant that it is called a small farmer cow due to its high production of milk, which reaches 20-25% of the cow's production and is characterized by small-sized particles of fat,

Which makes it suitable for breastfeeding children, as it makes from it expensive types of cheese and makes from its skins the finest types of leather products. (elaard.com).

In Sudan, the export of animals is very important activity for the state and herders because of its great benefit. There is an annual census of exports of live animals, according to the Ministry of Animal. table 8 explain it .

table 8: The Percentage of export (sheep, goat, camel and cattle) from 2010 to 2018

Year	Sheep (%)	Goat (%)	Cattle (%)	Camel (%)
2010	85.88	5.71	0.24	8.15
2011	89.08	5.29	0.68	4.93
2012	90.59	4.29	0.69	4.40
2013	91.72	4.83	0.27	3.16
2014	90.25	6.33	0.38	3.02
2015	88.66	7.24	0.74	3.34
2016	88.15	5.42	0.2.01	4.40
2017	87.40	5.45	2.24	4.89
2018	89.54	4.68	2.12	3.64

(Ministry of animal 2018)

2.6. The economic impact of brosellosis:

Brucellosis is a zoonotic disease globally affecting mainly domestic animals causing genitourinary infections leading to abortion (Brooks *et al*., 2012).

Brucellosis is characterized by abortion, non-viable offspring birth in female, and orchitis and epididymitis in male animals (Radostits *et al*., 1994; Seifert, 1996). Abortion is the major feature that is manifested in camels (Al-Khalaf and El-Khaladi, 1989). The disease is also associated with infertility and prolonged calving intervals, and has considerable impact on camel production. Chronic inflammation of epididymis, of the joints, tendon sheath and synovial bursae especially at the carpus may also occur in camels (Abbas and Agab, 2002; Wernery and Kaaden, 2002). The disease also have an impact on export and import of animals constraining livestock trade.(Afzal and Sakkir 1994) have suggested that sub clinical brucellosis can pose problems in racing camels by reducing the performance and productivity

of these animals in the Arabian Peninsula where camel racing is highly popular The disease can also have an impact on export and import of animals constraining livestock trade (Abu damir *et al*., 1989).

Many tribes in different parts of the Sudan depend entirely on camels for their livelihood. Camel meat is consumed throughout the country and the animals contribute effectively to the economy by their use in agricultural practices and exportation. However, brucellosis has emerged as a major cause of abortion, hence a constraint to their breeding (M.M. Omer *et al*., 2010), and has had a negative impact on the export of camels.

In goats It can cause abortion, retained placenta, and swelling of the testicles. Abortions usually occur in late pregnancy in sheep, in goats, mastitis and lameness may be seen. Fever, depression, weight loss, diarrhea.

Brucella from contaminated feed, pasture, or water after ingestion spreads through the blood and becomes localized in the lymph nodes, udder, uterus, testes, and spleen.

2.7. Brucellosis and international Trade:

Before oil was discovered in Sudan, export of livestock and livestock products were the country's most important foreign exchange earner. It is currently the second most important source of foreign exchange after oil (Animal Resources Services Company, 2014). Brucellosis is one of the most common zoonotic diseases in the present time in both developed and developing countries alike according to the classification of the global organizations WHO, FAO, OIE (Mohamed Refai, 2011) and it affect national and international trade .

According to reports of the General Directorate of Veterinary Quarantine and Meat Hygiene (The Ministry of Livestock2014) brucellosis one of infectious diseases that have an impact on exports of animals in Sudan

The importance of this disease is due to the high economic losses as well as the danger to human health and safety (General Administration of livestock - Kassala State, 2012).

The exporting and importing nations have prior agreements on how to interpret and handle positive tests, both for the individual animal and the cohort group, for all of the diseases tested.

Regional Economic Communities (COMESA, EAC and IGAD) need to agree on standards to which the exporting and importing nations subscribe and to which the quarantine stations and veterinary authorities on both sides of the trading equation agree and adhere. Disease control and import decision making must be uniform and science based.

At export and import quarantine stations, a standardized regimen of response to presumptive screening tests and a standardized regimen of responses to subsequent supplemental confirmatory tests are suggested for Brucellosis.

the main cause of return ships is due to Brucella test . The test performed by RBPT and may has a false positive which cause return of the ships and make economic loss. (Ministry of animal2018) explain the different reasons of returned ships of exported animals by a table.

table9**:** The Reasons of Returned Shipment from 2010 to 2018

Diseases	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**
Brucella	13%	14%	88.5 %	60%	61.5 %	92.8 %	1.4%	86.9%	0.5%
External parasite	0	0	9.8%	20%	21.6 %	0.7%	0	0.19%	0
Pox	67%	43%	2.1%	20%	9.0%	0.4%	0.5%	0.24%	0
Suspicion of FMD	3%	3%	0	0	0	0	7.1%	11.7%	0
T.B	0	0	0	0	22.5 %	0.2%	0.3%	1.2%	0
Lack of documents	17%	0	0	0	6.5%	3.3%	90.0 %	0.19%	0
H.S	0	0	0	0	0	0	0	0	99.4%

(Ministry of animal 2018)

2.7.1 Quarantine procedures for food animals and wild life that are for export as is written in (The Veterinary Quarantine laws in Sudan (2003):

General consideration:

1-The food animals cannot enter veterinary quarantines without health certificate and road document from Inspection and Vaccination Centers.

2-The animals must be kept for 21 days in quarantine or any other period determined by quarantine authority.

3-The responsibility of animal feed, care and safety are taken over by the owners of animals .

4-Export animals should be isolated from other animals in transport units and road.

5-Export animals must be given international health certificate and other test Certificates that are required by the importing countries.

6-Transportation units of export animals should be in conformity to the OIE Requirements and the Sudanese standard criteria.

Exported animals (sheep , goat and camel) are tested for *Brucella*, as required by the veterinary services of the importing country (Saudi Arabia). Rose-bengal test is carried out in the Central Veterinary Research Laboratories in Khartoum.

2.7.2 Quarantine laws for export :

Quarantines are defined by their duration and by the activities and procedures practiced to assess health status.

The first law for exporting animals in the Sudan was set in 1913. That law Decline three quarantine processes which depend on three elements; namely appropriate infrastructure and budgetary, well defined quarantine procedures and trained personnel.

2.7.2.1. Quarantine process has three stages:

The first stage

in the vaccination and Inspection which comprised of inspectio of animals and rejection of those disqualified for export, vaccination of animals selected for export and ear-tagging them and re-inspection before animals transported for the second stage. The inspection and vaccination is done only by, and under the supervision of the responsible veterinarians from the Ministry of Animal Resources Every transporting unit given a separate road document, and animals are kept in the centres for 7 days to monitor the reaction of vaccine.

The second stage

is internal veterinary quarantine, here animals are kept for 7-10 days in those quarantines, which are represent by quarantines that are not at country borders like, Elkadro, Elshwak, Elrahad, Elkhewai, Kassala and Kosti.

The animals during quarantine are subjected to the following:

- Inspection of animals when entering and reject the unqualified
- Monitoring the animal daily
- No vaccination or treatment with any drug
- Animals examined for Brucella
- Ensuring that transportation units are suitable and comfort for the animals .
- Every transportation unit given a separate Road Document.

The third stage

is the Terminal quarantine, which is the final stage and located at the borders or the airports like Port Sudan quarantine on the red sea, Halfa quarantine at the borders with Egypt and Khartoum airport quarantine .

Procedures applied consist of:

a- Inspection of animals when entering.

b- Verification of animals document and Certificates.

c- Keeping animals for 21 days for monitoring in the quarantine.

d- No vaccination or clinical therapy only monitoring, isolation and rejection to outside the quarantine.

e- It is ensured that transportation units are qualified for export animals, as well as ventilation, cleanness, antiseptics and sufficient light .

Animals are given at this final stage, the international health certificate.

2.8.General Obligations for exportation:

Safety of international trade in animals and animal products depends on a combination of factors which should be taken into account to ensure unimpeded trade, without incurring unacceptable risks to human and animal health . Because of differences between countries in their animal health situations, various options are offered by the Terrestrial Code. The animal health situation in the exporting country, in the transit country or countries and in the importing country should be considered before determining the requirements for trade. To maximize harmonization of the sanitary aspects of international trade . Veterinary Authorities of OIE Members should base their import requirements on the standards of the OIE. The Veterinary Authorities of the importing and exporting countries should enter into a formal agreement recognizing the compartment. Biological tests and/or vaccinations required by the importing country should be carried out in accordance with the in the Terrestrial recommendations Code and Terrestrial Manual, as well as disinfection and disinfestation procedures. Quarantine programs are designed to both facilitate the detection of communicable diseases and to make accurate assessments of the overall health status of individuals and/or groups entering a new population. Prudence dictates that for public health and safety the infectious disease status of all incominganimals is considered at best uncertain.

2.9. Control Programs for Livestock Export:

For national disease control programs, the choice of what laboratory tests to use is at the discretion of the program managers and there is no requirement to use OIE approved protocols.

a. For livestock in export trade and any other animals moving internationally, all laboratories testing must use OIE approved tests or other tests as agreed to between exporting and importing nations.

2.9.1. International trade of lives animals according to regulations of OIE

Safety of international trade in animals and animal products depends on a combination of factors which should be taken into account to ensure unimpeded trade, without incurring unacceptable *risks* to human and animal health.

Because of differences between countries in their animal health situations, various options are offered by the Terrestrial Code. The animal health situation in the exporting country, in the transit country or countries and in the importing country should be considered before determining the requirements for trade. To maximise harmonisation of the sanitary aspects of international trade, Veterinary Authorities of Member Countries should base their import requirements on the standards of the OIE. These requirements should be included in the model certificates approved by the OIE

2.9.2. Certificates should be drawn up in accordance with the following principles:

1) Certificates should be designed so as to minimize the potential for fraud including use of a unique identification number, or other appropriate means to ensure security. Paper certificates should bear the signature of the certifying veterinarian and the official identifier (stamp) of the issuing Veterinary Authority. Each page of a multiple page certificate should bear the unique certificate number and a number indicating the number of the page out of the total number of pages. Electronic certification procedures should include equivalent safeguards.
2) Certificates should be written using terms that are simple, unambiguous and as easy to understand as possible, without losing their legal meaning.

3) If so required, certificates should be written in the language of the importing country. In such circumstances, they should also be written in a language understood by the certifying veterinarian.

4) Certificates should require appropriate identification of animals and animal products except where this is impractical

5) The signature and stamp should be in a colour different from that of the printing of the certificate. The stamp may be embossed instead of being a different colour.

6) Only original certificates are acceptable.

2.9.3. Responsibilities of the exporting country as OIE 2019 manual

1) **An exporting country should, on request, supply the following to importing countries:**

a) information on the animal health situation and national animal health information systems to determine whether that country is free or has zones or compartments free from listed diseases, including the regulations and procedures in force to maintain its free status

b) regular and prompt information on the occurrence of notifiable diseases;

c) details of the country's ability to apply measures to control and prevent the relevant listed diseases;

d) information on the structure of the Veterinary Services and the authority which they exercise in accordance regulations of exporting procurers

e) technical information, particularly on biological tests and vaccines applied in all or part of the national territory.

2**) Veterinary Authorities of exporting countries should:**

a) have official procedures for authorisation of certifying veterinarians, defining their functions and duties as well as conditions of oversight and accountability, including possible suspension and termination of the authorisation;

b) ensure that the relevant instructions and training are provided to certifying veterinarians.

c) monitor the activities of the certifying veterinarians to verify their integrity and impartiality.

3) The Veterinary Authority of the exporting country is ultimately accountable for veterinary certification used in international trade.

Exporters of live animals in general must provide the following documents:

1 Veterinary health certificate declaring the animals to be free from epidemic or contagious diseases. The certificate is signed by a qualified veterinary officer from the Federal Ministry of Animal Resources and endorsed by a senior veterinarian.

2 Form EX from any commercial bank indicating the quantities to be exported and the amount to be paid according to the price and type to be exported from the Ministry of Trade.

3 A commercial license issued by the Ministry of Commerce.

4 Tax identification number.

5 Registration in the Chamber of Commerce.

6 Quality certificate from the General Organization for Standardization and Metrology.

7\ An export license from the Ministry of Foreign Trade or a valid sales contract between the exporter and the supplier authenticated by the Ministry of Commerce.
8\ Ministry of Commerce form (red image).
9\ An import permit from the importing country stating the type of animals to be imported, the purpose of the import and any requirements required.

2.10. Public Health Importance:

Moreover, the main species affecting humans are *B. abortus* and *B. melitensis*, which cause brucellosis, also known as Malta fever (Khamesipour . F *et al*.,2013). In humans, the disease, which is often referred to as 'undulant fever' or 'Malta fever' is a serious public health problem. Human brucellosis remains one of the most common zoonotic diseases worldwide, with more than 500,000 new cases annually (WHO and FAO1985).

Brucella melitensis and *B. abortus* are the two species most commonly found in human cases, and *B. melitensis* is responsible for the most serious infections (Schulze et al 2002) .*Brucella* infections in pregnant women in early pregnancy may lead to high rates of fetal loss (up to 40%) and infection in men can lead to orchitis and epididymitis. *Brucella melitensis* DNA persists in human blood for many years after infection despite appropriate treatment and apparent recovery (Vrioni *et.al.*, 2008). In humans, the symptoms are not specific and are easily confused with other fever causing diseases such as malaria, typhoid fever, rheumatic fever, and arthroses (Makita et al 2011). Furthermore, there is reduced work capacity due to illness of the affected people (Mangen *et.al.*, 2002) . The source of infection for humans are infected domestic animals, wild animals and their products The disease is an occupational risk for farmers, veterinary surgeons, and workers within the meat industry (WHO 2015).

2.11. Transmission to Human:

This disease is also known as Rock fever, Cyprus fever, Gibraltar fever, Malta fever, and Undulant fever (Arnold2007) because the fever typically rises and falls like a wave (Medicine 2001).

The etiologic agents of Brucellosis in human are several different species, *Brucella melitensis, abortus, suis* and *canis* (Ovine and Caprine 2001). Consumption of *Brucella* in infected food e.g. milk and meat has led to a high number of human brucellosis cases and is a serious public health issue . The situation is even more grave as farmers from rural areas think that raw camel milk has a healing effect on the digestive system (Gwida *et,al.*, 2011).

The clinical picture is not specific in animals or humans and diagnosis needs to be supported by laboratory tests . Effective treatment is available for the human disease but prevention is the ideal , through control of the infection in animals and by implementation of hygienic measures at the individual and public health levels (WHO and FAO2006) .

Humans can become infected when they come into contact with infected excretions of anmal, foetuses or abortions, foetal membranes or with infected carcass material in abattoirs. Brucellosis vaccines should be handled with care. Humans can also be infected by ingesting infected unpasteurized, unboiled milk. The disease in humans caused by *B. abortus* is also called undulating fever.

table10: Classification of the genus *Brucella* and pathogenicity to humans

Species	**Biovar(s)**	**Colony morphology**	**Host(s)**	**Pathogenicity to humans**
B. melitensis	1-3	smooth	goats, sheep, cattle, wildlife	high
B. abortus	1-6, 9	smooth	cattle, sheep, goats, wildlife	average
B.Suis	1 et 3 2 4 5	smooth smooth smooth smooth	pig pig, reindeer, hare rodents caribou	high not average high
B. neotomae	-	smooth	desert rat	not
B. ovis	-	rough	sheep, experimentally in goats	not
B. canis	-	rough	dog	low
B. cetaceae	-	smooth	cetaceans	average
B. pinnipediae	-	smooth	pinnipeds	average

B. ovis and *B. canis* are rough strain *brucella* and are diagnosed with the same serological tests as *B. melitensis* and *B. abortus* which are smooth strains.

2.12. Etiology of Brucellosis:

Causative agents of Brucellosis are gram negative bacteria, belonging to genus *Brucella*, five out of ten known species have potential zoonotic significance (Godfroid *et al.,* 2005; Seleem *et al.,* 2010) . Gram –negative coccobacillus or short rod . This organism is a facultative intracellular pathogen. *B. melitensis* contains three biovars (biovars 1, 2 and 3) . All three biovars cause disease in small ruminants, but their geographic distribution varies .

Brucella abortus and *Brucella suis* infections also occur occasionally in small ruminants, but clinical disease seems to be rare . Camel is known to be susceptible for both *B. abortus* and *B. melitensis*, but it is not considered as a primary host (Gwida *et al*., 2011; Wernery and Kaaden, 2002) .

Epidemiological reviews on camel brucellosis have shown a cosmopolitan prevalence (Abbas and Agab, 2002; Gwida *et al*., 2012; Seleem *et al.,* 2010; Sprague *et al*., 2012).

table11:The main species of *Brucella* and their hosts

Species	**Smooth/rough**	**Hosts**
Brucella abortus	smooth	cattle, camels, wild ungulates, humans
Brucella melitensis	smooth	sheep, goats, cattle, camels, humans
Brucella ovis	rough	sheep, red deer (New Zealand)
Brucella suis	smooth	swine, cattle, humans
Brucella canis	rough	dogs, humans
Brucella neotomae	smooth	wood rats

(Godfroid 2002)

Brucella can be classified into smooth (*B. abortus, B. melitensis, B. suis* and *B. neotomae*) and rough (*B. ovis* and *B. canis*) strains. Smooth strains are generally more virulent than rough strains and more pathogenic in humans . This distinction refers to the structure of the lipopolysaccharide (LPS) in the bacterial cell wall. While S-LPS consists of three components, lipid A, core oligosaccharide, and O-antigen, in R-LPS the O-antigen is either absent or reduced to only a few sugar residues There are 2 types of smooth lipopolysaccharide (sLPS) surface antigens, designated A and M. A antigen predominates in *B. abortus* and *B. suis*, while M is the major antigen in *B. melitensis*. Numerous outer and inner membrane, cytoplasmic and periplasmic proteins have also been characterized. The sLPS from *B. abortus* is 100 times less potent than that of *E. coli* and *Salmonella* in inducing TNFα from macrophages as well as oxidative metabolism and lysozyme release by neutrophils. This feature of sLPS is supposed to contribute to the survival of *B. abortus* within phagocytic cells. In addition, Brucella sLPS is not susceptible to the actions of polycationic molecules, suggesting that smooth *Brucella* can resist the cationic bactericidal peptides of the phagocytes. The sLPS also conferes antiphagocytic properties to *Brucella* and does not activate the alternate pathway of the complement cascade (K MANISH et.al 2013)

Apart from *B. abortus*, seven other species are known, including *B. melitensis, B. suis, B. neotomae, B. ovis and B. canis.* . Their importance with respect to infection and their incidence *B. melitensis* causes abortion in sheep and goats, and Malta fever in humans. It also occasionally causes abortion in cattle and wildlife. Cattle are affected when they live in close contact with infected sheep and goats.

Serological tests cannot differentiate between *Brucella* species and hence a positive serological result can indicate any of the following causative smooth strained *Brucella* species: *B. abortus, B. melitensis, B. suis*.

B. canis causes brucellosis in dogs. People can become infected. *B. ovis* causes epididymitis in rams and infertility in ewes. Sheep brucellosis has been found in all sheep breeds in South Africa. Goats and other animal species are apparently not affected. This is not a controlled disease. *B. suis* is a pathogen of pigs that can also infect humans, dogs and horses *B. neotomae* causes brucellosis in desert rats (Bovine Brucellosis Manual2016).

2.13 Pathology of Brucellosis :

The infection occurs via the mucous membranes, including oralnasopharyngeal, conjunctival and genital mucosa, and also through cutaneous abrasions. Animals become infected through feed, water, colostrum, contaminated milk and, especially, by licking or sniffing at placentas and aborted fetuses. The spread

of brucellosis during sexual activity plays a subordinate role. The primary shedding routes of *Brucella* organisms remain uterine fluids (lochia) and placenta (Alani *et,al*.,1998 , Wernery 2014). Little is known about the pathological changes caused by *Brucella* organisms in camelids. These bacteria have a predilection for the pregnant uterus, udder, testicles, accessory male sex glands, lymph nodes, joint capsules and bursae. Lesions may be found in these tissues.

2.14. Immunology of Brucellosis :

2.14.1.B-Lymphocytes (B-Cells):

These cells mediate humeral immunity. The major function of B lymphocytes is the production of antibodies in response to foreign protein (antigen) of bacteria, viruses or tumor cells. Antibodies are specialized proteins that specifically recognize e and bind to one particular antigen, usually a protein, polysaccharide or

lipopolysaccharide. B-lymphocytes contact antigens via Antigen Presenting Cells (APC) and split to form memory cells and plasma cells. Plasma cells produce antibodies that bind and inactivate pathogens and memory cells enable the immune system to react quicker when exposed to the same pathogen in future.

For every foreign antigen, there are antibody molecules specifically designed for that particular antigen. Antibody production and binding to the foreign substance or antigen is critical as a means of signaling other cells to engulf, kill or remove that substance from the body. There are five major classes of antibodies or immunoglobulins (Ig): IgG, IgA, IgM, IgE and IgD. On the first exposure to an antigen, IgM production levels are much higher than IgG levels. On subsequent reactions, IgG levels will be higher than IgM levels.

2.14.2. Brucellosis Immunology :

Brucella abortus antigenic stimulation of the host immune system includes the Lipopolysaccharides (LPS) of its gram negative cell wall. Field strains and the S19 vaccine strains have an O-side chain LPS. The RB51 vaccine strain does not have an O-side chain. 8 different biovars exist and may be identified on phage biochemistry with monospecific antiserum. Cross reactions to other bacteria may occur (e.g. *Yersinia, Chlamydophila, Coxiella*). The brucella bacteria typically enters the body through the mucous membranes (nose, mouth, conjunctiva), where the reticulo-endothelial system (macrophages) picks it up and drains it to the local lymphnodes. From here a bacteremia usually ensues which may be recurrent. The organism typically targets the synoviae of joints, the testes and seminal vesicles, the udder and the gravid uterus (erythitol sugar) which includes the endometrium, placenta and foetus. Cell destruction and inflammation occurs which may lead to abortion. *Brucellae* are intracellular bacteria that stimulate both the cellular and

humoral immune systems.

2.14.3. The humoral immune component

is driven by B-lymphocytes. Antigen Presenting Cells (macrophages) present brucella specific antigens to B-lymphocytes. Memory B-cells are formed as well as active Plasma cells that produce specific antibodies to neutralize *Brucellae*. These plasma cells die after a few days and the antibody titre starts dropping. Memory cells retain the immunity for future recognition of the pathogen. IgM is produced first (primary response) during the natural immune response, followed by IgG after a short lag period which reaches higher concentrations. IgM declines once IgG starts spiking. During vaccination IgM antibodies persists longer and reaches a greater peak than IgG (compared to normal infection). This phenomenon may be useful in differentiation of infected versus vaccinated cattle if paired serum samples are collected for serology (SAT test). Experience is needed in interpreting results.

2.14.4.The cellular immune component

consists of macrophages and neutrophils. APC's present *Brucellae* antigens to T- lymphocytes. T-Memory cells are formed, as well as active T-helper/ killer cells that are able to recognize *Brucellae* infected cells. These T-helper/ killer cells then attach to the infected cells and secrete mediators to attractk phagocytic cells (macrophages) to destroy the infected body cells. Cellular mediated immunity is a typeIV hypersensitivity reaction (principle that the brucellin intradermal test is based on, that works in similar fashion to the tuberculin intradermal test). (Bovine Brucellosis Manual 2016)

2.15. Diagnosis of Brucellosis

Accurate diagnosis is the key to prevent the spread of and to control brucellosis. However, diagnosis of brucellosis is frequently difficult to establish.

This is not only because the disease can mimic many infectious and infectious diseases, but also because the established diagnostic methods are not always sensitive enough. Although serological tests have been used as diagnostic tool for screening of camels brucellosis, they are neither adequately sensitive nor specific due to an insufficient immune status of the host or serological cross reactivity (Morgan and Mackinnon, 1979; Farina, 1985), as well as most tests have been directly transposed, without validation for camels. The most specific diagnostic test is isolation of the causative agent; however, it is time consuming and low sensitive especially in the chronic stage of the disease (Alton *et.al.*,1988) . Because of these difficulties, the development of new diagnostic tests for direct detection of *Brucella* species is increasingly drawing interest.

The morphology of the *Brucella* bacterial colonies is associated with the presence of lipopolysaccharides (LPS) in the external membrane of the bacterium.

Smooth (S-LPS) and rough (R-LPS) phenotypes are differentiated. The S-LPS phenotype is found in most *Brucella* species, only *B. canis and B. ovis* possess the R-LPS. Some proteins of *Brucella* are responsible for serological cross-reactions between *Brucella* spp. and other bacterial species (Wernery U.et al 2014). Cross-reactivity exists to:

- *Yersinia enterocolitica* O:9
- *Escherichia hermannii*
- *E. coli* O:157
- *Francisella tularensis*
- *Stenotrophomonas maltophilia*
- *Vibrio cholera* O:1
- *Salmonella* serotypes group N

Therefore, difficulties may arise in the diagnosis of brucellosis. Abortion and reduced fertility in the camel frequently have other causes, such as salmonellosis, trypanosomosis, or infections with *Campylobacter* or *Tritrichomonas fetus* (Wernery U.*et al*., 2014), making laboratory testing essential. An incorrect diagnosis of brucellosis may occur when based on serology alone.

2.15.1.Serological tests

Because no serological test is 100% accurate, generally, diagnosis is made based on the results of two or more tests. Thus initial testing is commonly done using a screening test, a test with high sensitivity and perhaps of less specificity. The screening tests are usually relatively inexpensive, fast and simple to perform. If a positive reaction occurs in a screening test, a confirmatory test is performed. The confirmatory test is a test which provides good sensitivity but higher test specificity, thereby eliminating some false positive reactions. Most confirmatory tests are more complicated and more expensive to perform.(Fernando Poester *et.al.*,2010).

Consideration should be given to all factors that impact on the relevance of the test method and test results to a specific diagnostic interpretation or application. the serological methods described represent standardized and validated methods with suitable performance characteristics to be designated as either prescribed or alternative tests for international trade. This does not preclude the use of modified or similar test methods or the use serological tests detect antibodies produced against lipopolysaccharides (LPS) of both smooth and rough *Brucella* spp. The smooth species; *B. abortus*, *B. melitensis* and *B. suis* which contain the O-polysaccharide (OPS) as part of the lipopolysaccharides (LPS) are diagnosed serologically using either a whole cell antigen or smooth- lipopolysaccharide (S-LPS) prepared by chemical extraction, while the rough species; *B. canis* and *B. ovis;* which contain no

detectable OPS, are mainly diagnosed using rough-lipopolysaccharides (R-LPS) or protein antigens.

Various serological tests are used to detect specific antibody in serum and milk following infection. These tests remain the most practical diagnosis of *brucellosis* (WHO, 2006; Lyimo, 2013). These include: Serum Agglutination Test (SAT), Complement Fixation test (CFT), Rose Bengal Plate test (RBPT), Buffered Acidified Plate test (BAPA), Enzyme Linked Immunosorbent Assay (ELISA) and Milk Ring Test (MRT) which is used for testing animals only (Radostitis *et al*., 2007).

2.15.1.1.Rose Bengal Plate Test (RBPT):

The RBPT is a simple, rapid and spot test and can be performed in field.

The low pH prevents some agglutination by IgM and encourages agglutination by IgG1 thereby reducing non-specific interactions this test is internationally acknowledged as the choice for the screening of brucellosis. However, due to cross reactivity between these antigen with other bacterial species including Yersinia enterocolitica O:9 and E. coli serotype O:57 RBPT may suffer higher rates of false positive results than other serological tests. Still many workers reported RBPT to be a routine and reliable test and could not be replaced for the diagnosis of brucellosis .(K MANISH *et.al*., 2013).

Serum samples may be screened using the Rose Bengal plate agglutination test .serum is mixed with an equal volume of antigen, and then observed for agglutination. Any visible reaction is considered to be positive. The test is very sensitive and 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 positive results occur in vaccinated animals. False negative results are common in sheep, goats and pigs .

2.15.1.2. Buffered acidified plate antigen test (BAPA) :

This test is used for screening livestock, wildlife and human population. It is recognized by OIE as a screening test for cattle, bison and swine to detect immunoglobulins IgG_1 and IgG_2. The specificity and sensitivity are 65-99% and 70-99% respectively (Hennager, 2013). It is a simple spot agglutination where 80 µl serum and 30 µl of antigen are dispensed onto a clear glass plate and mixed with a stirrer, 10 – 12 minutes incubation time is needed while rocking. Any resulting visible agglutination signifies a positive reaction (Hennager, 2013).

2.15.1.3. Serum Agglutination test (SAT)

Historically, the SAT has been recognized as the principal serological test used for the diagnosis of brucellosis. IgM isotypes of antibody is the most active agglutinin at neutral pH (Nielsen *et al.*, 1984). Therefore, SAT is susceptible to false positive reaction by cross-reacting antibodies (Nielsen 2002). because the SAT may yield both false negative or false positive results. The efficacy of test is useful only when it is used at herd level. also recognized that not every *Brucella* infected animals show a diagnostically significant titre. The presumptive diagnosis provided by the serological tests is usually accepted as indication of brucellosis; although it can only detect IgM and IgG2 and fails to detect IgG1. However, chronic carriers produce mainly IgG1 that block the agglutinating activities of IgG2 which may result in lower detection rates.(Hafez et al. 2011) reported lower number of positive samples detected by SAT in comparison to RBPT and ELISA.

The SAT has been the most widely used serological test for the diagnosis of brucellosis in animal and man. It has been used for the control and eradication of

brucellosis in most countries of the world such as Britain, United States of America, Canada and Germany (Dahoo *et al.*, 1986).

It can be performed in tubes. According to reports of FAO/WHO Expert Committee on Brucellosis (1986), It has an international standardization. (Falade, 1978), compared RBPT, SAT and MRT for the diagnosis of Brucellosis in caprine and concluded that SAT offered a better serological result, (Morgan *et al.*,1969) mentioned that a proportion of sheep, goats and humans bacteriologically and serologically positive for Brucellosis failed to react to the SAT. This proved the inferiority of SAT compared to the other conventional tests .

2.15.1.4. Competitive Enzyme Linked Immunosorbent Assay (cELISA) :

ELISA offers 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. Owing to these properties ELISA is extensively used in the diagnosis of brucellosis in every species of animals. The smooth lipopolysaccharide (sLPS) is commonly used as the antigen for ELISA (OIE 2004). The sensitivity and specificity of ELISA was found higher by using LPS as diagnostic antigen instead of OPS (Nielsen *et al*., 1996).

The competitive enzyme immunoassays were developed in order to eliminate some, but not all of the problems arising from residual vaccinal antibody, and from crossreacting antibodies, the assays are carried out by selecting a monoclonal antibody with slightly higher affinity for the antigen than most of the vaccinal/crossreacting antibody, but with lower affinity than antibody arising from infection (Munoz *et al.,* 2005; OIE, 2009; Poiester *et al.,* 2010, B.Y Kaltungo

et,al.,2014). The specificity of the competitive enzyme immunoassay is very high and is able to detect all antibody isotypes (IgM, IgG1, and IgG2 and IgA) (Nielsen, 2002, B.Y Kaltungo *et,al*.,2014).

ELISA Besides its higher sensitivity than other conventional tests, ELISA is found to detect sera as positive about 2 to 4 weeks earlier (Gameel *et al.,* 1983). It can also be used both for screening and confirmatory tests (Bekele 2004).

2.16. False Positive Serological Reactors :

False positive results are a major problem which made serological diagnosis of brucellosis difficult in some cases. false positive reactions sometimes occur. These may be the result of cross-reactions between antigens of *Brucella* spp and unrelated organisms, for example Yersinia enterocolitica or they may result from the presence of nonspecific agglutinins distinct from antibodies, which are present in certain sera

As described above, many modifications of various serological tests have been made to overcome the problem, some with limited success, some a little better(Jungersen G.*et.al*,2006).

Virtually all serological tests for antibody to smooth *Brucella* sp. use LPS, part of LPS or whole cells as the antigen. The immunodominant epitope on the surface of the smooth cell is OPS the outermost portion of LPS. OPS is a homopolymer of 4-formamide-4,6-dideoxymannose. Most of the problems but not all arise from an immune response of the animal to another microorganism which shares epitopes with *Brucella* sp. OPS. (Fernando Poester *et.al* , 2010).

2.17.Prevention ,Hygiene and control of animal brucellosis:

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:

• 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 .

• Isolation of purchased replacements for at least 30 days . In addition a serological test prior to commingling is necessary.

• Prevention of contacts and commingling with herds of flocks of unknown status or those with brucellosis .

• If possible , laboratory assistance should be utilized to diagnose causation of abortions , premature births , or other clinical signs . Suspect animals should be isolated until a diagnosis can be made .

• Herds and flocks should be included in surveillance measures such as testing of slaughtered animals with simple screening serological procedures such as the RBT.

• Proper disposal (burial or burning) of placentas and non-viable fetuses . Disinfection of contaminated areas should be performed thoroughly.

• 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 .

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.

2.17.1.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.(WHO, FAO and OIE2006).

Because of the grave medical and economic consequences of brucellosis, serious efforts have been made to prevent the infection through the use of vaccines. In OWCs, both inactivated and attenuated *Brucella* vaccines have been used successfully. Dromedaries were vaccinated with *B. abortus* strain S19.(Radwan *et al.*, 1997).

It is often recommended that vaccination with strains 19 and Rev.1 should be limited to sexually immature female animals . This is to minimize stimulation of postvaccinal antibodies which may confuse the interpretation of diagnostic tests and also to prevent possible abortions induced by the vaccines . However, field and laboratory studies have demonstrated that conjunctival administration of these vaccines makes the vaccination of the herd or flock a practical and effective procedure . Rapid herd immunity is developed and application costs are minimized. The lowered dose results in lower antibody titres and these recede rapidly. Several diagnostic tests have been developed which are useful in differentiating antibody classes . Of these, the complement fixation test and ELISA are currently the most widely used .

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.

The worldwide trend towards more animal commerce and larger populations, along with limited resources, have made the control of brucellosis very difficult in many countries . Evaluation of the procedures used for the prevention and control of animal brucellosis should be performed. This should include surveillance of animals and humans and investigations of outbreaks . Procedures, including case definition and diagnostic tests, should be standardized and should be flexible enough to allow modification when new information becomes available.(WHO, FAO and OIE2006)

The live attenuated *Brucella melitensis* Rev.1 is considered the best available vaccine for use in sheep and goats. (Vemulapalli *et al*., 2004; FAO 2010).

Effective vaccines have played an important role in reducing the incidence of brucellosis in many countries.

2.17.1.1. Brucella abortus strain 19

The most widely used vaccine for the prevention of brucellosis in cattle is prepared from *B. abortus* strain 19. It is an attenuated (live) vaccine.

2.17.1.2. B. melitensis Rev- 1

The live *Brucella melitensis* Rev- 1 strain is considered the best vaccine available for the prophylaxis of brucellosis in small ruminants . The vaccination of pregnant animals with full standard doses of Rev- 1 administered subcutaneously is followed by abortion in most vaccinated animals

2.17.1.3. Brucella abortus rough strain RB51

“R” standing for “rough” and “B” for *Brucella;* 51 does not stand for number of passages which were necessary to select strain RB51; it refers to an internal laboratory nomenclature used at the time it was derived. Strain RB51 turned out to be essentially devoid of the O- chain, its roughness being very stable after multiple passages in vitro and in vivo through various species of animals (Bricker and Halling, 1995).

Vaccination is often the first step in the control of Brucellosis.

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.(WHO and FAO2006)

The Ministry of Animal Resources, Fisheries and Range (MARFR) and through its authorized department and referenced institutions for example Animal Research Corporation, Animal Central Research lab (ACR) is doing utmost efforts to control and alleviate those diseases for the safety of the Sudanese people and for the sake of animal wealth export.

CHAPTER THREE

Materials and Methods

3.1 Sterilization

Test tubes, bottles, flasks and pipettes were sterilized in the hot air oven at

160°C for one hour.

3.2 Sources and type of samples

Blood samples were collected from camels and goats intended to exportation from different areas of Sudan and different age groups (range of age groups from2 to 5 years).

A total of 5700 blood samples were collected, 2700 from camels, and 3000 from goats.

3.3 **Collection of blood samples**

Blood samples were collected for sera from camels and goats by vena -puncture of the jugular vein using evacuated tubes with needle holder or by using disposable plastic syringe after clipping the hair and disinfecting the skin with alcohol. 5ml blood was collected from each animal. Tubes containing blood samples were placed in racks inside a small ice box on the top of ice and after clotting they were transported to the laboratory, and the sera were separated by centrifugation and placed in small sterile Bijou bottle. The sera were tested in the same day of collection for *brucella* antibodies using RBPT.

3.4. Serological tests

Four serum tests were conducted on the samples .

3.4.1 Rose Bengal Plate Test (RBPT):

This test is a simple spot agglutination test using antigen stained with Rose Bengal dye and buffered to a low PH, usually 3.65±0.05, this antigen was obtained from Central Veterinary Research Laboratories (CVRL), soba.

The test was performed according to the OIE manual, (2012).

3.4.1.1. Test procedure

Thirty microlitre (30µ) of antigen was placed on a white ceramic tile and the same volume of 30 µ (microlitre) test serum was placed beside the antigen. The two were mixed thoroughly using sterile applicator stick and rocked gently for 4 minutes after which they were observed for agglutination.

3.4.2 Buffer Acidified Plate Antigen (BAPA):

This test is prescribed by OIE for international trade. Tt is a quick easy presumptive test with in order to exclude negative samples from further serological testing.

3.4.2.1 Test procedure: Three drops of the serum were put, each one in a separate square of the plate. The first drop of the serum = 20 microlitre, the second drop of the serum = 40 microlitre, the third drop of the serum = 80 microlitre. Thirty microlitre of the antigen were added to each drop. They were mixed gently, rotated the plate and interpreted for agglutination within 8 minutes.

3.4.3. Serum Agglutination Test (SAT):

This was carried out using the agglutination method as described by Alton *et al.* (1988). The SAT antigen was obtained from Central Veterinary Research Laboratories (CVRL), soba.

3.4.3.1 Test procedure: Seven test tubes were required per sample was used. For the 1st tube, 0.8ml of phenol

saline was dispensed while 0.5ml was applied to the 2nd, 3rd 4th , 5th , 6th and 7th tubes using microtitre pipette fitted with corresponding tips. Similarly, 0.2ml of the test serum was added to the 1st tube and mixed properly. Serial dilution was then carried out by pipetting 0.5ml of mixture in the 1st tube to 2nd, then to the 3rd, then to the 4th until to the 7th tubes. The final 0.5 ml from the 7th tube was discarded. This process of doubling dilutions results in 0.5ml of dilutions 1:5 , 1:10 ,1:20 ,1:40 , 1:80 , 1:160, 1:320 .

0.5ml of antigen (diluted 1:12 with phenol saline) was added to all the tubes. The tubes were thoroughly mixed , thus giving final serum dilutions of 1:10 , 1:20 ,1:40, 1:80 , 1:160 , 1:320 , 1:640 , covered and incubated at 37^0C for 20 hours.

3.4.4 Competitive Enzyme Linked Immunosorbent Assay (cELISA):

The competitive enzyme linked immunosorbant assay (cELISA) was done and results were interpreted according to the instructions of the manufacture (SVANOVIR® *Brucella*-Ab c-ELISA, Svanova Biotech AG Uppsala, Sweden).

Samples, reagents and plate(s) were brought to room temperature prior to starting the test. 500 ml wash solution per plate was prepared by adding 25 ml PBST (Phosphate Buffered Saline Tween-20) to 475 ml distilled water. Serum samples were diluted 1:100 with sample dilution buffer.

Positive and negative controls were diluted 1:200 in dilution buffer. 50 µl of the prediluted controls and samples were added into each of the appropriate wells, the controls were run in duplicate. 50 µl of the sample dilution buffer was pipetted into two appropriate wells as conjugate control. 50 µl of mAb solution was added into every well used for controls and samples. The plate was sealed and all the reagents were mixed for 5 min. The plate was incubated at room temperature for 30 min. After that the plate was rinsed 4 times with PBS Tween buffer. 100 µl of the conjugate solution were added into each well. Then the plate was sealed and incubated at room temperature for 30 min. The plate was rinsed 4 times again with PBS Tween buffer. 100 µl of the substrate solution were added to each well and incubated for 10 min at room temperature. The time started after the first well was filled. The reaction was stopped by adding 50 µl from the stop solution to each well. The optical densities of the controls and samples were measured at 450 nm in a microplate photometer.

3.4.4.1 Test procedure:

The freeze-dried sLPS is reconstituted to 1 ml with distilled water and is further diluted 1/1000 (or to a dilution predetermined by titration against the OIE ELISA Standard Sera) in 0.05 M carbonate buffer, pH 9.6. To coat the microplates, 100 µl volumes of the diluted sLPS solution are added to all wells, and the plates are covered and incubated for 18 hours at 4°C. After incubation, the plates may be used or sealed, frozen and stored at –20°C for up to a year. Frozen plates are thawed for 30–45 minutes at 37°C before use.

Unbound antigen is removed by washing all micro plate wells with PBST four times. Volumes (100 µl) of serum diluted in the range of 1/50 to 1/200 in PBST, pH 6.3, containing 7.5 mm each of EDTA and ethylene glycol tetra-acetic acid (EGTA) (PBST/EDTA) are added to specified wells and incubated at ambient temperature for 30 minutes.

Test sera are added to the plates and may be tested singly or in duplicate. The controls, calibrated against the OIE ELISA Standard Sera, are set up in duplicate wells and include a strong positive, a weak positive, a negative control serum, and a buffer control.

Unbound serum is removed by washing four times with PBST (PBST containing EDTA/EGTA must not be used with HRPO as it inactivates the enzyme). Volumes (100 µl) of conjugate (MAb M23) specific for a heavy chain epitope of bovine IgG1 conjugated with HRPO and diluted in PBST (predetermined by titration) are added to each well and the plates are incubated at ambient temperature for 30 minutes.

Unbound conjugate is removed by four washing steps. Volumes (100 µl) of substrate/chromogen (1.0 mM H2O2 [100 µl/20 ml citrate buffer] and 4 mM ABTS [500 µl/20 ml citrate buffer]) are added to each well, the plate is shaken for 10 minutes and colour development is assessed in a spectrophotometer at 414 or 405 nm. If required, 100 µl volumes of 4% SDS may be added directly to all wells as a stopping reagent.

The control wells containing the strong positive serum are considered to be 100% positive and all data are calculated from these absorbance readings (between 1.000 and 1.800) using the equation:

Per cent positivity (%P) = absorbance (test sample)/absorbance (strong positive control) × 100

CHAPTER FOUR

4 -The Results

4.1.1 Rose Bengal plate test (RBPT) results:

The formation of distinct pink granules (agglutination) was recorded as positive while the absence of granule formation was recorded as negative. Sera positive to RBPT were classified into four categories:

+1 or weak positive: when very weak fine agglutination occurred, this could be hardly seen by unaided eyes.

+2 or positives: when the agglutination was fairly visible.

+3 or positives with rim formation: when the agglutination appeared prominently in the periphery forming a rim.

+4 or strong positives: where there was a granular agglutination occurring very rapid and large clumps occurred, leaving only clear fluid.

2700 of Camel's serum samples (males and females) were tested with (RBPT) and 3000 Goat's serum samples also were tested and the results were illustrated in Table no 8 below:

Table No. 12: RBPT results

Camel males 2000		**Camel females 700**		**Goats males 3000**	
420 Positive	1580 Negative	182 Positive	518 Negative	420 Positive	2580 Negative

From the results of RBPT in tab12 we calculated the percentage of Camels male.

Table 12.1: RBPT percentage of Camels male

		Frequency	Percent
Valid	+ve	420	21.0
	-ve	1580	79.0
	Total	2000	100.0

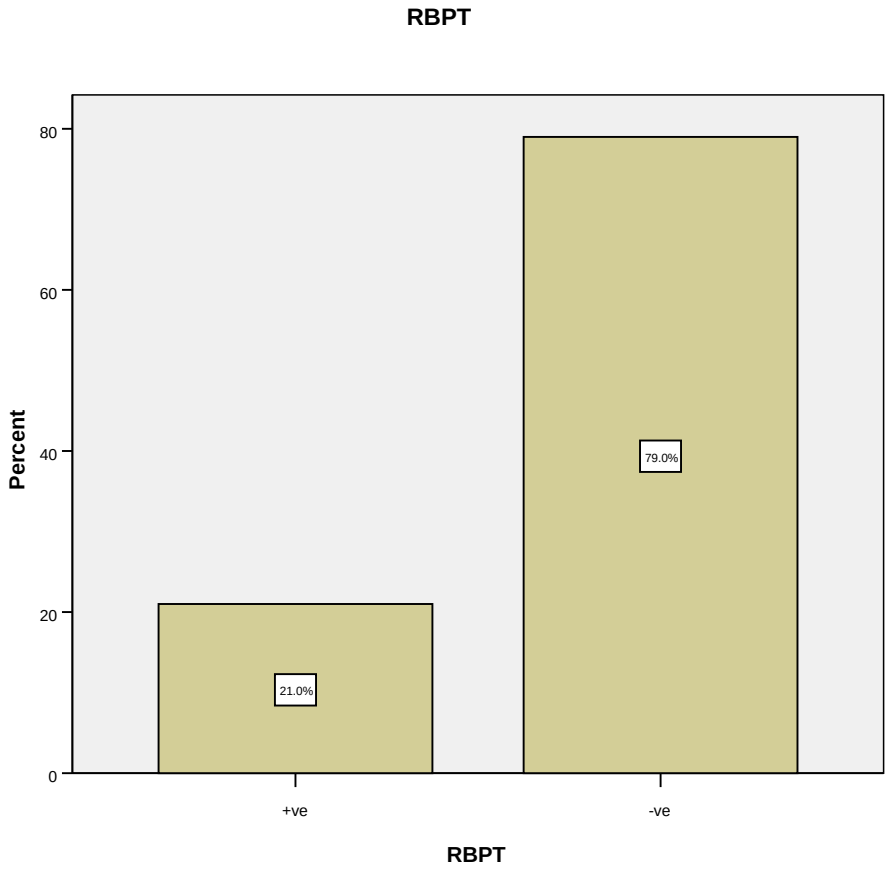

Figure 1: percentage of positive RBPT for camels male.

From the results of RBPT in tab12 we calculated the percentage of Camels female.

Table 12.2 :RBPT percentage of Camels female

		Frequency	Percent
Valid	+ve	182	26.0
	-ve	518	74.0
	Total	700	100.0

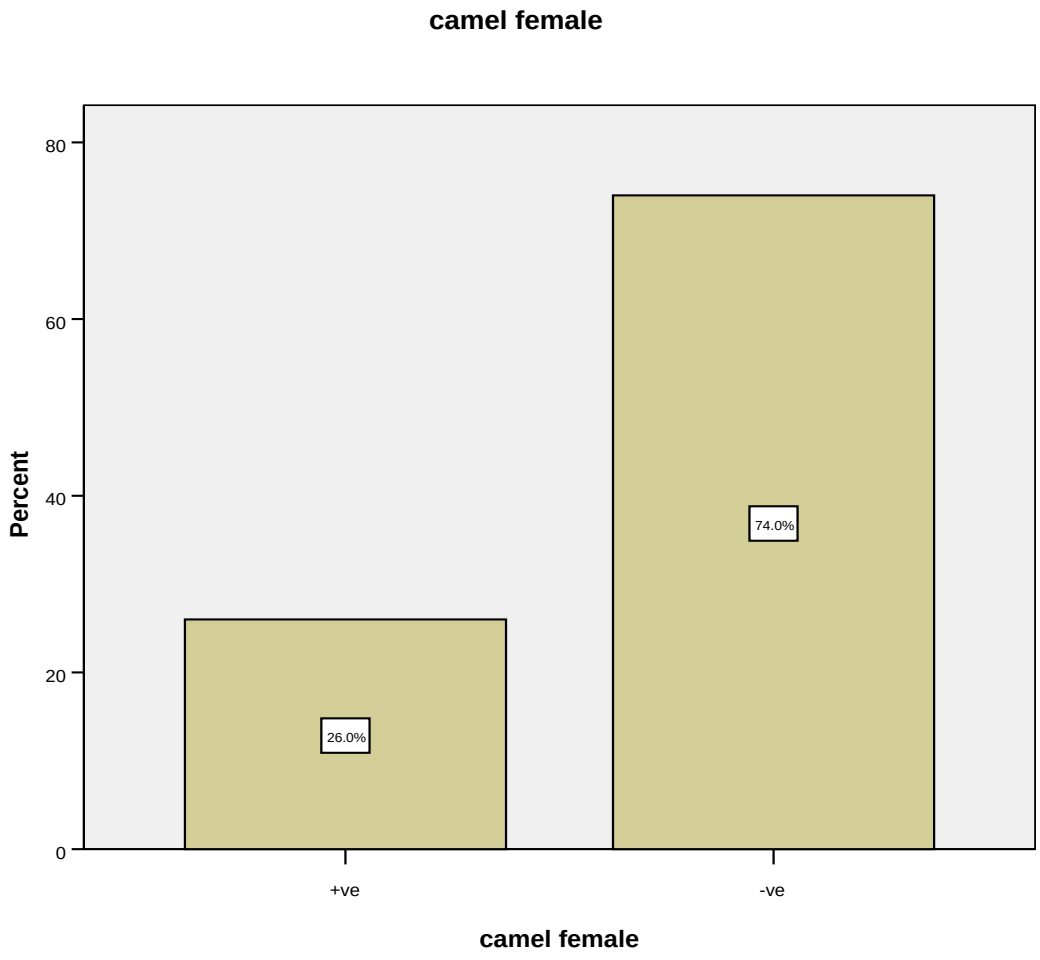

Figure 2: percentage of positive RBPT for camels female

From the results of RBPT in tab12 we calculated the percentage of Goats.

Table 12.3: RBPT percentage for Goats

		Frequency	Percent
Valid	+ve	420	14.0
	-ve	2580	86.0
	Total	3000	100.0

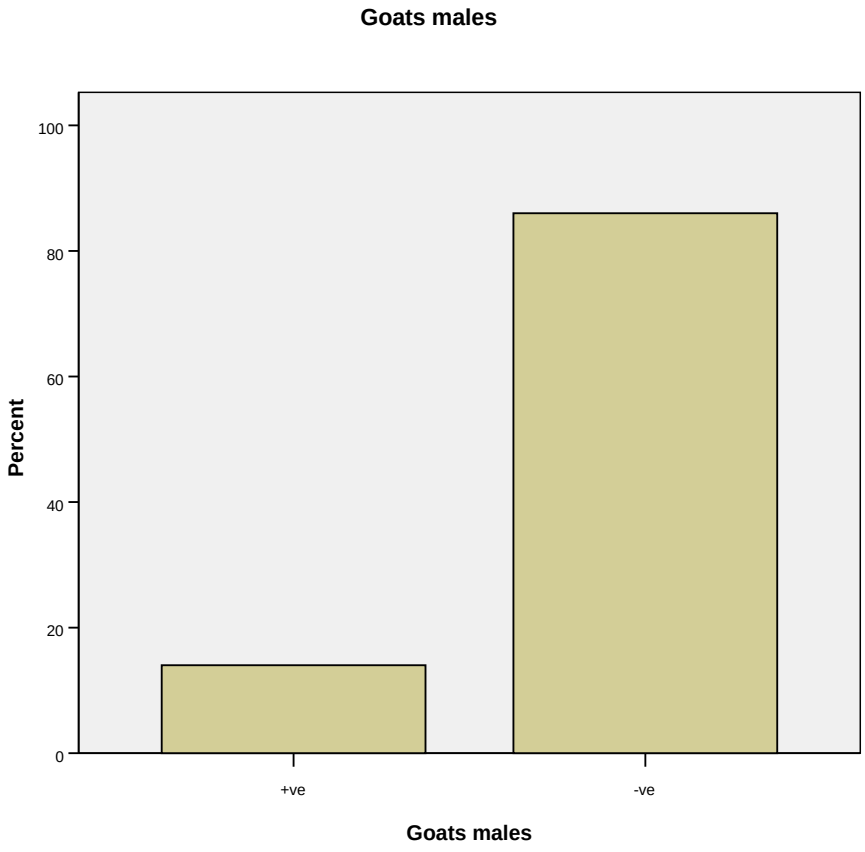

Figure3: percentage of positive RBPT for Goats

4.1.2 Buffer Acidified Plate Antigen (BAPA) results:

In positive case, the agglutination occurs taking ring shape surrounding the sample inside the plate (OIE Terrestrial Manual, 2012).

We were tested the positive (RBPT) to (BAPA) and the results were illustrated in Table 13.

Table no13:BAPA test

Camel males 420		**Camel females 182**		**Goats 420**	
260 Positive	160 Negative	102 Positive	80 Negative	106 Positive	318 Negative

From the results of BAPA test in table No 13 we calculated the percentage of Camelsmale.

table13.1:BAPA percentage for Camel males

		Frequency	Percent
Valid	+ve	260	61.9
	-ve	160	38.1
	Total	420	100.0

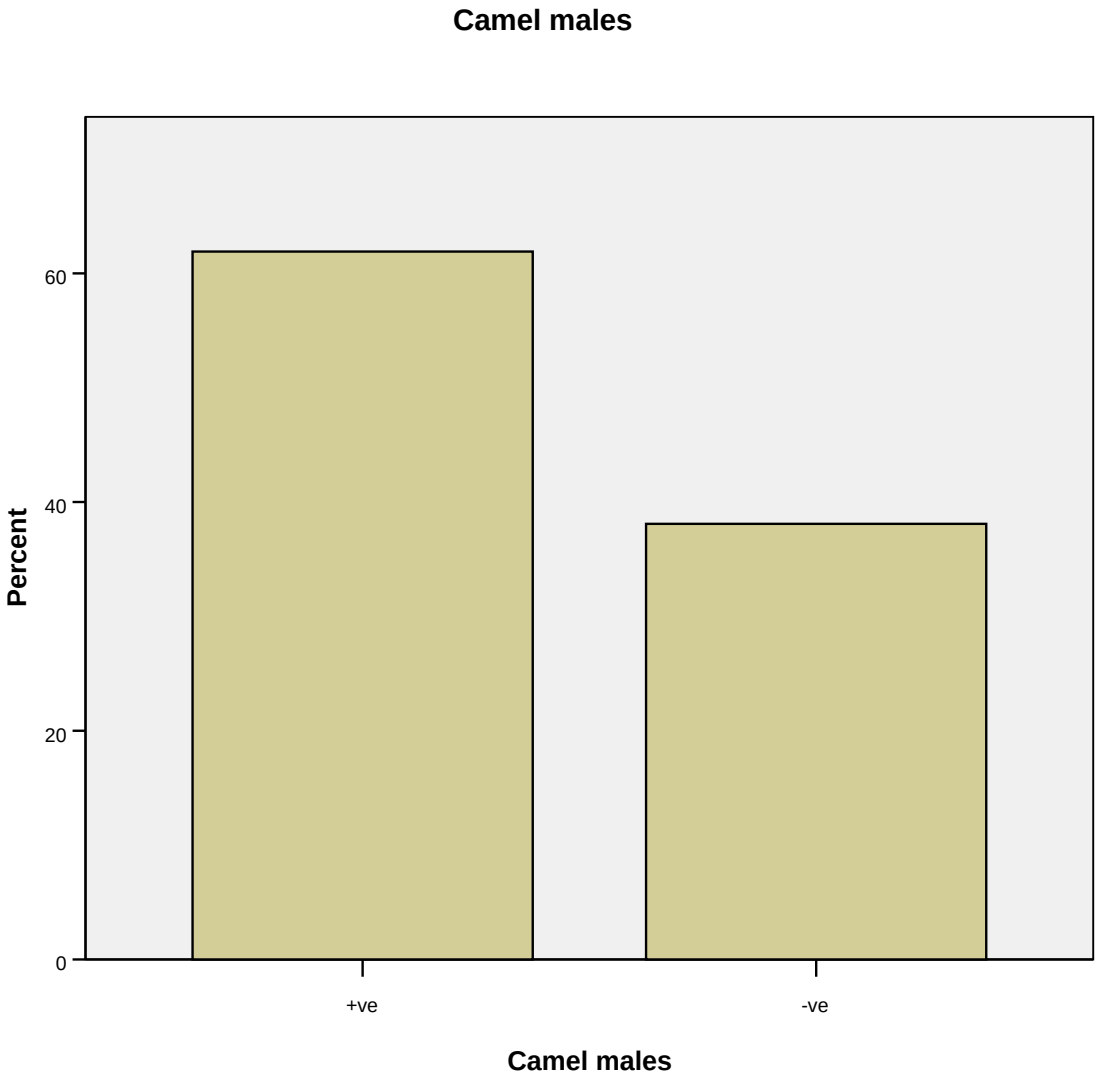

Figure4: percentage of BAPA Camels male

From the results of BAPA test in tab13 we calculated the percentage of Camels female

table13.2: BAPA percentage for Camel females

		Frequency	Percent
Valid	+ve	102	56.0
	-ve	80	44.0
	Total	182	100.0

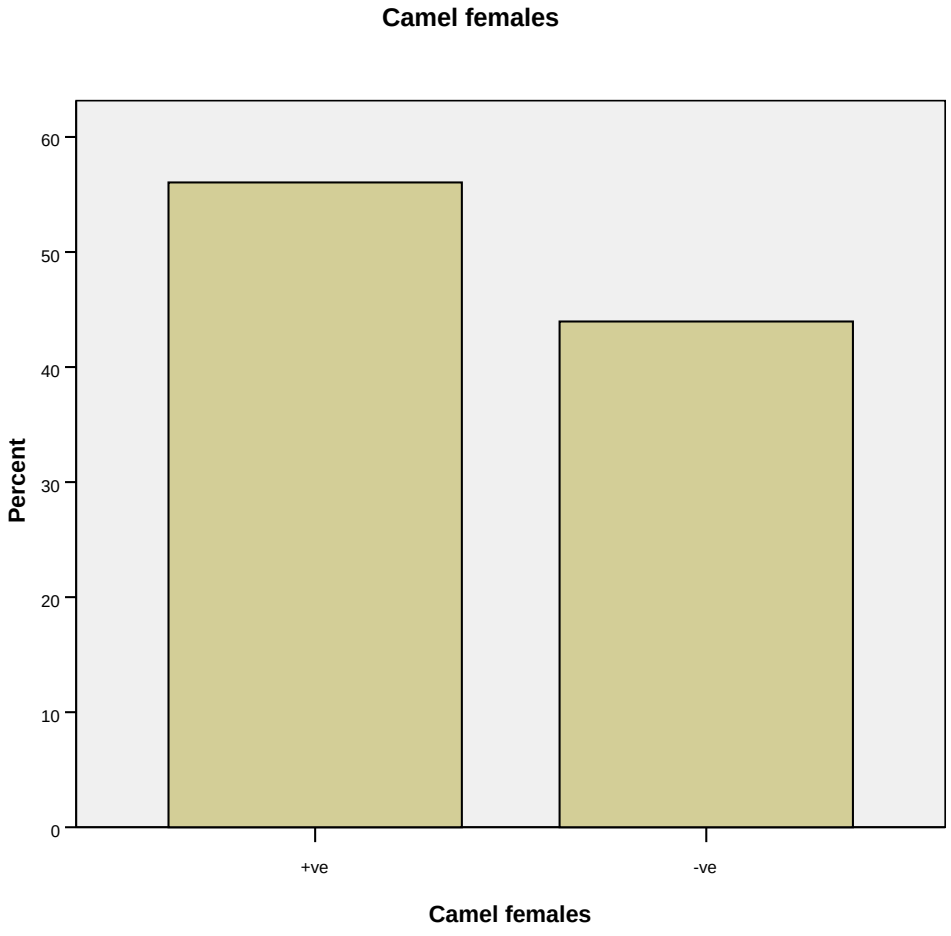

Figure 5: percentage of positive BAPA Camels Female

From the results of BAPA test in tab13 we calculated the percentage of Goats

table13.3:BAPA percentage for Goats

		Frequency	Percent
Valid	+ve	106	25.2
	-ve	314	74.8
	Total	420	100.0

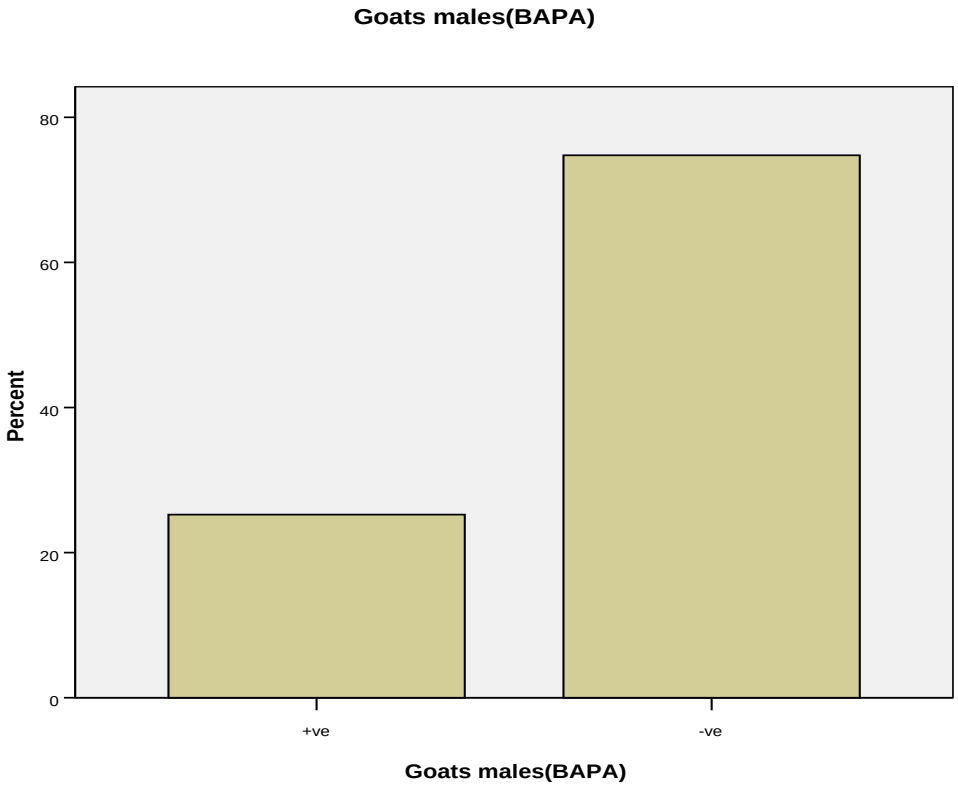

Figure 6: percentage of positive BAPA Goats

4.1.3 Serum Agglutination Test (SAT) results:

The test was read by examining the tubes against a black background with light coming from behind the tubes. A positive reaction is one in which the serum – antigen mixture was clear and agglutinated antigen appeared at the bottom of the tube. Gentle shaking does not disrupt the floculi. This was considered a complete agglutination and was recorded as ++++. In partial agglutination serum-antigen mixture was partially clear and gentle shaking does not disrupt the floculi, this was recorded as +++ or ++. Some sedimentation as + and no clearing as negative reaction (Alton, 1975).

We were tested the positive (RBPT) to (SAT) test and the results were illustrated in Table No 14.

Table No14:SAT

Camel males 420		**Camel females 182**		**Goats 420**	
254 Positive	166 Negative	102 Positive	80 Negative	109 Positive	311 Negative

From the results of SAT in table14 we calculated the percentage of Camel males

table 14.1:SAT percentage for Camel males

		Frequency	Percent
Valid	+ve	254	60.5
	-ve	166	39.5
	Total	420	100.0

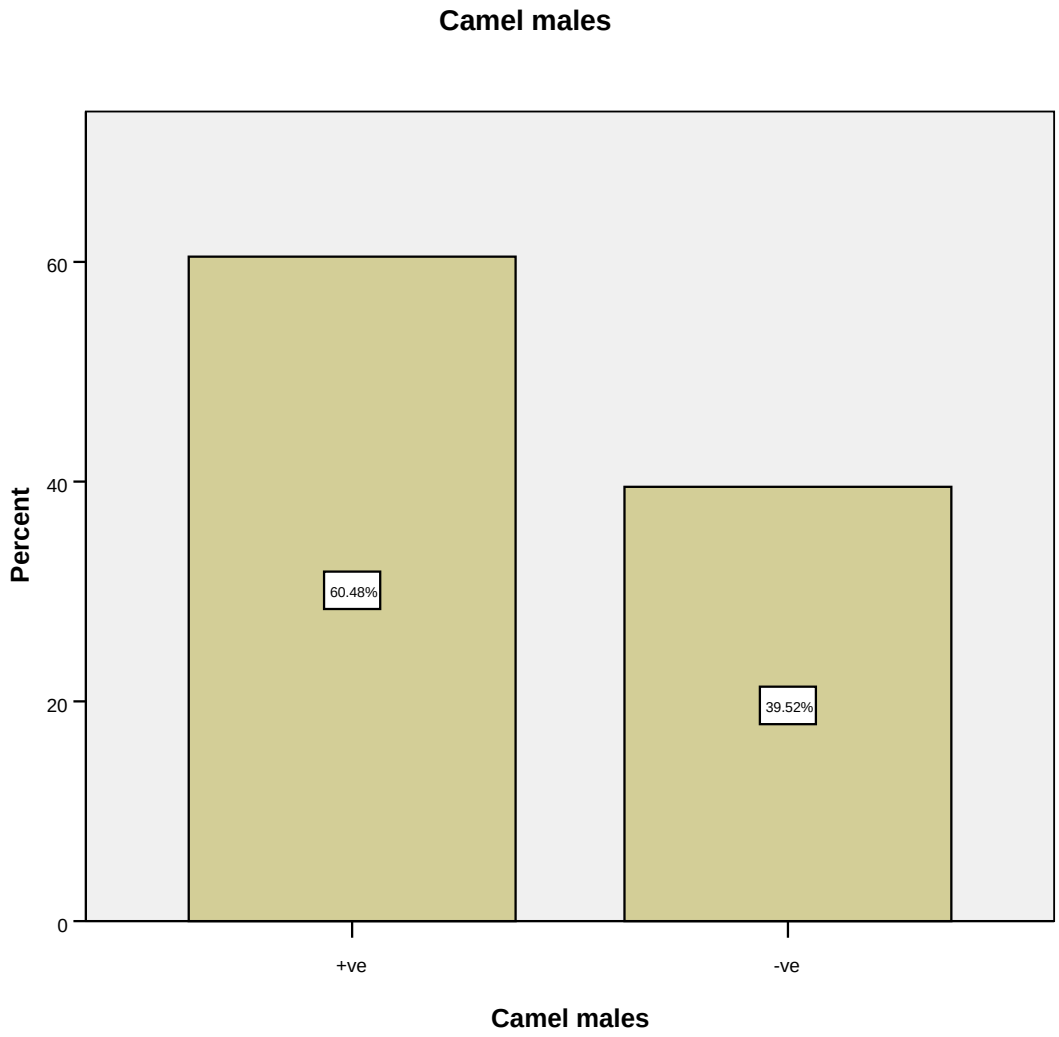

Figure7: percentage of positive Camel males SAT

From the results of SAT in table14 we calculated the percentage of Camel females

table 14.2: SAT percentage for Camel Females

		Frequency	Percent
Valid	+ve	102	56.0
	-ve	80	44.0
	Total	182	100.0

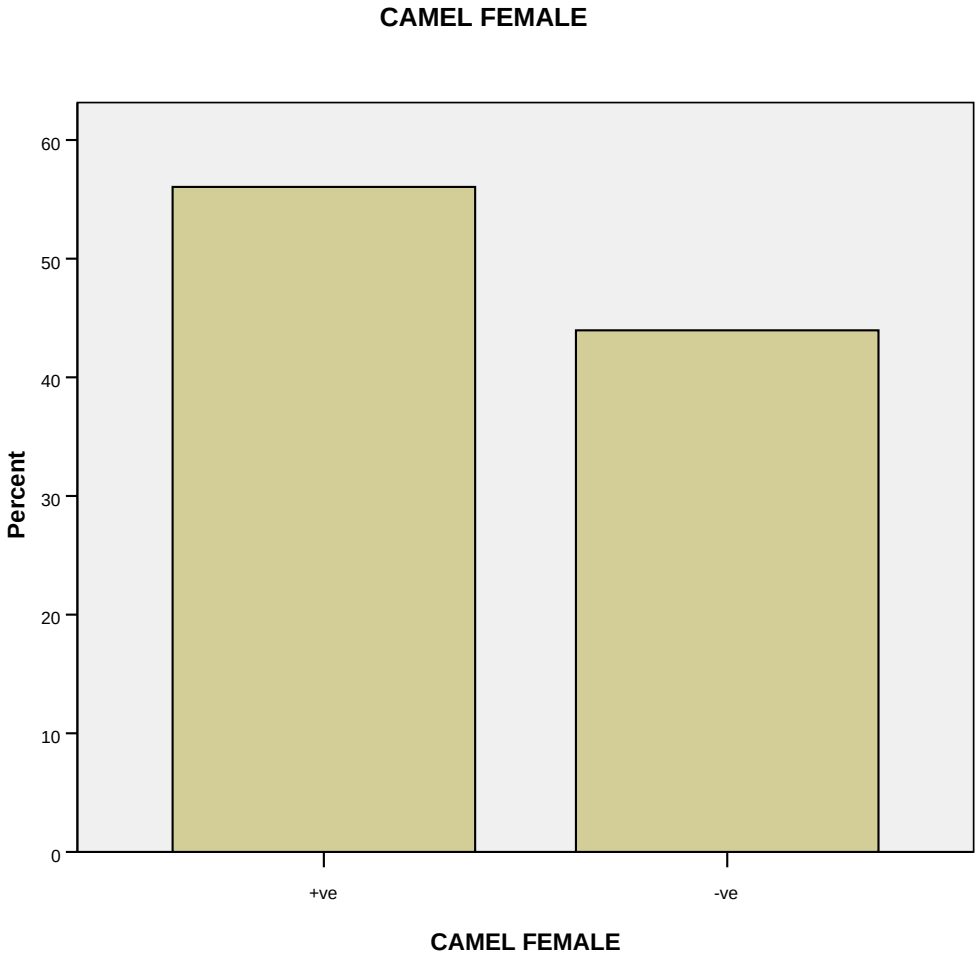

Figure 8: percentage of positive SAT Camels Female

From the results of SAT in table14 we calculated the percentage of Goats

table14.3: SAT percentage for Goats

		Frequency	Percent
Valid	+ve	109	26.0
	-ve	311	74.0
	Total	420	100.0

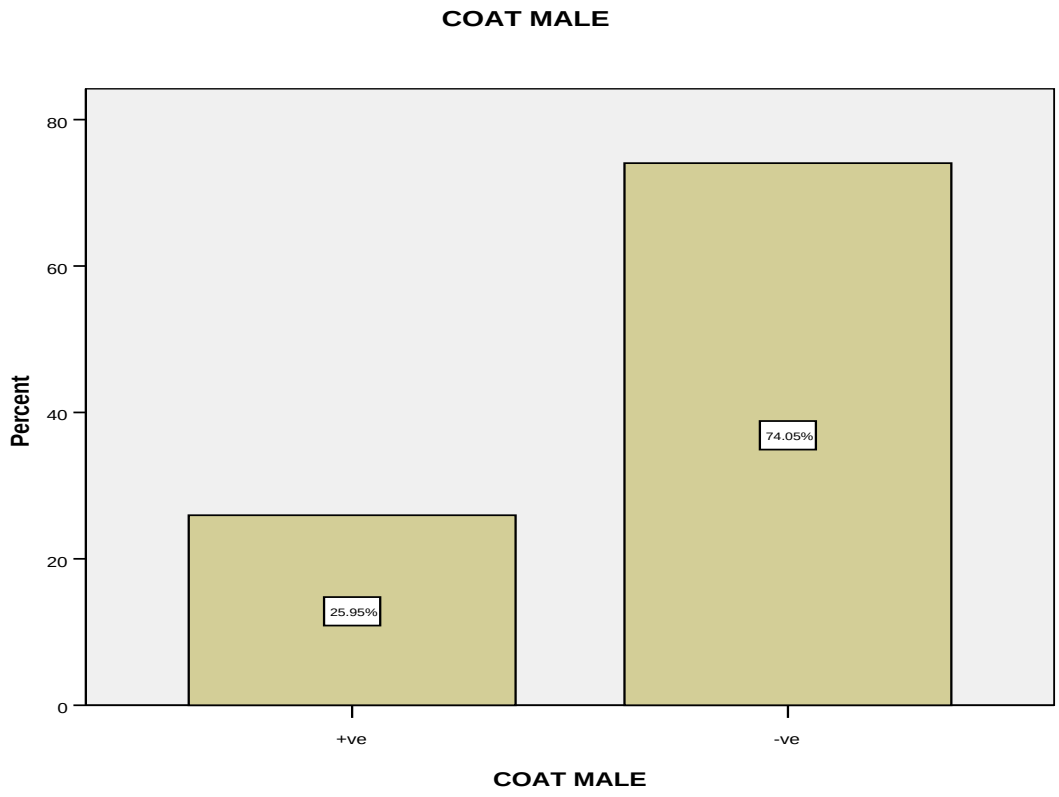

Figure9 : percentage of positive SAT Goats

4.1.4. cELISA results:

This test was calibrated against the OIE ELISA Standard Sera, the diagnostic sensitivity should be equal to or greater than that of the BBATs (RBT/BAPA) in the testing of infected animals . However, like all other serological tests, it could give a positive result because of S19 vaccination.

We were tested 528 of strong positive samples of (BAPA) test with (cELISA) and the results were illustrated in Table No 15.

Table No 15: cELISA results

Camel males 260		**Camel females 122**		**Goats 146**	
147 Positive	113 Negative	52 Positive	60 Negative	45 Positive	101 Negative

From the results of cELISA in table15 we calculated the percentage of Camel males

table 15.1: cELISA percentage for Camel males

		Frequency	Percent
Valid	+ve	147	56.5
	-ve	113	43.5
	Total	260	100.0

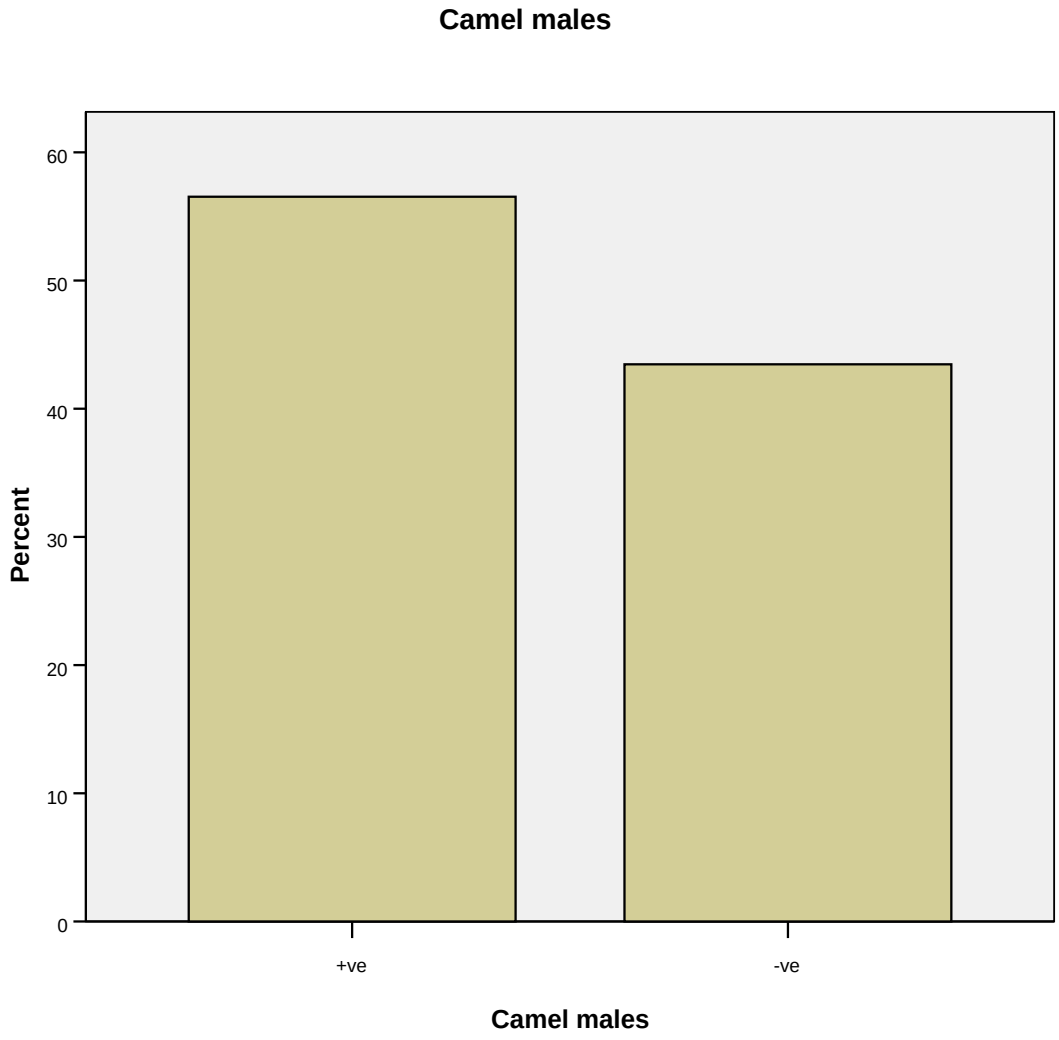

Figure 10: percentage of positive cELISA Camels male

From the results of cELISA in table15 we calculated the percentage of Camel fmales

table 15.2: cELISA percentage for Camel females

		Frequency	Percent
Valid	+ve	52	42.6
	-ve	70	57.4
	Total	122	100.0

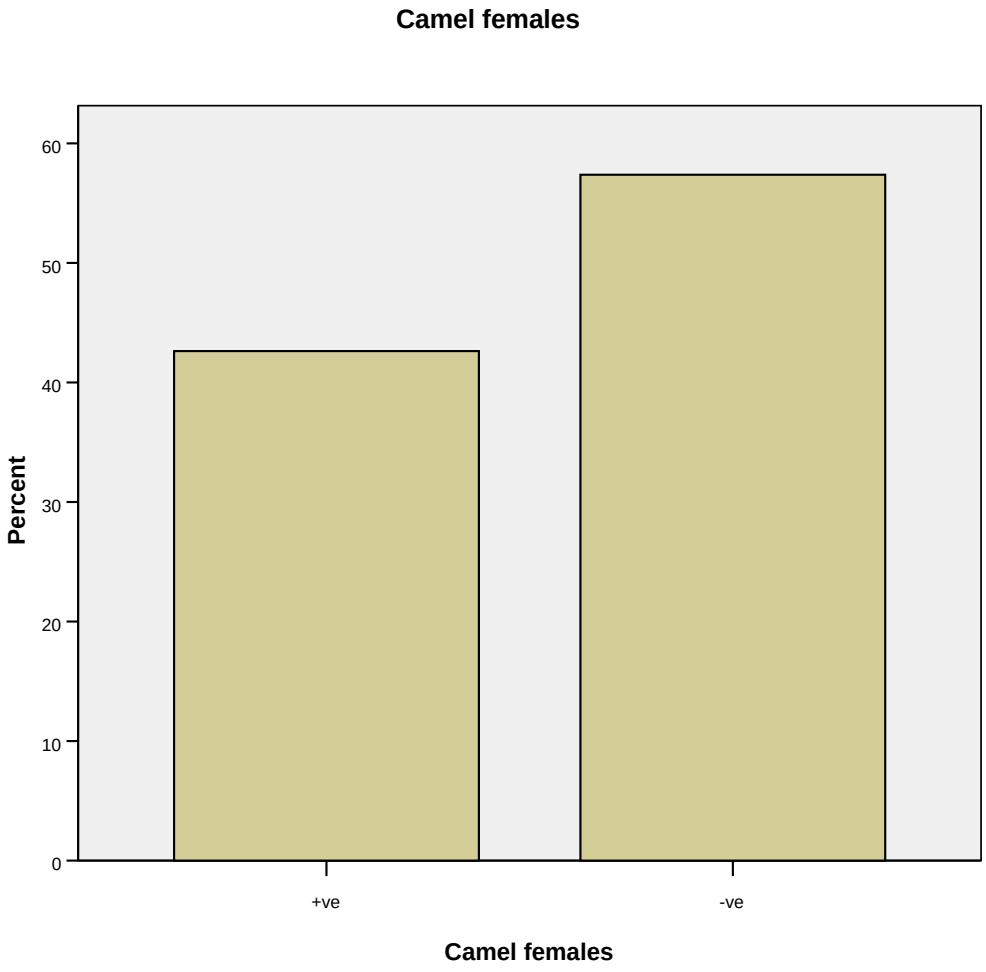

Figure 11: percentage of positive cELISA Camels female

From the results of cELISA in table15 we calculated the percentage of Goats

table 15.3: cELISA percentage for Goats

		Frequency	Percent
Valid	+ve	45	30.8
	-ve	101	69.2
	Total	146	100.0

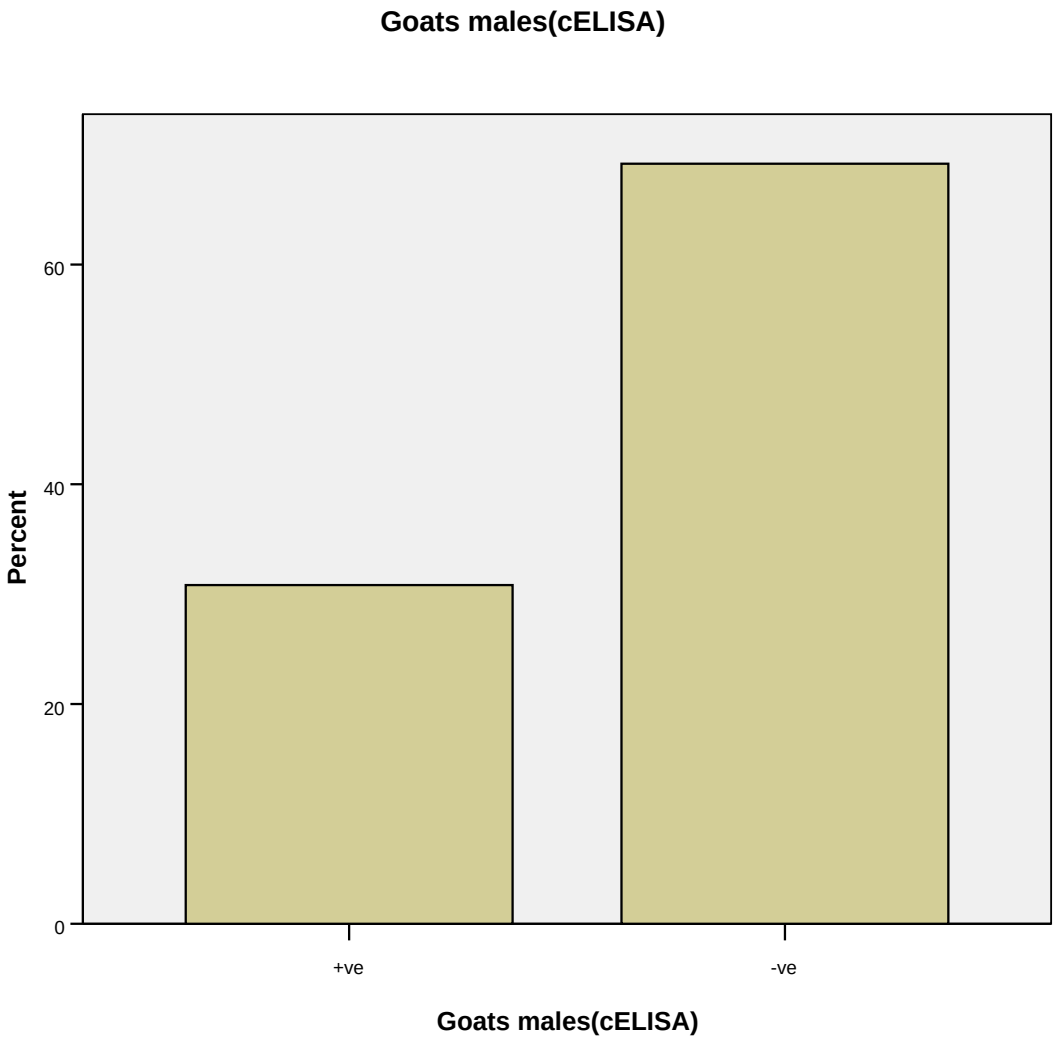

Figure12: percentage of positive cELISA Goat

We can group the results in one table to compare between them

Table No 16: the comparative results of the four serological tests

	RBPT		**SAT**		**BAPA**		**cELISA**	
	+	–	+	–	+	–	+	–
Camel males 2000	420	1580	254	166	260	160	147	113
	2000 out of 2000		420 out of 2000		420 out of 2000		260 out of 2000	
Camel females 700	182	518	102	80	122	60	52	60
	700 out of 700		182 out of 700		182 out of 700		112 out of 700	
Goats 3000	420	2580	109	311	146	274	45	101
	3000 out of 3000		420 out of 3000		420 out of 3000		146 out of 3000	

The four serological tests for Goats can be collected in one figure as bellow

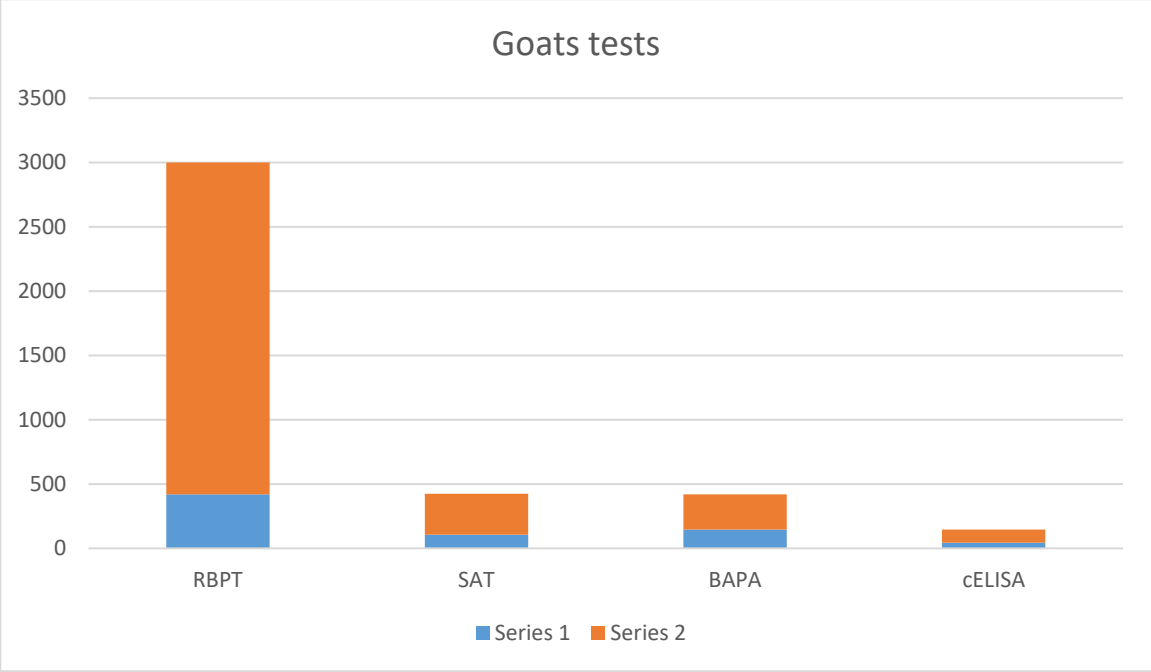

Figure 13: explain the four tests for Goats

The four serological tests for Camel males can be collected in one figure as bellow

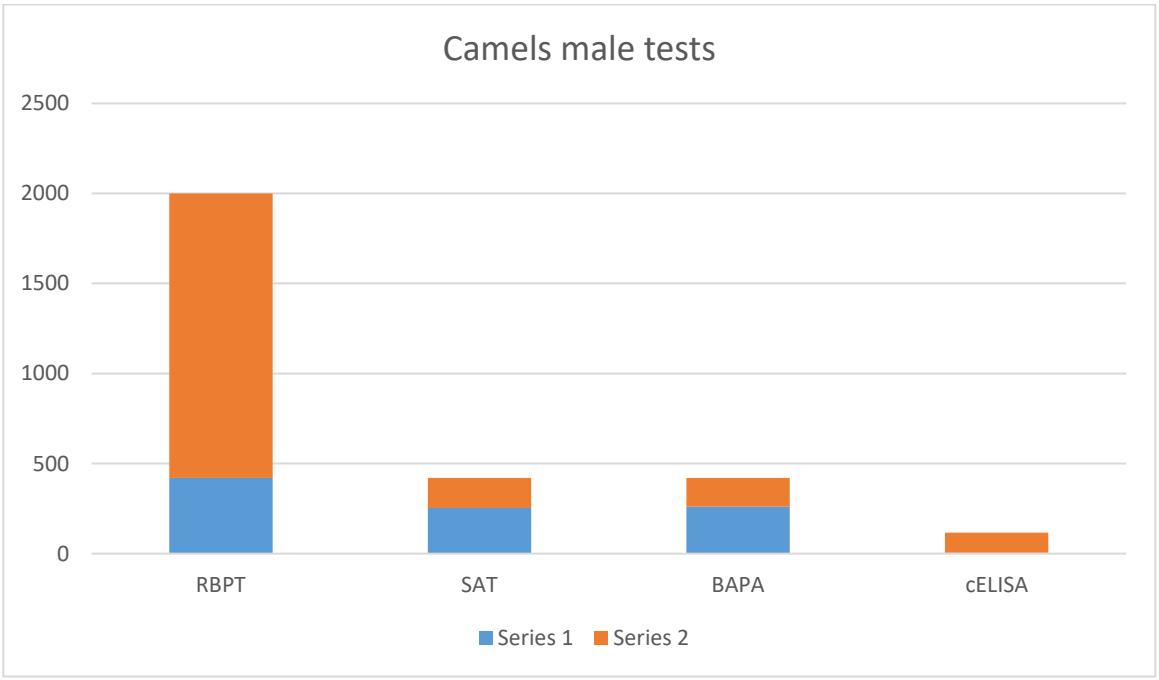

Figure 14: explain the four tests for Camels male

The four serological tests for Camel females can be collected in one figure as bellow

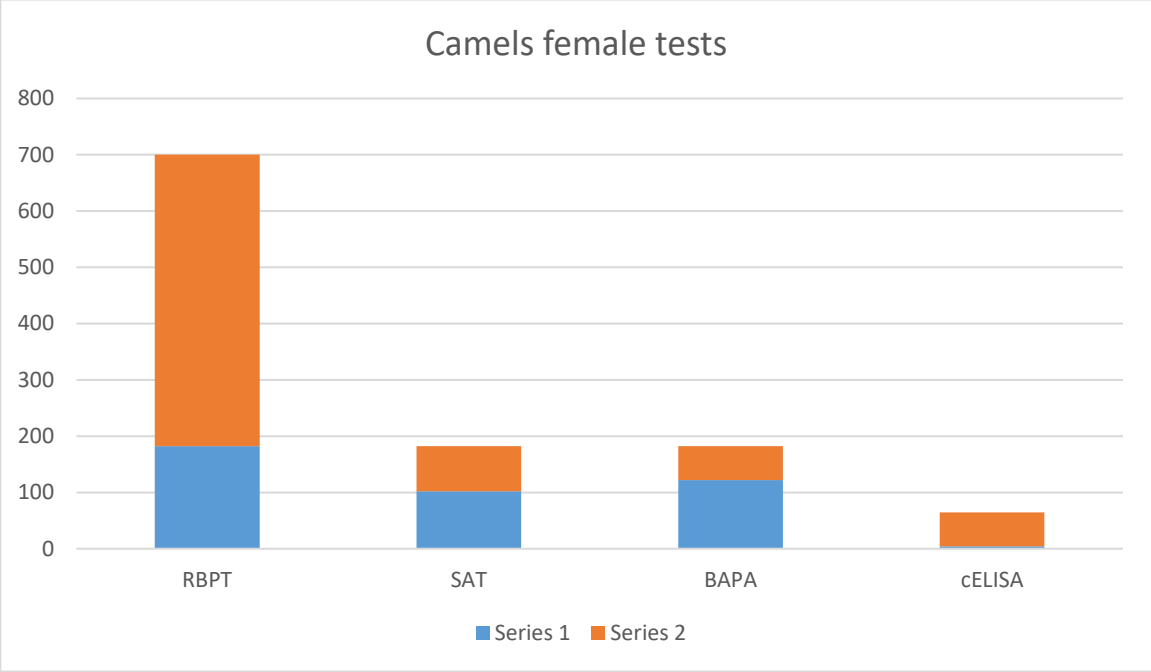

Figure 15: explain the four tests for Camels female

4.2: Statistical analysis and comparing the results :

Statistical analyses were carried out using the Statistical Package for Social Sciences SPSS for Windows® version 14.0. Was used for all appropriate statistical analyses. Descriptive statistics of the variables were obtained including frequencies, prevalence and cross-tabbing were computed. Associations in the Chi-square test and logistic regression model were deemed significant when $p \leq 0.05$.

Differences in the seroprevalence and the coexistence of the disease agents among the investigated animal species were analyzed by Chi-square test. The differences were considered statistically significant when ($p \leq 0.05$). The same test was used to analyze the association between their seropositivity.

The prevalence proportion was calculated as the number of animals testing positive by RBPT , SAT , BAPA and c-ELISA divided by the total number of animals. Correlation among factors and outcome variables were assessed using Chi-square tests. For all analyses, $p<0.05$ was considered significant

Camel males (RBPT) * Camel males(SAT) Cross tabulation Count.

		Camel males(SAT)		
		+ve	-ve	Total
Camel males (RBPT)	+ve	254	166	420
Total		254	166	420

Test Statistics

	Camel males (RBPT)	Camel males(SAT)
Chi-Square(a,b)	672.800	18.438
df	1	1
Asymp. Sig.	.000	.000

The value result is .000
The test considered significant because the p<0.05.

Camel females (RBPT) * Camels female (SAT) Cross tabulation Count

		Camel females(SAT)		
		+ve	-ve	Total
Camel females (RBPT)	+ve	102	80	182
Total		102	80	182

Test Statistics

	Camel females (RBPT)	Camel females(SAT)
Chi-Square(a,b)	161.280	2.659
df	1	1
Asymp. Sig.	.000	.003

The value result is .000 - .003
The test considered significant because the p<0.05.

Goats males (RBPT) * Goats males(SAT) Cross tabulation Count

		Goats males(SAT)		
		+ve	-ve	Total
Goats males (RBPT)	+ve	109	311	420
Total		109	311	420

Test Statistics

	Goats males (RBPT)	Goats males(SAT)
Chi-Square(a,b)	1555.200	97.152
df	1	1
Asymp. Sig.	.000	.000

The value result is .000
The test considered significant because the $p<0.05$.

Camel males (RBPT) * Camel males(BAPA) Cross tabulation Count

		Camel males(BAPA)		
		+ve	-ve	Total
Camel males (RBPT)	+ve	260	160	420
Total		260	160	420

Test Statistics

	Camel males (RBPT)	Camel males(BAPA)
Chi-Square(a,b)	672.800	23.810
df	1	1
Asymp. Sig.	.000	.000

The value result is .000

The test considered significant because the p<0.05.

Camel females (RBPT) * Camel females(BAPA) Cross tabulation Count

		Camel females(BAPA)		
		+ve	-ve	Total
Camel females (RBPT)	+ve	102	80	182
Total		102	80	182

Test Statistics

	Camel females (RBPT)	Camel females(BAPA)
Chi-Square(a,b)	161.280	2.659
df	1	1
Asymp. Sig.	.000	.003

The value result is .003
The test considered significant because the p<0.05.

Goats (RBPT) * Goats (BAPA) Cross tabulation Count

		Goats (BAPA)		
		+ve	-ve	Total
Goats (RBPT)	+ve	106	314	420
Total		106	314	420

Test Statistics

	Goats (RBPT)	Goats (BAPA)
Chi-Square(a,b)	1555.200	103.010
df	1	1
Asymp. Sig.	.000	.000

The value result is .000
The test considered significant because the $p<0.05$.

Camels male (BAPA) * Camels male (cELISA) Cross tabulation Count

		Camels male (cELISA)		
		+ve	-ve	Total
Camels male (BAPA)	+ve	147	113	260
Total		147	113	260

Test Statistics

	Camels male (BAPA)	Camels male (cELISA)
Chi-Square(a,b)	23.810	4.446
df	1	1
Asymp. Sig.	.000	.0035

The value result is .0035

The test considered significant because the $p<0.05$.

Camel females(BAPA) * Camel females(cELISA) Cross tabulation Count

		Camels female (cELISA)		
		+ve	-ve	Total
Camels female (BAPA)	+ve	52	50	102
	-ve	0	20	20
Total		52	70	122

Test Statistics

	Camels female (BAPA)	Camels female (cELISA)
Chi-Square(a,b)	2.659	2.656
df	1	1
Asymp. Sig.	.003	.003

The test considered significant because the $p<0.05$.

Goats (BAPA) * Goats (cELISA) Cross tabulation Count

		Goats (cELISA)		
		+ve	-ve	Total
Goats (BAPA)	+ve	45	61	106
	-ve	0	40	40
Total		45	101	146

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	24.547(b)	1	.000

The test considered significant because the $p<0.05$.

CHAPTER FIVE

Discussion

The existence of brucellosis in the Sudan was detected for a long time, early in the last century. Since then, lack of eradication programs resulted in a wide spread of the disease. In country where brucellosis exist, it poses a serious economic problem especially in export of animals because the main condition of exporting live animals is performing of Rose Bengal plate test (RBPT)(Frank *et al*.,2018) . Most gulf area asked for that test (Saudi Arabia, UAE and Qatar) .

The RBPT is the test recommended from the Oie for exporting animals (OIE2000). It is an approved examination as a quick field scan with acceptable accuracy for large numbers as it does not take more than four minutes and is inexpensive, and a person with medium training can perform it and it is a screening test .

False-negative reactions occur rarely, mostly due to prozoning and can sometimes be detected by diluting the serum sample or retesting after 4–6 weeks. Nevertheless RBT appears to be adequate as a screening test for detecting infected herds or to guarantee the absence of infection in brucellosis-free herds(OIE 2018).

It was found that we found that the prevalence of Rose Bengal Plate test was 14% in Goats and it is near to (Gasim 2009) who reported the prevalence rate of brucellosis was 12.4% in goats in Khartoum state but our ratio is too far from (Ream 2013) who found the prevalence rate in Khartoum was 2% and in Red See was 0.45% and in Kasala State was 2.1% data for brucellosis were available and collected from the records of Federal Ministry of Health, Khartoum Sudan .

also another result disagree with us by (Hatim 2004) in red sea he found (0.4%) from herds in the side country . another study disagree with our result (Rias 2004) who found the prevalence rate was (0.3%) of goats tested were positive from farms in different areas of nyala .

Another study near to our result by (Eman ,*etal*.,2018) in Khartoum state she found prevalence rate was (11.4%) . Our result is agree with Azza (2006) who found 16.57% by the RBPT during her work in Omdurman area and it is higher than the result reported by (Nisreen 2006) in the same year but in another place showed the prevalence rate of goats was10.5% in Khartoum state . (Khuzaima *et al.*,2018) found the prevalence was10.8% in El-Gedarif state . 6% found by (Youssif 2010) in three different places West Darfur state , El Geneina locality and Furbranga locality collected randomly .

The prevalence of brucellosis in the Northern area of Sudan was found 9% for goats by (Zein2015) from different localities were collected.

Prevalence of goat brucellosis by RBPT was higher too in another countries, in UAE Alain region was 11.5% and 7.5% and 7.3% in Abu Dhabi and the western regions respectively . Different country have the same study but with very high result (Abeer 2013) in Egypt found the prevalence among goats was (26.6%) selected from private farms and suspected to suffer from brucellosis from different localities in Gharbiya governorate. After one year (Lobna *et al.*, 2014) found that the occurrence of brucellosis in goats was (7.5%) but very high scary result in the same year but in another region in Egypt by Ashraf (2014) he found the prevalence rate was (73.33%) in Qalyoubia, El-Behera, El-Sharkia, El-Garbia and El-Fayoum selected from farms suffer from brucellosis .

In was found that the prevalence of camels male and females were21% and 26% respectively by RBPT in red sea state, another researcher agree with us (Hatim 2004) who found (19.2%) were females sera and (19.06%) were males in

Khartoum state .In another region of Sudan in Darfur by another researcher we had a very high result 30.5% by (Omer *et al.*, 2007) from Central Veterinary Research data .but after one year (Musa *et al.*, 2008) agree with us by 23.8% in Darfur . Our results agree with (Ream 2013) who found the prevalence rate in Red See was 19% and in Kasala State was 19.4% but it less in Khartoum was 6.2% data for brucellosis were available and collected from the records of Federal Ministry of Health, Khartoum Sudan. another result disagree with us in Khartoum by (Saad 2013) reported lowest rates of camel brucellosis of 5.3% and 5.8% in Darfur and Khartoum States respectively, also another research disagree with our result by (Elamir 2014) who said the prevalence rate was 5.8%in Khartoum State , camel population is distributed in the seven localities of the state. The Prevalence of brucellosis in the Northern area was more high 13.8% for camel by (Zein2015) from different localities were collected .

In Africa there were many results in Camels tested by RBPT as (Berhanu 2013) found the sero prevalence was (2.76%) in male animals and in females 2.34% from animals randomly selected camels in order to estimate seroprevalence is in agreement with the previous reports of (Omer *et al*., 2000) who found 3.1% Camels are typically kept by pastoral groups with goats and sheep ,but (Teshome *et al.*, 2003) found a little less result 2.8% from camels in three arid and semi-arid camel-rearing regions of Ethiopia and moreless by(Megersa *et al*., 2005) they found 1.8% from Ethiopia . Another African country had results near to Ethiopia, they found prevalence rate 3.1% By (Gahanem Tilahun *et al*., 2009) from Somalia camels were classified according to their age into four groups the higher seroprevalence observed was in age group > 4 years old.

In Egypt 7.3% (El-Boshy *et al.*,2009) camels aged 30– 42 months were used in this study were raised in an open yard in a government quarantine station (Nobaria City) with free access to food and water ad libitum for three weeks. The prevalence of

Brucella antibodies in camels was also observed in Alain region (5.6%) followed by lower prevalence's of (5.2%) and (4.9%) in Abu Dhabi and the western regions respectively. camels were randomly collected from 267 mixed farms (izba) located in three regions of Abu Dhabi emirate by (Momamed A. *et al.,* 2013).

In Pakistan few available studies regarding brucellosis in camel reported 2.0 % by (Aslam 2009) and a little high rate 3.07% of prevalence by(Gul *et al*., 2014) both sexes were selected randomly from different Government farms in Punjab, Pakistan. (Asim 2017) study Prevalence of brucellosis in Punjab Pakistan for camels was 3.41%. However, relatively higher sero prevalence of camel brucellosis has been recorded in Jordan 19.4% (Dawood, 2008) .

in Iraq (Alatabi *et al.,* 2020) found that the seroprevalence rate of brucellosis in examined camels was (6.97%) by RBPT camels of both sexes were selected randomly from different places in Al-Najaf province camels were involved in this study with ages between 3 to 9 years.

(12.90%) were positive for RBPT by **(**Ahmed M *et al*., 2017) estimate the sero-prevalence of brucellosis in camels in Shalateen city, in Egypt. Sera were collected from apparently healthy dromedary camels.

(15.5%) were found positive for *Brucella* antibodies by RBPT in camel serum samples by (Microorganisms 2020) from different parts of Egypt .

It was found that the prevalence of Serum Agglutination Test (SAT) for Goats was 3.5%.(Mohamed Abdelhmeed *etal.*,2012)was study the prevalence of Brucellosis in sheep and goat intended for export and local consumption in Khartoum state (0.74%) of live export was positive and (0.74%) for the local slaughter was positive also to (SAT) test.

SAT showed (20%) positive sample in cattle in Eldein area, Eastern Darfur state, Western Sudan by (African Journal2012)

(Eman Mohamed *et al.*, 2018) collected serum samples from both sexes of goats in four different localities in Khartoum state and testing for brucellosis using serum agglutination test (SAT) found 11.4% were positive.

(0.2%) positive by the serum agglutination test (SAT) by (Nahla Ahmed 2018) who worked on exported sheep from Alkadaru quarantine in Khartoum state.

the seroprevalence of camel brucellosis in Banadir region of Somalia was 10.1% by SAT (Ahmed A 2017) apparently healthy one-humped camels above two years old with no history of vaccination against brucellosis.

*(*A.M. Montasser2011*)* at South Provinces of Egypt he found the percent of positivity in animals by SAT test for Cattle 90.6% and for Sheep 65.1% and Goat 100%. All animals tested were Egyptian native breeds from farms with a known history of brucellosis according to the directorate of veterinary medicine (Assiut) the samples were taken from slaughtered animals under strict hygienic conditions.

Another study in Egypt by(Basyony *et al.*, 2012) he found positive results for SAT test in Buffalo(43.42%) and (35.14%) in different localities , Cattle were (61.76%) and sheep were(56.54%) different investigated animals, both apparently healthy animals and suggestive infected cases (suffering from abortion), from different farms. the study focused primarily on serodiagnosis of suspected cases .

another study in in the Punjab, Pakistan by (Gul ST *et al.*, 2014)found the prevalence of brucellosis in food animals buffaloes (8.49) , cattle (7.57) Goats (9.57) and sheep (2.14).

It was found that the prevalence of Serum Agglutination Test (SAT) for Camels male was 12.7% and Camels females was 14.5%

(Gwida *et al*., 2011) fond in Sudanese camels serum samples (70.6%) positive by SAT test, samples were collected from apparently healthy camels.
(M. A. Eltayeb 2020) collected serum for agglutination test (SAT) in Sudanese citizens in Gezira state, central Sudan. The city is surrounded by nomadic areas and owners of cattle, and the Sudan is one of the countries endemic for Brucellosis. the SAT scored(13.8%) positive results .
serum agglutination test (SAT) were used as screening tests, and overall seroprevalence of brucellosis was found to be as 21% SAT,Seroprevalence was higher in females (26%) than in males (16%) by SAT , samples from camels of three districts of Sindh province of Pakistan to determine the seroprevalence of Brucella infections in camels and also to identify potential risk factor associated with seropositivity by (Abdul Sattar Baloch 2017).
another study by (Abd-El Halim *et al.*,2017) was carried out to control the brucellosis in buffalo farm in Assuit Governorate, Egypt 13.22% positive for SAT.

It was found that the prevalence of Buffer Acidified Plate Antigen (BAPA) for Goats was 3.5%

*(*A.M. Montasser2011*)* at South Provinces of Egypt he found very high percent of positivity in animals by BAPA test for Cattle 100% and for Sheep 91.3% and Goat100%. All animals tested were Egyptian native breeds from farms with a known history of brucellosis according to the directorate of veterinary medicine(Assiut)
(El-Hady *et al.*, 2016) during routine diagnosis and control program Sera were collected from animals in Al Sharqia Governorate, East of Cairo The results of

screening tests Buffer acidified plate antigen test (BAPA) 4.42% in Private cattle farms and 8.93% in Individual animals.

another study by (Abd-El Halim *et al.*, 2017) was carried out to control the brucellosis in buffalo farm in Assuit Governorate, Egypt 14.51% positive for BAPA.

In another study BAPA (37%) in buffaloes 10 Governorates from farms and villages in Upper Egypt (emarefa.net2018).

another study in a village called Beni-Suef in Egypt, (Alexandria Journal 2019) collect serum samples were examined for brucellosis from cows , buffaloes and human using buffered acidified plate antigen test (BAPA) and the results were 8.71% 8.23% 6.5% respectively .

It was found that the prevalence of Buffer Acidified Plate Antigen (BAPA) for Camels males was 13% and Camels females was 14.5%

(Ahmed M *et al.,* 2017) estimate the sero-prevalence of brucellosis in camels in Shalateen city, in Egypt. Sera were collected from apparently healthy dromedary camels. And serologically tested by buffer acidified plate antigen test (BAPA) found the positive were (11.60%)

Buffered acidified plate antigen test (BAPA) for exported Sheep from Alkadaru quarantine prevalence at a very low percentage (0.2%) by (Nahla Ahmed 2018) in Khartoum state .

serum samples were collected from slaughtered camels in Egypt for BAPA (15.2%) were positive by (Hosny Ahmed 2017) camels from Daraw abattoir. These animals had no history of vaccination against Brucella, they were apparently healthy and clinically normal.

Specific test called (cELISA) It is a test intended for Brucella bacteria . The test needs a long period of time to complete and it is has many steps, as it is time

consuming and cannot be done by non-specialists because of the multiple steps in addition to the high prices of the test and the required devices.

It was found that the prevalence of Competitive Enzyme Linked Immunosorbent Assay (cELISA) for Goats was 1.5%

it is very low rate compairing with screening tests which reduce the amount of exported animl and reduce the hard currency another researcher had a low rate with cELISA prevalence for goats in West Darfur state, El Geneina locality and Furbranga locality by (Youssif 2010) who found 3%, 2% and 4% respectively as we see it is low rate in comparing with screening tests which is take us to our thought the screening tests are un fear for exporting animals in Sudan.

The same different appear in camels with prevalence rate in cELISA 7.4% 2.36% of camels were seropositive with cELISA in Punjab Pakistan for camels (Asim2017) samples from farms of animals with history of recent abortion with consent of the farmer to have collection on positive samples for molecular analysis from different animal species including cattle, buffalo, sheep, goat and camel from different districts of Punjab, Pakistan Significant animal level data, including age, breed, breeding method, lactation status, pregnancy status and other reproductive disorders were also collected from the animal records.

(Mohamed Abdelhmeed *et al.*,2012) was study the prevalence of Brucellosis in sheep and goat intended for export and local consumption in Khartoum state there were no serum samples positive(0.0%) by competitive enzyme linked immuno sorbent assay (c ELISA) as confirmatory .

(Eman Mohamed *et al*., 2018) were collected serum samples from both sexes of goats various age groups and breeds that are either kept in farms or houses in four different localities in Khartoum state and testing for brucellosis using competitive enzyme-linked immunosorbent assay (cELISA) found 11.4% were positive .

in Bahr el Ghazal region, South Sudan. animal (cattle) and human sera respectively were examined Competitive Enzyme Linked Immuno Sorbent Assay (c-ELISA) were used to detect *anti-Brucella* antibodies and the results were 31% and 33.3% (PLoS Negl 2018) for brucellosis sero-prevalence in South Sudan The study population consisted of cattle and herders from cattle camps belonged to herders in the areas of Aweil, Gogrial, Tonj, and Wau states. Majority of cattle is owned by pastoralists who migrate throughout the dry season looking for pastures in small groups of families or in large groups of villages.

(M. A. Eltayeb 2020) collected serum for competitive Enzyme-linked immunosorbent assay (cELISA) in Sudanese citizens in Gezira state data from people at risk with brucellosis. The city is surrounded by nomadic areas and owners of cattle, the positive results (10%).

A cross-sectional epidemiological study was carried out to determine the seroprevalence and risk factors of bovine brucellosis in dairy and traditional cattle herds in Kibaha district of Tanzania. c-ELISA dairy cattle was 6.4 %. All tested dairy goats (*0.0%*) were negatively by (Justine A 2015).

It was found that the prevalence of Competitive Enzyme Linked Immunosorbent Assay (cELISA) for Camels males was 7.4% and Camels females was 7.4% . Alder research by (Gwida *et al.*, 2011) found in Sudanese camels serum samples (68.8%) were positive by cELISA,

(cELISA) were used as screening and confirmatory tests the sero positive for male were (3.35%) and for female were (5.88%) apparently healthy dromedary camels males and females were serologically examined in Egypt by (Hosein *et al.*, 2016) . in Iraq (Alatabi *et al.*, 2020) found that the seroprevalence of brucellosis in examined camels was (4.65%) by cELISA, camels of both sexes were selected

randomly from different places in Al-Najaf province camels were involved in this study with ages between 3 to 9 years.

camel serum samples were found positive for *Brucella* antibodies by c-ELISA (20.2%) from different parts of Egypt by (Microorganisms 2020).

brucellosis in camels in Pakistan (95%) cELISA positive test samples by (Shahzad A. *et al*., 2017) the primary objective of the study was to detect *Brucella* species involved in the causation of disease in camels .

in Somalia for detection of *Brucella* antibodies in camel sera collected from camel herds reared in Mogadoshu, The camel population in the study area has never been vaccinated against brucellosis of both sexes, mixed ages and from Comparative evaluation of serological tests for detection of *Brucella* infection in Somali camels the seropositivity was 3.9% by cELISA (A.M.Ibrahim2018).

in Pakistan Only 2 % of the camel sera were cELISA positive (Sana 2016) sera were collected using random and multistage cluster sampling from different areas.

competitive-ELISA (c-ELISA) were used as screening tests, and overall seroprevalence of brucellosis was found to be as 13% by c-ELISA tests in Pakistan by (Abdul Sattar Baloch 2017) to determine the seroprevalence of *Brucella* infections in camels and also to identify potential risk factor associated with seropositivity.

in Dubai Arab Emarats (Mayada M Gwida *et al.*, 2011) found positive SAT (70.6%) from samples were collected from apparently healthy camels *(Camelus dromedaries)* which were imported from Sudan .

the RBPT is less accurate than others because of the false positive results . it is very harmful to animal export due to preventing animals from exported and loss of hard currency .The other tests are doing well with brucella specially the cELISA but it is

very expensive and consuming more time we cannot recommended it for export .The most suitable for export is PABA because it is fast easy and not expensive and the most important is more accurate than RBPT.

CHAPTER SIX

Conclusions and Recommendation

Conclusions

1- Diagnosis of brucellosis in any species is not a trivial matter. Because of inherent problems with bacterial isolation, inefficiency, cost, danger and other factors, most laboratories prefer to use other, more cost effective methods as Molecular biology as a diagnostic tool is advancing and will soon be at the point of replacing actual bacterial isolation. It is rapid, safe and cost effective and time saving.

2- The Rose Bengal Pate test is a screening test and as we found in our results it has a very high prevalence rate more than ELISA which affect the exportation of animals.

3- The positive samples with RBPT examined by a more accurate examination which is SAT test then BAPA test and finally with the specific test cELISA .

4-RBPT examination excluded from the exported animals the higher rate compared to other tests.

5- RBPT is a fast field survey of acceptable accuracy for large numbers, and does not take more than four minutes.

6-The SAT test examination is a time consuming requires 24 hours to read it

7-BAPA test is accuracy and speed. It is a one-step examination such as RBPT but it is high efficient and not expensive.

8-cELISA which is a test specific for Brucellosis but it is not suitable for export procedures.

Recommendation

- It is important to study brucellosis in humans in pastoral communities because they are contact with animals and do not have adequate health care or health awareness of the importance of zoonotic diseases and their severity and impact on fertility and production.

- It is necessary to know the level of prevalence of brucellosis in the national herd to determine the country's need for a vaccination or treatment program and disposal of infected animals.

- More researches about brucellosis in Sudan to know the real situation of the disease.
- A Control program for Brucellosis is highly needed.

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