Introduction

Brucellosis is an important and widely prevalent zoonotic disease of man and animals (cattle,buffaloes, sheep, goats, dogs etc) caused by *Brucella* organisms (Schelling *et al.*, 2003). The main species of genus Brucella affecting domestic animals are, *Brucella abortus, Brucella suis , Brucella melitensis, Brucella canis and Brucella ovis* (Gillespie and Timoney, 1981). Dogs can be infected by four of the six species *Brucella (Brucella canis, Brucella abortus, Brucella melitensis, Brucella suis,* (Bruce *et al*, 2006). Some of these organism can potentially cause human infections, resulting in one of the most important and widespread bacterial zoonosis in the world. The zoonotic nature of brucellosis was demonstrated in 1905 by isolating ***B****rucella* from goat milk. The recent isolation and characterization of non-classical species of ***B****rucella* demonstrates that in spite of brucellosis being an old disease, there is still several aspects of these organisms and their associated diseases that remain unknown.

Dogs may serve as direct source of infection for their keepers or households with *Brucella canis,* or reservoir for other *brucella* spp that can infect food animals and man.

There are no pathognomic signs of brucellosis in animals at individual level. Therefore, serological tests are the usual identifying possible infected animals. The common serological test used for the diagnosis of brucellosis is Rose Bengal plate test (RBT).

The aim of this investigation was to detect the presence of antibodies *brucella* in dogs as a preliminary investigation.

CHAPTER I

LITRTURE REVIEW

1.1.**Taxonomy**

The genus ***Brucella*** belongs phylogenetically to the a-proteobacteria, a group that contains bacterial species with a wide variety of lifestyles, including symbionts of animals and plants (***Wolbachia***, ***Sinorhizobium***), as well as obligate or facultative intracellular and extracellular pathogens, such as ***Rickettsia***, ***Brucella*** and ***Agrobacterium***. Six species are currently recognized within the genus ***Brucella***: ***Brucella melitensis***, ***Brucella abortus***, ***Brucella. suis***, ***Brucella ovis***, ***Brucella canis***, and ***Brucella neotomae***. This classification is mainly based on differences in biochemical characteristics, pathogenicity and host preferences .Each of these species of ***Brucella*** is adapted to a specific host, but not exclusively (Marinana et al., 2009). Although *Brucella* species can be differentiated by conventional phenotypic tests, These species display a high egree of DNA homology in DNA-DNA hybridization assays (>90% identity), including the recently recognized marine mammal strains . Therefore, it has been proposed that the genus *Brucella* should be a monospecific genus, with *Brucella m*elitensis as the sole species and the other species should be considered as biovars . Conversely, several molecular genotyping methods have been developed and applied to characterize *Brucella* species, indicating that significant DNA polymorphisms occur between species, which favor the current multi-species classification of *Brucella*. Importantly, comparison of genome sequences of *Brucella suis* and *Brucella melitensis* demonstrated that exist clusters of genes that are

unique in both species (designated genetic islands). It is reasonable to hypothesize that these unique genes may contribute to the differences in host specificity between *Brucella* species

Furthermore, recent studies based on comparative whole genome analysis of several *Brucella* species indicate that there is limited divergence with a large number of pseudogenes. Interestingly, these genomic analyses do not clearly explain the host preferences of *Brucella* spp. One of these studies indicates that at the ***Brucella ovis*** is the basal lineage to the rest of the ***Brucella*** spp., and that apparently most ***Brucella*** species diverged from their common ***Brucella ovis*** ancestor in the past 86,000 to 296,000 years.It is noteworthy that the International Committee on Systematics of Prokaryotes, Subcommittee on the Taxonomy of ***Brucella*** has taken a clear position recommending a taxonomic classification that includes different species within the genus, either classical or new, which are still considered as individual species. Therefore, the genus currently group nine species, namely ***Brucella melitensis***, ***Brucella abortus***, ***Brucella suis***, ***Brucella ovis***, ***Brucella canis***, ***Brucella neotomae***, ***Brucella ceti***, ***Brucella pinnipedialis***, and ***Brucella microti*** . There is one newly isolated ***Brucella*** species from baboons that has not yet been classified nor included in the above mentioned list. (Marinana *et al*., 2009).

1.2. Brucella melitensis

Brucella. melitensis is the most important etiologic agent of brucellosis in small ruminants, although cattle and other ruminants may also be infected. This species has three different biovars and it has the higher zoonotic potential within the genus, and thus it is recognized as the most important agent of human brucellosis. This pathogen is widespread in several parts of the world, particularly the biovar 3 in Mediterranean and Middle Eastern countries. Parts of Latin America are also seriously affected with biovar 1, especially Mexico, Peru and Northern Argentina. Importantly, ***Brucella melitensis*** have never been isolated in Brazil, where it is considered a foreign disease. In goats and sheep, ***Brucella. melitensis*** infection causes abortion, reduced milk yield, and orchitis. Both sexually mature genders are equally susceptible. The predominant sign of acute infection is reproductive failure with abortion and birth of weak offspring. Abortions occur mostly during the last two months of gestation. Generally, transmission in sheep and goats occurs through materials excreted from the female genital tract. In goats, approximately two thirds of acute natural infections during pregnancy lead to infection of the udder and milk excretion of the bacteria during the subsequent lactation. Persistent infection of the udder is accompanied by intermittent shedding of the agent in milk. Inflammation of the mammary gland reduces milk production. However, clinical signs of mastitis are seldom detectable in naturally infected goats. (Marinana *et al.*, 2009).

Brucella melitensis is the most virulent ***Brucella*** for humans with a few organisms (10 to 100) being sufficient to cause a debilitating chronic infection. Humans acquire brucellosis mainly through ingestion of contaminated milk and unpasteurized dairy products. Contact of mucosa and skin abrasions with fluids and tissues from aborted fetuie of infected animals are also important sources of ***Brucella*** transmission. Furthermore, people may be infected by inhalation of

contaminated dust or aerosols. Thus, ***Brucella*** is one of the most common laboratory-acquired pathogens worldwide and is included in the potential biological weapon list. Human infections with ***Brucella melitensis*** may have variable clinical manifestations and can become life threatening. Although the majority of patients present with general symptoms, such as fever, malaise, sweats and lymphadenopathy and/or hepatosplenomegaly, a more severe form of the disease can be accompanied with osteo-articular signs (spondylitis, arthritis and osteomyelitis) or genitourinary tract changes (orchitis, epididymitis, glomerulonephritis and kidney abscesses). More severe complications comprise, in descending order of frequency, neurobrucellosis, liver abscesses, and endocarditis (Marinana *et al*., 2009).

1.3 Brucella abortus

Brucella abortus has seven different biovars, namely biovars 1-6 and 9. Cattle is the preferential host for ***Brucella abortus***, but the organism can be transmitted to buffaloes, camels, deer, dogs, horses, goats, sheep, and man. In Brazil, bovine brucellosis due to ***Brucella abortus*** is the most prevalent ***Brucella*** infection.

Brucella abortus causes primarily a disease in cows, being isolated from the udder, uterus, and lymphoid organs .Outbreaks of brucellosis in dairy herds result in decreased milk production, increase somatic cell count in milk, occurrence of abortions and post-partum metritis. Late abortion is associated with necro-hemorrhagicplacentitis.and fetal lesions, particularly fibrinouspleuritis and pericarditis and pneumonia .Infected cows usually abort only once, and subsequent gestations may generate calves that may be born weak or healthy. Some infected cows will not exhibit any clinical symptoms of

the disease and give birth to normal calves. Transmission occurs mainly after abortion or parturition of infected cows via contaminated fetus, fetal membranes, and uterine secretions. Bulls can be infected but they do not readily spread the disease. ***Brucella abortus*** is a common cause of orchitis that is often associated with a vesiculitis and epididymitis. Infection in males may result in either temporary or permanent infertility, depending on the intensity of the lesions (Marinana *et al*., 2009).

1.1.3. Brucella suis

Porcine brucellosis is an emerging disease caused by ***Brucella suis*** biovars 1, 2 and 3. It is mainly a disease of domestic and wild pigs but it can also affects other species such as cattle, horses, rabbits, dogs, and humans. Biovars 1 and 3, which have pathogenic potential for humans, occur in Europe, North, South and Central America, Southern Asia and Pacific islands. In Brazil, only the biovar 1 has been isolated, and there are just a few reports of ***Brucella suis*** in the country, with a seroprevalence of 0.34% in recent surveys (Brazil, 2000). Prevalence is very low in industrial swine production systems, but it may be quite high among backyard pigs slaughtered without sanitary inspection in Brazil. Porcine brucellosis is a herd problem. Pigs of all ages can acquire the infection, but the disease primarily occurs in adults. ***Brucella suis*** is excreted in

large numbers, for long periods in the semen and urine as well as in uterine discharges and milk being transmitted by both venereal and oral routes. ***Brucella suis*** infection in pigs often does not result in clinical signs, and therefore clinical diagnosis is very difficult. ***Brucella suis*** causes primarily a genital disease with abortions, but it also affects other organs, especially bones and joints .Brucellosis is the only disease in which reproductive failure in sows is accompanied by orchitis in boars and osteo-articular disorders such as arthritis, osteomyelitis, spondylitis and paralysis. The most important clinical signs in sows are infertility, irregular estrus, abortion in any stage of gestation and birth of weak piglets with a high neonatal mortality rate. Although orchitis and epididimitis are the most common lesions in boars, in some cases the infection is restricted to sexual glands and may not result in impaired fertility but can be an important source for shedding the organism in the semen. (Marinana *et al*., 2009).

1.5. Brucella canis

Canine brucellosis is caused by ***Brucella canis*** that infects domestic dogs, wild carnivores and rarely other domestic animals. It is especially common in Central and South America .Infection of dogs with ***Brucella canis*** is widespread in Brazil, with prevalence ranging between 0.84 to 58.3% and it is concentrated mostly in the Southeast and South regions of the country. Humans are susceptible to ***Brucella canis***, but infections are uncommon and they are usually mild. Most natural human infections have been acquired through close contact with infected dogs. Laboratory infections have also been reported. Natural infections occur most commonly after ingestion of contaminated placental tissues or aborted fetuses, vaginal secretions from infected bitches, and during breeding. The organism may be shed for long periods in vaginal secretion after abortion and semen (Marinana et al., 2009) .

In females, the most prominent clinical sign is abortion after 45-55 days of gestation in about 75% of the cases. Early embryonic death and reabsorption, or abortion 10-20 days after mating, may occur in some cases. In males, the main sign is epididymitis and orquitis, which may be unilateral or bilateral, and often results in infertility. Semen from infected males usually contains large numbers of abnormal sperm and inflammatory cells, especially during the first three months after infection. Chronically infected males may have no sperm, or reduced numbers of immature sperm (marinana et al., 2009).

A particularity of ***Brucella canis*** infection is a prolonged bacteremia. Therefore, blood culture is a valuable diagnostic approach in this case

1.6 Brucellosis

1.6.1Definition

Infection by *Brucella* spp is diffused worldwide in several animal species and in humans. The most clinically relevant *Brucella* species, *Brucell aabortus, melitensis, Brucella. Canis* and *B*rucella. *suis,* tend to be host-adapted, although infections of other animal species, including humans, may occur sporadically. (Keid *et al*., 2017). It was named brucellosis after Bruce (1887) who was the first one to isolate the organism and recognized the disease. In animals, the disease is characterized by bacteraemia followed by localization of organisms in the reproductive organs, reticuloendothelial tissues and sometimes joints (Gillespie and Timoney, 1981). The disease in man is known as Malta fever and is characterized by undulant fever, chills, headache, pain in legs, large joints and lumber regions, profuse neutral sweating, insomnia, sometimes laryngitis and bronchitis (Van Der Hoeden., 1964).

1.6.2 **History of Brucellosis**

Bruce isolated *Brucella melitensis* (*Micrococcus melitensis*) in 1887 from the spleen of a British soldier who died from a febrile illness (Malta fever) common among military personnel stationed on Malta. For almost 20 years after isolation of *Brucella. melitensis,* Malta fever remained a mystery and was thought to be a vector-borne disease until the mistocles Zammit accidentally demonstrated the zoonotic nature of the disease in 1905 by isolating *Brucella. melitensis* from goat's milk (Wyatt, 2005).The first case of Brucella suis infection in a dog was published in 1931. Outbreaks of canine abortions had been reported in 1963, but it was not until 1966–1967 that Brucella canis was isolated from canine tissue and vaginal discharge(Bruce *et al*., 2006). Canine brucellosis, due to *Brucella. canis,* was first recognized in 1966 in USA from episodes of abortion and reproductive failure in kennels since then, the disease has been reported in several countries (Corrente *et al*., 2010). *Brucella abortus* was identified in uterine discharge of apparently healthy bitch and queen with open pyometra housed on a cattle farm (Wareth *et al*., 2016).

1.6.3 Transmission

The main sources of infection are vaginal fluids of infected females and urine in males. Routes of entry are venereal, oronasal, conjunctivae mucosa and placenta (Wanke , 2004). Brucella abortus can infect dogs that ingest aborted or fetal tissue from infected livestock. Intact dogs have the potential to harbor and transmit this organism through natural mating, oronasal contact and ingestion of contaminated tissue or fluid (Bruce *et al.*, 2006) . all dogs, had a history of being fed foetuses from cows (Cadmus *et al*., 2011) The infection may be transmitted in dogs either by the venereal or oral route (Corrente *et al*., 2010).Pregnancy is an important physiological condition for the transmission of infectious agents to puppies and transplacental transmission may be

epidemiologically relevant in the spread of the *brucella canis* (Taques *et al.,* 2016) .

1.6.4 Causes and Pathogenesis

Brucella canis a gram-negative coccobacilus (Wanke , 2004). It is a facultative intracellular pathogen that preferentially infects members of the Canidae family, report the genome sequencing of two *Brucella canis* strains isolated from humans and one isolated from a dog host (Viana *et al.,* 2017). The bacteria attach to an exposed mucous membrane, penetrate the tissue; thereafter, more bacteria attach, phagocytosis continues, and virulence increases. These bacterial inclusions travel to the lymph nodes, replicate and start a bacteremia within 7–30 days. These intracellular Brucella canis bacteria target reproductive (steroid-dependent) tissue. In the male, these are the prostate, testicle, and epididymides. The female has the fetus, gravid uterus, and placenta. Bacteria are found in fetal stomach contents, suggesting that puppiedds swallow the amniotic fluid and bacteria in utero. The aborted placenta has focal coagulative necrosis of the chorionic villi, necrotizing arteritis, and numerous bacteria in trophoblastic epithelial cells. Other body systems are seeded by the blood-borne bacteremia such as the intervertebral disks, and kidney or form antigen–antibody complexes in the anterior uvea of the eye. Histopathologic findings are reticular cell hyperplasia in lymph nodes and a granulomatous response in skin, testis and organs. Bacteremic episodes can last for years. Experimentally infected dogs remained positive in blood cultures for 5.5 years. In the first 3–4 months of infection, bacteremia declines, and titers reflect persistent bacteremia and/or organisms in sequestered organs or targeted gonads. The cellular damage from inflamed epididymides induces a sperm granuloma from leakage of antigenic material into the surrounding tunic that stimulates antisperm antibodies, humoral and cellular immune responses. Consequently, the semen consists of sperm with abnormal morphology, agglutination or absence of sperm. The antibody is directed against sperm and not Brucella canis. Spontaneous recovery

can occur naturally 1–5 years after the initial infection. The dog becomes abacteremic with low agglutination titters of 1:25or1:50 that suggest clearance of the bacteria. The titer does not rise again if challenged, and no re infection occurs because of a developed cellular immunity from this natural recovery. Negative blood culture correlates with decreased serum agglutination titter, even in some cases where *Brucella canis* persists in body tissue (Bruce *et al*, 2006).In pregnant bitches, the infection localises in the reproductive tract where it causes placentitis with subsequent abortions and stillbirths A virulent Brucella canis strain isolated from those outbreaks readily replicated in different organs of mice for a protracted period. However, the levels of tumor necrosis factor alpha, interleukin-6 (IL-6), and IL-12 in serum were close to background levels. Furthermore, *Brucella canis* induced lower levels of gamma interferon, less inflammation of the spleen, and a reduced number of granulomas in the liver in mice than *did Brucella. abortus*. When the interaction of *Brucella canis* with cells was studied ex vivo, two patterns were observed (Chacón-Díaz *et al*., 2015).

1.6.5 Clinical signs

Clinical signs that suggest brucellosis: lymphoid hyperplasia, scrotaldermatitis, enlarged testicles or epididymis, abortion, stillbirths, and vaginal discharge (Keid *et al*., 2008).

1.7 Canine brucellosis (*Brucella canis*)

Brucella canis is a pathogenic bacterium for dogs and its zoonotic potential has been increasing in recent years, the etiologic agent of canine brucellosis is an important cause of reproductive failure in dogs. Because of the lack of prominent clinical signs (Keid *et al*., 2008) range from asymptomatic, lymphadenapthy, orchitis and epididymitis, embryonic loss, to abortion and testicularatrophy (Bruce *et al.*, 2006) .early embryonic deaths and resorption can occur a few weeks after mating and may be mistaken for failure to conceive, Epididymitis, orchitis, testicular atrophy, poor sperm quality and infertility and loss of *libido* have been reported in male dogs. Despite being infected, many dogs in most cases remain asymptomatic and appear to be healthy , but severe lymphadenitis involving the retropharyngeal and inguinal lymph nodes may be found. symptoms are late abortions in bitches, epididymitis in males and infertility in both sexes, as well as generalized lymphadenitis, discospondylitis and uveitis (Wanke *et al*. , 2004).

1.7.1 Epidemiology

Members of the canidae family are the reservoir hosts of Brucella canis. Historically, infection was originally associated in the mid 1970 with the beagle breed .This may have been accentuated because of this breed's popularity as research animals and in field trials. The list of breeds now includes Labrador Retrievers, Cocker Spaniels, German Shepherds, Boston Terriers, Poodles and more. Despite a higher prevalence in purebred dogs, the mixed breed mongrel or any sexually mature, reproductively active dog is susceptible. In the environment, stray and feral dogs remain predominant reservoirs. A predominant route is venereal transmission where the likelihood for spread remains high due to large numbers of organisms shed in reproductive secretions. One study suggested that dogs do not seem to infect the same gender when housed in close contact. However, other reports had male kennelmates becoming infected by being housed for an extended time in close quarters with a shedding male. Urine may be a less important route in its natural spread but does not contain a low amount of bacteria during the first weeks to three months following infection. The urine becomes the contaminated vehicle by the close anatomical connection of the bladder to the secretory prostateand epididymis. *Brucella canis* infection has been diagnosed in many geographical areas, with a particular prevalence for rural south eastern United States. It occurs in wild dog packs, new untested animals, kennels, puppy mills and even backyard mistakes. A study of stray dogs in Tennessee demonstrated a greater than three-fold rate of infection versus non-stray dogs. Reports document worldwide outbreaks from Alabama, Mexico, Britain, Europe, Brazil , Texas , Colorado, Illinois and Wisconsin , Michigan, Ontario and Quebec Canada, Japan, China, and Georgia Asymptomatic dogs harbor*B. canis* organisms for prolonged intervals. The time from initial exposure to a bacteremia is approximately 3 weeks and then the organisms localize in targeted genital tissues to seed a continuous or recurrent release that can last from months to years. The male prostate and epididymides

serve as effective sites for bacterial emissions. These two tissues are focal sites for widespread dissemination if the male remains actively breeding. Initial semen sampling has a higher concentration of bacteria for the first two months post infection, followed by a sporadic output of lower numbers for years, and the host displaying no apparent illness. In a kennel environment, the aborting bitch is at high risk for the spread of infection. A characteristic, lengthy vaginal discharge of infective uterine secretions persists for 4–6 weeks following a single abortion. Large numbers of organisms are present in the aborted placental tissues and fluids; two million colony forming units in an infective dose or ‘‘1010 organisms/ millilitre in discharge which constitutes 500 oral infective doses/ mL .*Brucella canis* is also found in the milk of infected lactating bitches that might lead to the potential infection of nursing pups. Artificial sources for transmission are blood transfusions, vaginoscopy, AI, and contaminated syringes (bruce *et al*., 2006).Outstanding was the severity of the epidemic, localisation of the infection in the genitourinary tract, the chronicity of the disease producing a hyperplasia of the lymphatic tissue of many organs, and isolation of pure 19*S*and 7*S*agglutinating antibody(Richard et al 1969)Brucellosis is a highly infectious zoonotic disease but rare in Sweden. Nonetheless, an outbreak of canine brucellosis caused by an infected dog imported to Sweden was verified in 2013. (Kaden *et al*., 2013).Epidemiology of brucellosis in Nigeria considering the conducive human-animal interface and ecological factors responsible for the transmission of the disease.(Ayoola *et al*., 2016)

1.8 Diagnosis

1.8.1 Bacteriological methods

The isolation and identification of *Brucella* offers a definitive diagnosis of brucellosis and may be useful for epidemiological purposes and to monitor the progress of vaccination programme. It should be noted that all infected materials present a serious hazard and they must be handled with adequate precautions during collection, transport and processing .

1.8.2 Microbiological analysis

A fragment of the inter-vertebral disk, heparinised blood and urine collected by cistocentesis from the dog were subjected to bacteriological investigations. Five ml of urine and 5 ml of blood were inoculated into Tryptose soy broth (TSB) supplemented with 7% of equine serum. The samples were also streaked on Columbia blood agar and MacConkey agar and all media were incubated in aerobic, microaerophilic and anaerobic conditions at 37°C. Because of the small sample size, the disk was split into two portions. One was used for inoculation into TSB and incubated in aerobic conditions; the second portion was analysed by PCR. Subcultures in Tryptose soy agar with 7 % equine serum of all the TSB-cultured samples were made after 3, 5, 7 and 14 days of incubations. The subcultures were monitored daily for 1 month before being discarded. All the media and reagents were purchased from Liofilchem (Corrente *et al.*, 2010).

1.8.3 Blood culture

Blood culture was performed according to the manual monophasic blood culture methods,using 2 mL of blood collected with sodium citrate. Tryptose Phosphate Broth and Tryptose Agar (Oxoid, Hampshire, UK) were used as culture media. Genital samples (vaginal swab and semen) were cultured directly on Tryptose Agar plates with antibiotics. The samples were incubated at 37_ C at aerobic atmosphere and *Brucella. canis* colonies were identified. (Keid *et al*., 2008).

1.8.4 Serological methods

Some animals with localized male genital tract infections could not be judged as infected solely by serological tests. (Flores *et al.,* 1977). Brucella canis has a rough and not a smooth cell wall antigen as *do Brucella*. suis, *Brucella* abortus and B. melitensis. The serology examines the agglutinating reaction to that cell wall or cytoplasmic protein antigen. Results may be negative during the first 3–4 weeks of infection, although the dog is undergoing a bacteremia by 2 weeks. Possible tests are the rapid slide agglutination test (RSAT and ME-RSAT), the tube agglutination test (TAT), indirect fluorescent antibody (IFA), agar gel immunodiffusion (AGID), enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR). Blood culture is definitive proof of infection, and it may yield positive results as early as 2–4 weeks post infection. The dog can remain positive for several years. A delay in serum titer is possible for 8–12 weeks after exposure to the antibody; the titer can fluctuate even with a persistent bacteremia. The magnitude of the titer does not reflect the stage of disease.(bruce *et al*., 2006) .

1.8.4.1Rapid slide agglutination test (RSAT)

The RSAT or card test is a rapid screening test developed in 1974 [68] and is commercially available and practical with results within 2 min. Agglutination is detectable 3–4 weeks after the onset of infection The patient's serum is mixed with a rose-bengal stained heat-killed Brucella ovis suspension on a card. If the precipitate on the test side is similar to the known standard, a positive interpretation is made and a more specific test ordered (bruce *et al.*, 2006).

1.8.4.2 Modified rapid slide agglutination test (ME-RSAT)

The modified RSAT (ME-RSAT) adds 2-mercaptoethanol (2-ME) drops to inactivate IgM mentioned above and thereby increases the specificity of the test. ME-RSAT is another screening test and useful as a subsequent test for a positive RSAT serum . *Brucella. Ovis* antigen is replaced by *Brucella. canis* cells to reduce the number of false positives. It is semiquantitative. An animal can be positive for 30 months after becoming abacteremic, although false negatives can occur during the first 8 weeks post infection (bruce *et al.* , 2006).

1.8.5Molecular method

1.8.5.1 Polymeric chain reaction (PCR)

1.8.1.1.DNA extraction

Nucleic acids were extracted from all the sample susing the DNeasy Blood & Tissue Kit, following the manufacturer's instructions. DNA extraction was made either from the whole blood and from the buffy coat obtained by using a specific reagent. *Real-time PCR for Brucella spp. Quantification*A real-time PCR assay targeting the BCsp31 gene of *Brucella* spp. was used to

evaluate the bacterial DNA load in the samples, with certain modifications Absolute quantification was achieved by constructing a standard curve using plasmid DNA. To generate the standard DNA, a 630-bp fragment of the BCsp31 gene of *Brucella melitensis* strain 16 M was amplified by PCR using primers BCsp31P-F (5' GAGCTTTGCGGTTGCACA-3') and BCsp31P-R (5'-AGATCGGAACGAGCGAAATA3') and cloned into PCR blunt vector-TOPO. The reaction was performed on a 7500 Real-time PCR System (Applied Biosystems, Foster City CA) with iTaq TM Supermix added with ROX.

1.8.5.2PCR specific for *B. canis*

For characterization of *Brucella. canis*, a combined PCR protocol was used, with 4 sets of primers targeting the genes BCSP31, *omp*2and *omp*31. The protocol used is able to characterize Simultaneously *Brucella. abortus, Brucella. melitensis, Brucella. Cani s*and *Brucella. suis.* For amplification, theAccuprime TM Taq DNA polymerase was used

(Corrente *et al*.,2010) .Real-time PCR for the detection of Brucella. canis and its first successful use in an outbreak investigation (Kaden et al 2013).

1.8.6 Diagnostic imaging

Radiographic findings can be suspected lesions of Brucella. canis. Unifocal or multifocal inflammation of inter vertebral disk (i.e., disk space) with unaffected vertebral architecture is visual evidence of

discospondylitis. Skeletal limb abnormalities may be indicative of osteomyelitis, or a soft tissue involvement diagnostic for stump pyometra. Any non reproductive lesion should be confirmed by antibody testing and/or culture. Thorough ophthalmological exams done either for visual deficit or breed certification can detect a uveitis and accompanying lesions. Real-time ultrasonography during the male breeding soundness evaluation identifies changes associated with inflammation or atrophy of the epididymides and testes, respectively. (bruce *et al*. , 2006).

1.8.7Differential diagnosis

Brucellosis should be considered in the differential diagnosis of back pain, discospondylitis, lameness, abortion, prostatic abscessation and testicular/epididymal enlargement in dogs, especially if there is exposure to feral pigs or consumption of uncooked feral pig meat. Euthanasia is the only guarantee of reducing the public health risk to zero (James *et al.*, 2017).

1.9 Zoonosis

Brucellosis is a zoonotic disease that is transmitted from animals to humans, and the development of a rapid, accurate, and widely available identification method is essential for diagnosing this disease (Kang *et al*., 2014)the first reported in the literature. This outbreak involved six persons (three children and three adults), a bitch and three puppies which had close daily contact with the family (Lucero *et al*., 2014).Brucella canis which is the main etiologic agent of brucellosis in dogs, can be transmitted to man. It causes mild or asymptomatic infection in human compared with other Brucella species. Brucella.canis can be transmitted to man either by laboratory accidents or contact with infected dogs (Yüksekkaya *et al* ., 2013) . Human Brucella canis infection incidence is unknown. Most identified cases are associated with pet dogs (Dentinger *et al*., 2015).symptoms, including fever, headache, night sweats, appetite loss, weakness, arthralgia and myalgia(Yüksekkaya *et al*., 2013) Brucellosis is highly contagious bacterial zoonosis affecting a wide range of domesticated and wild animals. In this study, Brucella abortus bv 1 was identified in uterine discharge of apparently healthy bitch and queen with open pyometra housed on a cattle farm. This study highlights the role of dogs and cats as symptomatic carriers and reservoirs for Brucella. To the best of our knowledge, this study represents the first report of feline infection with Brucella. abortus bv 1 globally. These pet animals may contaminate the environment and infect both livestock and humans. Surveillance and control programmes of brucellosis have to include eradication of the disease in dogs, cats and companion animals. (wareth *et al*., 2016).

1.10 Treatment

No single antibiotic should be used, but combination therapy has been helpful. However, With the possibility for relapse (even if neutered or spayed), the best treatment is removal from the facility or euthanasia (bruce *et al* , 2006) .

1.11 Control

Better management skills are imperative to avoid financial indebtedness from months of quarantine and lost income. Serological testing before entry and breeding will lower the anxiety among owners and also reduce the incidence in many breeds (bruce *et al*., 2006) . . Bacterial ghosts (BGs) are the empty envelopes of bacteria with no genome content inside, which emerge as a proper vaccine candidate due to its intact outer antigen. It is generally derived from a genetically engineered strain (Qian *et al*., 2017). Polymeric antigen BLSOmp31 is an immunogenic vaccine candidate that confers protection against Brucella canis in mice. The immunogenicity and safety of BLSOmp31 adsorbed to aluminum hydroxide gel (BLSOmp31-AH) were evaluated in Beagle dogs. In addition, the potential to elicit serum antibodies with complement-dependent bactericidal activity and/or to enhance phagocytosis by neutrophils were analyzed. Dogs were immunized three times with BLSOmp31-AH by subcutaneous route, followed by an annual booster. The vaccine elicited specific antibodies 3 weeks after the first immunization. Annual booster induced comparable antibody response as the primary series. Humoral immune response stimulated by BLSOmp31-AH did not interfere with routine agglutination test for canine brucellosis. Antibodies demonstrated a high complement-dependent bactericidal activity against Brucella. canis. (Clausse *et al*., 2017).Isolation until recovery (which may take up to 3 years) or euthanasia. Intensive antibiotic therapy may be of value; however, they should be followed by periodic serologic and/or bacteriologic monitoring following treatment. Transmission

probably can be interrupted by castration of infected males, or by spaying of females, followed by a course of antibiotic treatment. (Carmichael *et al*.,1976)

CHAPTER TWO

MATERIALS AND METHODS

2.1Study area

The study was conducted in Khartoum state, including different area namely Burri Western Elgeraif ,Alsafia, Hilt Khojaly , Kafory, Alsamrab, Bahri and Almurabaat. Khartoumstate is located centrally in Sudan between 15- 16 N and longitudinal 31.5- 34 E

2.2.Study population

A total of seventy seven dogs were surveyed for presence of antibodies (Ab) of *Brucell spp*, during the period from April to August 2017.

The surveyed dogs were forty five police caged dogs (PCD) with average animal age between one to three years, and body weight between 25-40kg. Thirty two of the surveyed animals were dogs owned by citizens (DOC) Husbandry and management of the police caged dogs (PCD). All these dogs were mature .

2.3. Husbandry and management of PCD

2.3.1 Housing:

The yard of barn was designed for at least 400 dogs based on about 6 square meters Cage/ dog. The designed total area is 3500 square meters (70 × 50).

2.3.2 Feeding

PCD eat one meal at 8:30am include spaghetti with meat. Different feeding programs were applied for feeding DOC.

2.4 Husbandry and management of DOC

Different housing and feeding program were applied for feeding DOC

2.5. Sampling

2.5.1Blood samples

2.5.1.1 Collection of samples for Rose Bengal test

From each dog 3-5 ml blood was collected from the cephalic vein of each dog in sterile disposable syringes. and transported in an iso-thermoflask to the laboratory. The sample was settled in a rack for a period of time to separate the serum. Serum was separated and centrifuged whenever it is needed. Centrifugation was done at speed of 30 cycles/sec. Rose Bengal test was immediately done.

2.6. Method for detection of Antibodies (Abs)

2.6.1Rose Bengal test

A simple spot agglutination test using antigen stained with Rose Bengal and buffered to a low PH, usually 3.65±0.05, this antigen was obtained from Lillidale, London. The test was performed according to the instructions of the manufacturer and OIE manual, (2004).

The serum samples and the antigen were brought to room temperature (22±4°C); Only sufficient antigen for the day's tests was removed from the refrigerator. The antigen bottle was shacked well, but gently, and an amount of

30 µl of the antigen was placed on disposable plat near. An equal volume of each serum sample was placed near the antigen drop.

Immediate thorough mixing of serum and antigen was performed (using a clean plastic rod for each test) producing a circular or oval zone approximately two centimetre in diameter. The mixture was rocked manually for five minutes. Agglutination was immediately read after the five minutes period had completed. Any visible reaction was considered positive .

CHAPTER THREE

RESULTS

Chapter III

Results

Forty five police caged dogs (PCD) and thirty two dogs owned by citizens (DOC) were examined for presence of antibodies of Brocella spp using Rose Bengal test (RBT) .
Fifty eight percent of the PCD were females and the rest were male (Fig.1.). one out of the forty five PCD were negative for Brucella antibodies .
The DOC were 50%males and 37% females (fig.1); there is no information of the sex in thirteen percent of the surveyed dogs (ND) due to technical error (Fig.1.)
Figure 3 . shows that 5 males , 7 females and 2 ND of the surveyed DOC dogs were negative for RBT; 11 males ,6 females and 2 ND dogs were found to be positive for RBT .

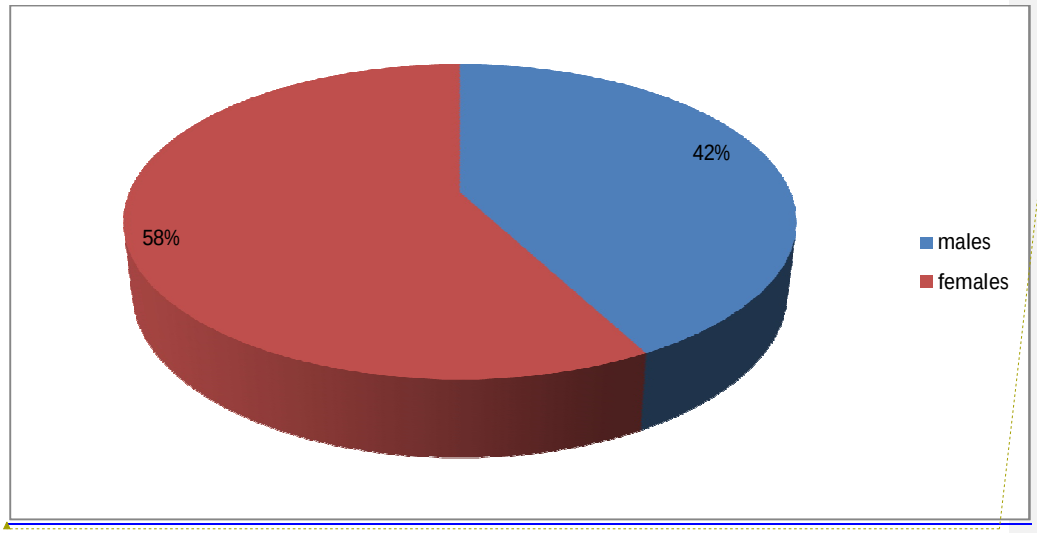

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Fig.1. male and female police caged dogs in Khartoum , sudan screened for brucellosis using Rose Bengal test.

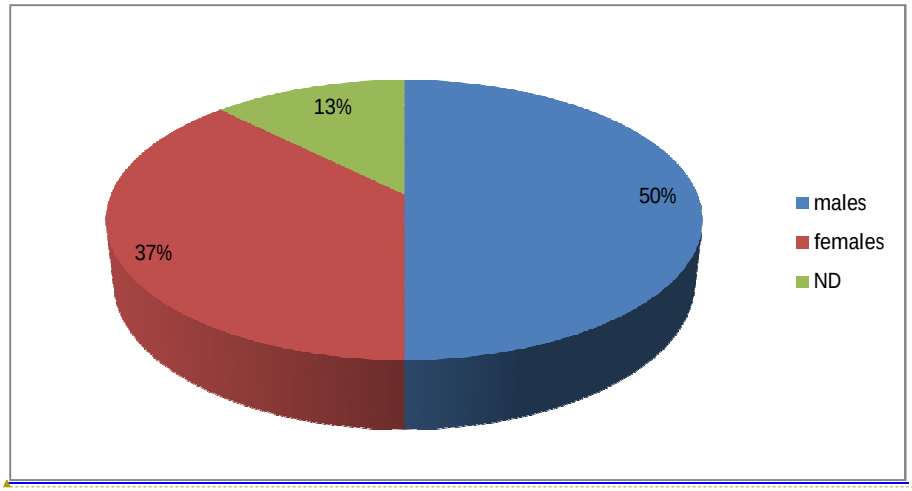

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Fig.1. male and female dogs owned by citizens in Khartoum , sudan screened for brucellosis using Rose Bengal test.

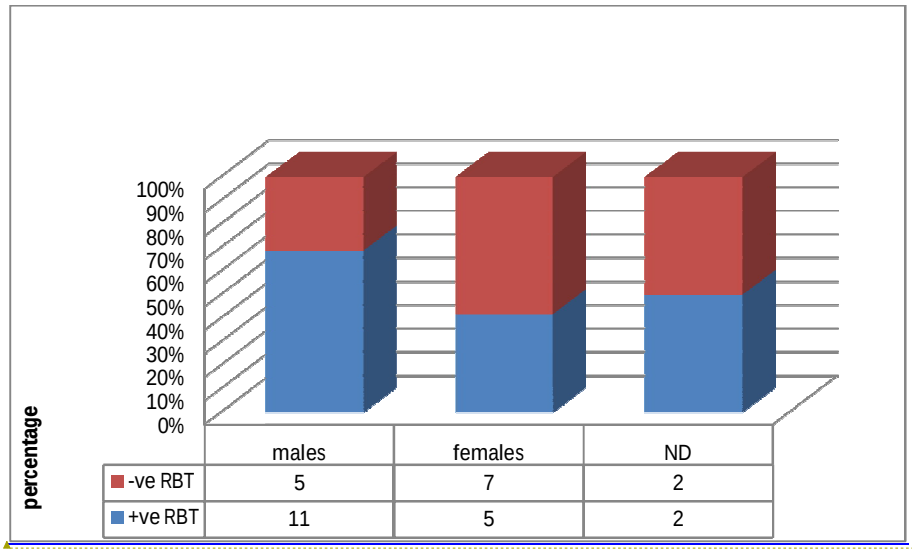

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Fig 3. Detection of antibodies against Brucella in dogs owned by citizens in Khartoum ,Sudan using Rose Bengal test .