T.R.
KAHRAMANMARAŞ SÜTÇÜ İMAM UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCE

DETECTION OF *BRUCELLA SPECIES* IN MILK AND SERUM OF SHEEP AND GOATS FROM KAHRAMANMARAŞ AND DUHOK

CHYAYİ JAFAAR AHMED

MASTER THESIS

DEPARTMENT OF BIOENGINEERING AND SCIENCES

KAHRAMANMARAŞ, TURKEY 2014

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DECLARATION PAGE

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KAHRAMANMARAŞ VE DUHOK'TAKİ KOYUN VE KEÇİLERİN SÜT VE SERUMLARINDA *BRUCELLA* TÜRLERİNİN SAPTANMASI

(YÜKSEK LİSANS TEZİ)

Chyayi Jafaar AHMED

ÖZET

Bu çalışma, Enzyme Linked Immunosorbent Assay (i-ELISA) kullanılarak Duhok (Irak) ve Kahramanmaraş (Türkiye)'deki bazı koyun ve keçi sütlerinde *Brucella* antikorunu araştırmak ve Rose Bengal Plate Test (RBPT) ile *Brucella* türlerini tespit etmek ve iki test sonucunu karşılaştırmak için yapılmıştır. *Brucella* antikorunun tespiti için toplam 180 serum örneği (140 koyun, 40 keçi) kullanılmıştır. Serum örneklerinin 30 tanesinde (%16.66) i-ELISA ile Brucella antikoru tespit edilmiştir. Koyunlara ait serum örneklerinde (25 pozitif; %17.85) keçilere oranla (5 pozitif; %12.5) daha fazla seropozitiflik gözlenmiştir. Süt örneklerinin 29 tanesinde (%16.11) *Brucella* antikoru süt-ELISA ile test edilmiş ve yüksek seropozitiflik koyun sütlerinde tespit edilmiştir (24 koyun sütü pozitif %17.14; 5 keçi sütü pozitif %12.5). Çalışmada bölgesel fark incelendiğinde Duhok'ta enfeksiyon oranı (%24.44) Kahramanmaraş'tan (%8.88) yüksek bulunmuştur.

Serum ELISA ve süt ELISA ile taraması yapılan 180 serum ve süt örneğinden 30 serum (%16.66) ve 29 süt örneğinde (%16.11) *Brucella* antikoru tespit edilmiştir. RBPT ve süt ELISA hassiyeti sırası ile %46.6 ve %93.33 olarak belirlenmiştir. Serum ELISA hassasiyeti ise %99.33 olarak tespit edilmiştir.

Key Words: Brucellosis, i-ELISA, Küçük ruminant, süt, serum

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DETECTION OF *BRUCELLA SPECIES* IN MILK AND SERUM OF SHEEP AND GOATS FROM KAHRAMANMARAŞ AND DUHOK

(M.Sc. THESIS)

Chyayi Jafaar AHMED

ABSTRACT

The aim of present study was to detect *Brucella* spp. via Brucella antibody in the serum and milk samples of sheep and goat from Duhok (Iraq) and Kahramanmaraş (Turkey) by enzyme-linked immunosorbent assay (i-ELISA) with the comparison of rose Bengal plate test (RBPT). One-hundred eighty serum samples (140 sheep; 40 goats) were tested for presence of Brucella antibody. The results showed that 30 (16.66%) serum samples were found to be positive for Brucella antibody by i-ELISA. Higher seropositivity was detected in serum of sheep 25 (17.85%) compared to goat 5 (12.5%). By milk-ELISA, 29 (16.11%) milk samples were found to be positive for Brucella antibody. Higher seropositivity was detected in the milk samples of sheep (24 positive; 17.14%) than goat (5 positive; 12.5%). The results indicated that the infection rates were higher in Duhok (24.44%) than Kahramanmaraş (8.88%).

Comparing with serum and milk ELISA tests for antibody detection within 180 samples, the ELISA detected antibodies in serum samples were higher (30; 16.66%) than milk (29; 16.11%). The sensitivity of RBPT and milk-ELISA was found to be 46.66 and 93.10%, respectively. However, the specificity was 99.33% in serum ELISA.

Key Words: Brucellosis, i-ELISA, Small Ruminant, Milk, Serum

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LIST OF SYMBOLS ABBREVIATIONS

FAO:	Food and Agriculture Organization
i-ELISA:	Indirect Enzyme-Linked Immunosorbent Assay
RBT:	Rose Bengal Plate Test
SAT:	Serum Agglutination Test
DNA:	Deoxyribonucleic Acid
ELISA:	Enzyme-Linked Immunosorbent Assays
STA:	Standard Tube Agglutination
MRT:	Milk Ring Test
2ME:	2-Mercaptoethanol Agglutination
°C:	Celsius degree
%:	Percentage
c- ELISA:	Competitive Enzyme-Linked Immunosorbent Assay
M- ELISA:	Milk ELISA
IgM:	Immunoglobulin M
IgG:	Immunoglobulin G
OIE:	Office International Des Epizooties
PCR:	Polymerase Chain Reaction
Mm:	Milimolar
WHO:	World Health Organization
rRNA:	Ribosomal Ribonucleic Acid
OD:	Optical Density
OPS:	O-Polysaccharide

CFT:	Complement Fixation Test
SLPS:	Smooth Lipopolysaccharide
S19:	Srain 19 Vaccine
Rev-1:	Rivanol
Rpm:	Rotation Per Minutes
µl:	Microliter

1. INTRODUCTION

Brucellosis is a bacterial disease caused by a member of genus Brucella. It is an important zoonosis and a significant cause of reproductive losses in animal. Brucella is usually caused by *Brucella melitensis* or *B. ovis* in small ruminants, *B. abortus* in cattle, *B. suis* in pigs and *B. canis* in dogs. Abortion, placentits, epididymitis and orchitis are the most common consequences of the disease (OIE, 2007). It is still an uncontrolled serious public health problem in many developing countries (Mantur and Amarnath, 2008) and continues to be a major health threat among human and domestic animals. Human Brucellosis has always been associated with animal disease. The principal members of the genus for human are *B. melitensis*, *B. suis* and *B. abortus*. In addition, some species of Brucella could be used in a bioterrorist attack (Quinn *et al.*, 2002). Animal Brucellosis is a serious problem worldwide and endemic globally. In areas where the disease is endemic, human Brucellosis is quite common but often not diagnosed (Ratushna *et al*., 2006).

Economic losses from Brucellosis are very significant. These include decreased productivity because of abortion, stillbirth and decreased milk production, as well as losing trade opportunities. *B. melitensis* is very contagious for human and the disease, unless diagnosed and treated both promptly and effectively, will become chronic, affecting multiple body systems. The infection is acquired by human following ingestion of contaminated dairy foods and from occupational exposure to infected living animals or carcasses during slaughter. While sheep and goats are the major reservoir of *B. melitensis* infection, there is an increasing evidence of emergence in cattle and camels (Young, 1995). In Iraq, *B. melitensis* biovar 3 is considered to the predominant species of Brucella isolated from aborted fetus of small ruminant (Al- Naqshabandy, 2012). Outbreaks in animal due to *B. melitensis* have become worldwide emerging problem. Particularly, controlling the disease is difficult due to the lack of knowledge on the epidemiology of host species and of an effective vaccine (Montasser, *et al.,* 2011).

The disease is still common in the Mediterranean region, Middle East, Central Asia and parts of Latin America. Management systems for small ruminants vary markedly in these areas and food hygiene practices are deficient. There are major gaps in the knowledge of many livestock producers, and the practices are not adequately focused on preventive measures (FAO, 2009).

Over the last ten years, the infection has reemerged, with high prevalence in sheep and goats, in other countries in particular in Eastern Europe, the Balkans and Eurasia. Of the three biovars of *B. melitensis*, biovar 3 seems to be the most common in these regions (FAO, 2009).

The diagnosis of Brucellosis in small ruminants requires the use of more than one serological test (Baum *el al*., 1995). However, the isolation of *Brucella species* is considered the only unequivocal method for the confirmation. The *B. melitensis* infection is the most serious one in sheep and goats. Therefore, successful control measures applied to these species should be sufficient to control the infection globally (Alton *et al*., 1988).

In this study, we aimed to detect the *Brucella* spp. in small ruminants in order to establish an epidemiological base for controlling and preventing the disease. The comparison between serological (Rose Bengal) and i-ELISA tests were performed for detecting Brucella antibody in the serum and milk of sheep and goat from Duhok (Iraq) and Kahramanmaraş (Turkey). The efficacy of serological tests (sensitivity, specificity and overall agreement) was also performed.

2. LITERATURE REVIEW

2.1. Definition and Etiology

Brucella is gram-negative, small, cocco-bacillary or short rods sizing of 0.5-0.7 μm by 0.6-1.5 μm. The bacteria is aerobic, non-motile, non-terminating and non-toxigenic, place in single or groups, non-sporulating, and non-encapsulated (Grimont, *et al*., 1992).

The genus of Brucella is known a causative agent in different animals. The members include *B. abortus* in cattle, *B. melitensis* in small ruminants and Malta fever in human. *B. ovis* in sheep and *B. suis* in pigs can also be transmitted to humans. *B. canis* and *B. neotomae* also infect dogs and rats, respectively. *B. pinnipedialis*, *B. inopinata*, *B. celi* and *B. microti* have also been identified in marine mammals (Cloeckaert, 2001).

The different species cannot be distinguished from each other morphologically. However, differentiation of *B. abortus, B. melitensis*, and *B. suis* is based on quantitative differences in several physiologic tests. Such include CO_2 requirement, H_2S production and growth in the presence of basic fuschin and thionin. Besides, within each of these species of Brucella, a number of strains have been recognized based on these tests and additional biochemical properties (Grimont *et al*., 1992).

The species can be differentiated based on susceptibility to specific phages, their metabolism, ultra-structural differences in cell wall and envelope components. These are correlated with their antigenic characteristics. The biotypes are differentiated serologically by mono-specific antisera A and M (Cherwonogradsky *et al*., 1987).

There is no single test by which a species may be recognized with absolute certainty. A combination of growth characteristics, colonial and cellular morphology, staining properties, agglutinating by mono-specific antisera and biochemical reactions allow an accurate identification (Bishop*, et al.,* 1994).

Pasteurization, usual disinfectant chalk solutions, caustic soda, 2% formalin and 1% lysol destroy the *Brucella* species. The Brucella survives in soft cheese for up to 6 months, in butter for up to 4 months, in milk for up to 6 weeks, in cooled meat for 14 days, and in ice cream for up to 30 days (Dajani *et al*, 1989).

The environmental resistance of the pathogens depends on whether they are protected against the radiation of sunlight or high temperatures. Neutral soil pH and moist

environment, which are rich in organic material, are favorable elements for survival. The Brucella survive for months in humid feces, up to 4 months in aborted fetus, 44 days in the dust of streets, 30 days in tap water, 51 days in sterile water, for 2-5 weeks in the soil of paddocks, up to 2 months in desert soil, and up to 2 years in frozen soil. Contaminated straw remains infectious for longer than a month (Weidmann, 1991).

2.2. Epidemiology

Brucellosis occurs worldwide in domestic and game animals and it is one of the major drug neglected diseases (Nicoletti, 1980). They create a serious economic problem for the intensive and extensive animal production systems of the tropics. The disease has been eradicated in some industrial countries, especially in Europe, through intensive schemes of control and eradication. However, the occurrence is increasing in developing countries in an even aggravating epizootiological situation. This depends on the policy of many developing countries for importing high producing breeds without having the required veterinary infrastructure. In addition, the increasing international animal trade causes an uncontrolled animal movements and therefore spreads and transmits the infection (Nicoletti *et al.,*1980).

Brucellosis has been shown to occur in 94 out of 153 countries in the world. Most of the other countries do not know about it or do not report its presence that is known to exist in 40 out of 49 African countries (Thimm, 1982).

Iraq is in both northern and eastern hemispheres. It is positioned in the Middle East, a recognized geographical region of southwestern Asia. The country is boarded by the Arabian Gulf, Kuwait, Saudi Arabia, Jordan, Syria, Turkey, and Iran. A review of serological investigations of Brucellosis among the farm animals and humans in northern Iraq from 1974 to 2004 showed that there were several attempts to determine disease incidence. Shareef *et al*, (2006) investigated the prevalence of Brucella agglutinins in animals and human in the Qaradagh district of Iraq. The results showed the percentages of positive goat and sheep were 3.36 and 1.34%, respectively. In a study of occurrence and epidemiology of *Brucella* spp. in raw milk samples from Basra province showed that 24.2% of 420 samples were sero-positive by the milk ring test. The overall Brucella prevalence of milk produced in the Basra province was 14.7% by culture isolation (Abbas and Al-Dewan, 2009). It was demonstrated that the prevalence of Brucellosis was higher in

the semirural area (29.3%) than the rural and urban areas of the Basra region (Yacoub *et al.,* 2006).

In 1977, the incidence of Brucellosis in Makah (Saudi Arabia) was found to be 0.8% in goats, 0.5% in sheep, 2.8% in camels and 3.6% in cows. In the Asir region, the incidence of Brucellosis increased to 18.2% in goats, 12.3% in sheep, 22.6% in camels and 15.5% in cows in 1987. The overall human infection rate was 1.6-2.6%, including both genders and all ages (El-Eissa, 1999). In another study evaluating milk samples from 120 seropositive milking camels, *B. melitensis* biovars 1, 2, and 3 were isolated from 41 camels (34%) (Radwan *et al.,* 1995). In 1998, Brucellosis was ranked as the number one reportable communicable disease (22.5%) in Saudi Arabian National Guard communities. Human Brucellosis cases increased sharply (4.9 to 69.5/100,000) during the period of 1985-1990. The highest rate (79.6/100,000) was recorded in 1988 (Memish and Mah, 2001).

In the Kingdom of Jordan, the estimation of Brucella antibody seroprevalence in sheep was 14.3% by RBT, 7.2% by ELISA, and 2.2% using both tests in series. Moreover, the overall incidence of abortion was 20% and the specific incidence due to Brucellosis was 13% (Al-Talafhah *et al.,* 2003). In camels, the incidence of Brucella-specific abortion was investigated in 7 camel herds located in different locations of southern Jordan and the Brucella-seropositive prevalence was 12.1%. In addition, thirteen herds (35.1%) had at least one positive camel (Al-Majali *et al*., 2008).

Studies performed in different regions of Turkey showed that *B. melitensis* was responsible for approximately 14-31% of the abortion cases in sheep (Ilhan *et al.,* 2008). In a study during the period of 2004-2006, the positive results of analyzed cattle serum were 32.92 and 34.64% by RBPT and SAT, respectively. The positive serums from farmers were 13.0, 14.22 and 17.88% by RBPT, SAT and ELISA, respectively. There was no significant gender difference for Brucella seropositivity. Of 28 serums from veterinarians, 13 (46.42%) were positive by the three serological tests. Moreover, a significant feature in patients with epididymoorchitis was 9.1% seropositive for Brucellosis (Yetkin *et al.,* 2005; Otlu *et al.,* 2008).

In Kuwait, a single serum sample was collected from each of 1,836 patients of different nationalities from January 2000 to December 2001. Four-hundred fifty five serum samples (24.8%) having a titer of 1:160 were presumptively diagnosed as cases of Brucellosis. The peak was during April and May when *Brucella* spp. were isolated from 123 blood cultures (74.1%). The isolation rate from blood was significantly higher in patients with a titer of 1:1,280 than in those with 1:160 ($p < 0.05$). The study revealed that Kuwaiti and Bangladeshi nationals were most affected. Significant titers on the STA test were detected in 24.8% of the serum samples (Za *et al.,* 1999).

In Iran, a study was conducted in north Khuzestan from March 2004 to June 2004 with 3,594 people by randomized cluster sampling. Two hundred twenty-eight out of 3,594 were positive for Brucellosis (6.3%). The study showed that the prevalence of Brucellosis among nomads was high due to their life style (Alavi *et al.,* 2007). In Baft (Iran), 1,350 camels were surveyed for Brucellosis. Serological examinations including the Rose Bengal Plate Test (RBPT), MRT and 2ME were performed on 1,123 camel serum samples. The positive results were obtained in 118 (10.5%), 96 (8.54%) and 89 (7.92%) camels, respectively. In another study, 102 blood samples from companion dogs were divided into two age groups (1-5 and >5 years) for determining the seroprevalence of *B. canis*. The overall prevalence was 4.90% (5 of 102) with the prevalence of 9.3 (4 of 43) and 1.69% (1 of 59) in dogs >5 years and dogs <5 years (Mosallanejad *et al.,* 2009). By using the geographic information system (GIS) for explaining the spatial distribution of Brucellosis in an endemic district of Iran, the annual incidence of human Brucellosis was 141.6 per 100,000 inhabitants. The highest risky villages were in the north and south of that district (Haghdoost *et al*., 2007).

Surveys conducted from different countries showed that brucella cases were 1.0% and 2.0 % in herds from USA and UK (William, 1975). Similarly 9.5% of herds and 1.3% of cattle were infected in Canada in 1963. The infection rates were 12, 23.5, 13.9 to 18 and 9.4% for cattle herds in Chad, southern Ghana, southern Sudan and Senegal, respectively (Hellmann *et al.*, 1984). According to the survey conducted in Northern Nigeria, the incidence was 8.6% in private Fulani cattle and 3.4% in the government herds in 1970.

2.3. Pathogenicity and Pathogenesis

The common route of infection in animals is the oral route which occurs via licking aborted fetus, infected placentas, and vaginal discharges or by ingestion of contaminated feed and water (Tun, 2007). Fully virulent Brucella is highly invasive and capable of penetrating the mucosa or skin of the nose, throat, conjunctiva, urogenital tract, teat canal, and abraded skin (Davis *et al*., 1990). Virulent Brucella organisms can infect both nonphagocytic and phagocytic cells but the mechanism of invasion of nonphagocytic cells is not clearly established yet. While invasion of nonphagocytic cells, Brucella tend to localize in the rough endoplasmic reticulum (Zhan and Cheers, 1995). After penetrating mucosal barriers, organisms may be engulfed by phagocytic cells (Tun, 2007). Specific receptors on macrophages appear to mediate attachment and uptake of Brucella. Brucella employs various mechanisms for allowing the survival rate inside phagocytic cells. They are capable of surviving and multiplying inside macrophages by inhibiting phagolysosome fusion. Adenine and 5'- guanosine monophosphate in crude supernatants from Brucella suspensions have been shown to inhibit phagolysosome fusion in neutrophils. Intracellular survival in macrophages and, to a lesser extent, neutrophils is enhanced by suppressing the myeloperoxidase-H_2O_2- halide system. Superoxide dismutase and catalase production may play a role in defense against oxidative killing (Walker, 1999). Stress proteins have been demonstrated in Brucella and could be a factor in intracellular survival in the host. These proteins are thought to play a role in protecting organisms from hydrolytic enzymes, oxygen radicals, and myeloperoxidase killing systems in the phagolysosome. The lipopolysaccharide of Brucella is directly associated with virulence and is thought to play a role in enhancing intracellular survival. It is believed that the variations in virulence observed among the *Brucella species* may be related to the greater ability of some species to avoid host defenses (Tun, 2007).

Following entry into the host, Brucella organisms both live free in the extracellular environment or in phagocytic cells, and localize to regional lymph nodes. They proliferate and infect other cells or are killed and the infection is terminated. Some animals appear to be innately resistant to infection. This resistance is related to the macrophage's ability to destroy the organisms. From the regional lymph nodes, Brucella disseminates hematogenously and localize in the reticuloendothelial system and reproductive tract (Walker, 1999).

Brucella species have the ability to resist for killing by professional phagocytes, such as neutrophils and macrophages, and are allowed to multiply in the same cells for maintenance of chronic infections (Ko and Splitter, 2003). It is hypothesized that the Brucella is able to replicate intra-cellular by induction of virulence genes, resulting in persistence of the organism in the host and subsequent occurrence of the disease (Kohler *et al*., 2003).

There are two documented ways for Brucella to manipulate this intracellular niche. The first one is accomplished through a recently identified virulence factor, the type IV secretion system, encoded by the VirB operon. It has been demonstrated that Brucella invade macrophage killing through VirB-dependent sustained interactions with the endoplasmic reticulum, preventing fusion of Brucella-containing vacuoles with lysosomes, which is necessary for intracellular survival and multiplication. Brucella VirB mutants exhibit reduced survival in mice due to the inability of the organism to prevent this fusion and subsequently replication (Celli and Gorvel, 2004). The second one (O antigen) is also involved in the avoidance of the endosome pathway that normally kills pathogens. It was shown that smooth strains of *B. suis* contained within phagosomes do not fuse with lysosomes, whereas rough versions fuse rapidly (Porte *et al*., 2003). Rough organisms have also been shown to be taken up by the macrophages in greater numbers than smooth organisms. It becomes cytopathic for the macrophages, potentially recruiting immune factors and aiding the spread of organism to neighboring cells (Pei and Ficht, 2004). The defined role of the O antigen in Brucella pathogenesis is unclear. However, it does hold an important role in virulence of the organism. The escape mechanism of intracellular killing by polymorphonuclear is not well elucidated. However, it has been reported that Brucella through the lipid peroxidation and control of antioxidant systems in the host cell continue to survive. It is also possible that Brucellosis will be related to increase free radical production and antioxidant depletion while oxidative stress may be implicated in the pathogenesis of Brucellosis (Mehdi *et al*., 2012).

There is preferable localization in the reproductive tract of the pregnant animals. Unknown factors in the gravid uterus, collectively referred to as allantoic fluid factors, stimulate the growth of Brucella. Erythritol, a four- carbon alcohol, is considered to be one of these factors. Experimental infection studies have demonstrated that Brucella localizes into the cisternae of the rough endoplasmic reticulum of trophoblasts of the placentome. Infection subsequently spreads to the fetus. The exact mechanism of abortion is unclear.

However, possibilities of abortion include interference with fetal circulation, endotoxin effect and fetal stress (Walker, 1999).

2.4. Clinical Manifestation of Brucellosis in Human

The clinical manifestations of human and animal Brucellosis mimic the features of other febrile illnesses (Corsby *et al*., 1984). In human, the most common symptoms are fever, chills, diaphoresis, headaches, myalgia, fatigue, anorexia, joint and back pain, weight loss, constipation, and dry cough. Physical examination often reveals no abnormalities and patients can look deceptively well. However, some patients in contrast are acutely ill with pallor, lymphadenopathy, hepatosplenomegaly, arthritis, spinal tenderness, epididymoorchitis, skin rash, meningitis, cardiac murmurs or pneumonia (Young *et al.,* 1983 and Corsby *et al.,* 1984). The fever of Brucellosis has no distinctive pattern but may exhibit diurnal variation (undulant fever). In general, infections of bone and joints, heart (cardiovascular complications), respiratory tract, gastrointestinal tract, genitourinary tract, central nervous system complications (neuroBrucellosis) and other manifestations like keratitis, corneal vices, uveititis, retinal detachment and endopthalmitis are also observed (Young, 1983).

In human, the incubation period of Brucellosis may be as short as 3 days but is sometimes several months in duration (Corsby *et al*., 1984). More commonly, there is a period of approximately 3 weeks after known exposure to organisms before the onset of symptoms. Organisms can enter through abraded skin, where they gain access to the lymphatics and lymph nodes. There is often lymphadenopathy with subsequent blood stream invasion secondary to the bacterial multiplication and dissemination from the primary node. The subsequent localization of the organisms occurs particularly in the reticulo-endothelial system and intracellular organisms are protected from antibodies and antibiotics. Infected tissues may have granulomas, microabscesses, in rare cases caesation and the spleen is heavily infected, and the bone marrow frequently has detectable granulomas. Hepatic involvement is very common, and bacteria may be present despite normal liver function tests. The microscopic examination most often reveals granulomas, but diffuse hepatitis or microabscesses may be present. In general, infection with Brucella takes place through the mucosa or the injured skin and oral ingestion of contaminated food. The invasion of the pathogen via the upper part of the intestinal tract and infection through

the mucosa of the respiratory system or the eyes also occurs frequently (Bishop *et al*., 1994).

Chills, sweats, and anorexia are seen in approximately three fourths of the patients. Acute illness and more than one-half of the patients report generalized muscle aching, headache and backache. Mental depression and increased nervousness may accompany these nonspecific symptoms (Corsby *et al*., 1984; Madkour *et al.,* 2001). Septicemia, oscillating fever, sweats, pains in the bones, joints and muscles, fever at 39°C to 40°C, and tachycardia characterize acute brucellosis. Hepatosplenomegally and generalized adenopathy also occur with a group of nodes gathered around a single larger one. However, sub-acute stage of brucellosis is manifested with focalization, localized foci of infection that evolves autonomously but mainly osteoarticular (sternocostal, knee, tibia, spine, sacro-iliac). Meningeal and encephalitic foci also occur. At this stage, brucellosis can mimic osteitis, tuberculosis, or meningitis. In chronic cases, low-grade fever, fatigue, vague pains, and sometimes-infectious foci (arthritis) are reported in patients. Weakness is common in the vast majority of patients and is the most outstanding complaint. Fatigue results in the inability to perform normal activities.

The findings on physical examination are minimal in contrast to the several complaints. More than 90% of the patients have fever, but only 10 to 20% have palpable splenomegaly or lymphadenopathy and organomegaly is more common in children. The fever tends to be intermittent, with characteristic diurnal variation. Illness may begin either insidiously or with a rather abrupt onset. The majority of patients with infection due to *B. abortus* have a self-limited disease. *B. melitensis* is the most invasive species in human, and illness due to *B .melitensis* or *B .suis* may be more severe or chronic (Young, 1983). Complications from Brucella infections are usually attributable to the granulomas that occur in various organs and tissues. Debilitating neuropsychiatric disorders, infection of a bone or joint (including the vertebral column), and in unusual cases endocarditis due to Brucella that has been seen. Other viscera, such as spleen and liver, and bone marrow may have an evidence of infection for a significant period of time (Young, 1983). Mostly, in humans organisms are acquired through contact with infected materials. For instance, within the United States 90% of Brucellosis is now due to contact with infected materials rather than to ingestion of contaminated fresh milk and milk products. The occurrence of disease principally in men between the ages of 20 and 50 years reflects the occupational hazard to persons in the meat processing industry (WHO, 1971). There is no adequate

means of detecting infected meat. Prevention is possible only by elimination of the infection in cattle and swine. Movement of animals allows the organism to be reintroduced into uninfected herds. Other workers are at risk because of animal contact that include veterinarians, livestock producers, farmers, dairy workers and laboratory personnel working with the organism (Wise, 1980).

2.5. Clinical Signs in Animals

The incubation period usually implies from exposure to infection time, which the clinical or serological evidence indicates that infection has occurred. The length of the incubation period of small ruminant Brucellosis varies considerably and to some extent is affected by infective dose, age, sex, stage of gestation and immunity of the infected animal (Bishop *et al*., 1994).

In small ruminants that eventually abort, the usual length of the incubation period varies according to the time at which infection has occurred. The first sign of disease in a susceptible herd of cattle is abortion at late stages of gestation (Godfroid *et al*., 2004a).

In rams, an acute to chronic uni- or bilateral orchitis and epididymitis, with frequent sterility may occur (Nielsen and Duncan, 1990). About 20% of animals do not abort, while 80% of the aborts are due to brucella infection. The placenta is not consistently retained after abortion, but if it occurs, metritis is common. Early abortion may result in a considerable reduction in milk yield (Godfroid *et al*., 2004a).

The presence of the disease in a susceptible herd of goats or flock of sheep is usually an abortion storm, during which a high proportion of pregnant animals abort generally late in gestation. In some cases, subsequent retention of the placenta and fetal membranes can occur. The milk yield following abortion is poor and quality of the milk may be reduced (Godfroid *et al*., 2004b). The disease is also manifested by acute orchitis, epididymitis and subsequent reduction in fertility (Bishop, 1994).

2.6. Immunity

2.6.1 Cellular immunity

After natural infection in both human and animals, there is an initial IgM antibody response, followed by an IgG antibody response. The agglutination test measures antibody directed at Brucella LPS antigens. Macrophages from immune system of the animals efficiently destroy the intracellular organisms and undoubtedly contribute to eradication of this infection by the host (Madkour *et al.,* 2001). Macrophage activation occurs when T-lymphocytes of the appropriate subset are stimulated to release lymphokines (interleukins). This is dependent upon recognition of the appropriate antigen by the T-lymphocyte and subject to regulation through the major histocompatibility complex. Nevertheless, Brucella organisms are less sensitive to killing by activated macrophages and this relative resistance to Brucella may contribute to the chronicity of infection (Tizard, 1992).

2.6.2. Humoral Immune Response

Following infection by natural exposure, a serological response can be expected within 2 to 4 weeks, but the response is variable and may be absent altogether. Invasion of the pregnant uterus can be expected to produce a large and persistent rise of antibodies, but this may be delayed until abortion or parturition. Invasion of the lactating udder causes a lesser serological response, and localization confined to a small number of lymph nodes may fail to stimulate any response at all, or only a minimal one.

The pattern of the serological response in terms of immunoglobulin production has not been extensively studied in sheep and goats. However, available information suggests close similarity in cattle. The production of IgM followed within a week or two by a predominance of IgG. Both types fall into a low level in the more chronic stage of infection. However, the IgG predominates. The serological response is transient and sometimes missing in young and sexually immature animals. As mentioned thereafter, when *B. melitensis* Rev.1 vaccine is applied under standard conditions, it may induce a long lasting serological response to the agglutination test that seriously interferes with serological screening for infected animals (Alton 1967; Elberg, 1981). There were no differences found between the diagnostic antigens from field strains of *B. melitensis* and those from the Rev.1 vaccine. Serological tests are capable of distinguishing antibodies arising from infection and vaccination.

2.7. Diagnosis

Diagnostic tests for Brucellosis are sub-divided into three groups for the detection (Alton *et al.,* 1988; Saegerman *et al.,* 1999; OIE Manual 2004). There is no single available test that completely covers all requirements for diagnosing brucellosis. The ideal diagnostic test should detect infection early during the long and variable incubation period, not influenced by the presence of non-specific antibodies, detect carriers, and differentiate between responses to vaccination and to field infection (Alton *et al.,* 1988; Nielsen 2002).

Because of variable incubation periods and the permanent subclinical nature of the disease in most animals, a definitive diagnosis should be based on isolation and identification of Brucella (Alton *et al.,* 1988; OIE Manual 2004). The isolation and characterization of the disease-causing agent by bacteriological methods are still considered the "gold standard" for the diagnosis (Bricker, 2002). However, the method has also drawbacks. Due to the biological properties of brucella*,* the cultural methods are time-consuming and when the level of infection is low, they may fail (Fekete *et al.,* 1990; Gallien *et al.,* 1998). Thus, highly sensitive diagnostic tests for detection of Brucella based on the polymerase chain reaction (PCR) have been developed and reported (Bricker, 2002).

The choices include fetal membranes, lung, stomach content, liver and spleen of aborted fetuses, uterine discharge from cows, and milk or colostrum. Other specimens may include supramammary lymph nodes, retropharyngeal, mandibular, iliac, or prescapular and parotid lymph nodes, udder tissue, fluid aspirated from hygromas, male sex glands, testes and semen or seminal plasma and external inguinal lymph nodes of infected bulls (Alton *et al.,* 1988; OIE Manual 2004).

2.7.1 Direct microscopic examination

Using Stamp's modification of Ziehl-Neelsen stain, Brucella stains red against blue background in tissue sections and smears. However, other organisms such as *Coxiella burnetii*, *Chlamydophila abortus* and *Nocardia* spp. are also acid-fast.

2.7.2 Isolation

Brucella grows on serum dextrose agar, but growth from primary isolation is seldom before 48 hours of incubation at which stage colonies are usually 0.5 to 1.0 mm in diameter. Although the growth of Brucella may be markedly slower, uses of selective media, such as Farrel's medium may substantially enhance the chances of isolation by inhibiting the growth of contaminants. For this reason, the cultures should be incubated for five days or longer before being discarded as negative. Most wild strains are fastidious and growing slow, require supplementary carbon dioxide (5 to 10 percent) for primary isolation at an optimal growth temperature of 36 to 38°C (Alton *et al.,* 1988; Quinn *et al.,* 1999).

2.7.2.1 Colony morphology

After 3-5 days incubation on selective serum agar, pinpoint, smooth, glistening, bluish and translucent colonies appear. As they aged, the colonies become opaque and 2-3 mm in diameter. Smooth colonies in a clear growth medium, such as serum-dextrose agar, are convex, entire-edged, having a smooth shiny surface and pale yellowish-brown when viewed under transmitted light. Smooth forms are often markedly pathogenic whereas the rough variants are usually less pathogenic (Quinn *et al.,* 1999).

2.7.2.2 Biochemical tests

For routine identification, a combination of growth characteristics, colonial and cellular morphology, staining properties, agglutinating antiserum and biochemical reactions allow an accurate identification (Quinn *et al.,* 1999). Brucella is non-motile, catalase-positive, oxidase-positive, give a rapid urease activity (except some *B. melitensis* strains), reduce nitrate and indole-negative.

2.7.3 Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) is highly sensitive, very specific, rapid, and easily adapted to high volume demand diagnostic tool to detect slow growing bacteria including Brucella (Bricker, 2002). These methods can detect a few bacteria in a sample and are as sensitive as classical culture-based techniques.

Furthermore, it is possible to detect dead bacteria reducing the necessity of careful sample conservation before analysis (Ouahrani-Bettache *et al.,* 1996). As long as careful attention is given to avoid contamination in the laboratory, the method is very reliable and highly reproducible at any properly equipped laboratory (Bricker, 2002).

PCR is the amplification of a DNA sequence to high copy number. This amplification involves two oligonucleotide primers that hybridize to opposite strands of the target sequence and repeated heating cycles to denature the DNA and subsequent annealing of the primers to their complementary sequences. This is followed by extension of annealing primers with DNA polymerase. A heat-stable polymerase (Taq polymerase) derived from a bacterium adapted to hot springs, *Thermus aquaticus,* has been used to extend the primers. The successive cycling doubles the amount of DNA synthesized to amplification greater than 105, which permits the detection of a small number of bacteria including brucella (Fekete *et al.,* 1990).

Numerous PCR based assays for Brucella have been developed and published. The assays were designed to exploit a single unique genetic locus that was highly conserved in Brucella targeting *BCSP31* or the 16S rRNA genes (Herman and De Ridder, 1992; Bricker 2002). The advantage of these assays is that they tend to have a simple design and are very robust. They are useful for screening or for identification when species or biovar designations are not critical (Bricker, 2002). In order to differentiate *Brucella species* and/or biovars, several PCR assays were developed. These assays are directed toward to genetic loci that are variable among species and/or biovars. Such targets are uncommon in Brucella since the genus is remarkably homogeneous and has been proposed a single species. While some large deletions and rearrangements have been reported within a species or biovar. The genetic differences consist of single nucleotide polymorphisms (Bricker, 2002). Differential PCR-based assays are particularly useful for epidemiological trace back, or for species-specific eradication programs. The different PCR assays that have been developed can be categorized as genus-specific and species-specific. Strategically, for the species-specific tests, three approaches were reported; 1) assays designed with highly specific primers and stringent assay conditions; 2) assays designed with semi-specific primers and mildly permissive assay conditions; and 3) assays based on amplification with random primers under the very permissive conditions (Bricker, 2002).

2.7.4 Serological tests

Serological tests are widely used for detection of infected animals. However, no serological test is appropriate in every epidemiological situation. Therefore, consideration should be given to all factors that has impact on the relevance of the test method and test results for a specific diagnostic interpretation or application (Dohoo *et al.,* 1986). Most of the serological tests, particularly those using whole-cell suspensions as antigen, such as the serum agglutination test (SAT), Rose Bengal test (RBT), complement fixation test (CFT), most enzyme-linked immunosorbent assays (ELISA) and milk ring test (MRT) have been developed against the O-polysaccharide (OPS) moiety of the smooth lipopolysaccharide (SLPS) (Nielsen, 2002).

ELISAs (iELISA and cELISA) are the methods that involve the immobilization of the active components on a solid phase. Since the conventional serological tests and iELISA cannot distinguish vaccinal antibody produced by strain 19 vaccine, a competitive enzyme immunoassay (cELISA) was developed. The main rationale for this assay was that vaccines induce the antibody of lower affinity due to the shorter exposure to antigen. Thus a competing antibody could be selected to inhibit binding of vaccinal but not field strain induced antibody. Because of their inherent supply and uniformity advantages, monoclonal antibodies were selected as competitive antibodies that should be specific for a common epitope of the OPS molecule. This allows its use for *B. abortus*, *B. melitensis* and *B. suis* (Nielsen, 2002) Serological tests may show cross-reactions with other Gram-negative organisms, such as *Salmonella* group N, *Eschericia coli* O:157, *E. coli* O:116, and *Pseudomonas maltophilia*. However, the most notable cross-reaction is between smooth lipopolysacharide (SLPS) found in Brucella and *Yersinia enterocolitica* O:9 making diagnosis difficult due to the sharing of antigenic determinants in the O-polysaccharide (OPS) molecule (Corbel *et al.,* 1983; Muñoz *et al.,* 2005; Nielsen *et al.,* 2006).

2.7.4.1 Rose Bengal test (RBT)

RBT is a spot agglutination test used to screen herds. The test is highly sensitive for individual diagnosis, especially in cattle vaccinated with strain 19 (Alton *et al.,* 1988; Nielsen, 2002; OIE Manual 2004) and can be performed in the field. The antigen stained with Rose Bengal stain is buffered at a pH of 3.65 (Alton *et al.,* 1988). At this level of activity, non-specific agglutinins are destroyed and IgG agglutinates strongly (Alton *et al.,*

1988; OIE Manual 2004). This test is prescribed by the OIE for international trade in cattle.

2.7.4.2 Complement fixation test (CFT)

The complement fixation test is very specific sensitive and is regarded throughout the world as being the confirmatory test of choice for serological detection of infected animals. According to Radostits *et al.* (1994a, b), the CFT rarely exhibits non-specific reaction and is useful in differentiating the titers of calf-hood vaccination from those due to infection. The reactions to the CFT recede sooner than those to the serum agglutination test after calf-hood vaccination with the strain 19 vaccine. The test has been modified, standardized and adapted to microtitre systems. Results are expressed in International Units (IU) and a cut-off point of 20 IU has been defined, which is rigorously applied where strain 19 vaccine has not been used for several years (Alton *et al.,* 1988). The CFT has been a valuable asset in control and eradication programs as a confirmatory test. The OIE Manual recommends it as a test prescribed for international trade.

2.7.4.3 Indirect enzyme-linked immunosorbent assay (i-ELISA)

Indirect ELISAs are the tests in which antigen is bound to a solid phase. If antibody is present in the sample, it binds to the immobilized antigen and may be detected by an appropriate anti-globulin-enzyme conjugate. It gives a colored reaction with a chromogenic substrate indicating the presence of antibody in the sample (OIE Manual 2004). The iELISA is a highly sensitive test but is not capable of differentiating between antibody resulting from S19 vaccination or other false-positive reactions induced by pathogenic Brucella strains. Therefore, it should be considered more as a screening test than a confirmatory test in the testing of vaccinated herds affected by false-positive results (Nielsen, 2002; OIE Manual, 2004). This test is prescribed by the OIE in cattle.

2.7.4.4 Milk ring test (MRT)

The MRT is used to detect antibodies in milk. The development of a positive reaction is dependent on two reactions: 1) aggregation of fat globules in the milk and 2) stained Brucella cells (antigen) which are added to the milk, and are agglutinated by the Brucella antibody-fat globule complexes which rise to form a colored cream layer at the top. This is a screening test used on bulk milk to detect infected animals on a herd basis or to monitor clean herds. Herds of which the MRT is positive should be examined by serological tests to identify the infected animals (Alton *et al.,* 1988). It is a relatively

insensitive test subject to wrong interpretation caused by various conditions, such as mastitis, colostrum and milk at the end of the lactating cycle. It is recommended by the OIE as a screening test for small ruminant Brucellosis.

2.7.5 Test to demonstrate an allergic reaction to Brucella

The Brucella skin test is an alternative immunological test which can be used to screen unvaccinated herds. A purified, free and smooth LPS antigen is used in the test. This test has a very high specificity, such that serologically negative animals that are positive to the brucellin test should be regarded as infected animals (Bercovich and Muskens 1999; Saegerman *et al.,* 1999; Godfroid *et al.,* 2004a). Results of this test may also aid the interpretation of serological reactions due to infection with cross-reacting bacteria, especially in Brucellosis-free areas (Godfroid *et al.,* 2002). Although the brucellin intra-dermal test is one of the most specific tests for brucellosis, diagnosis should not be made solely on the basis of positive intra-dermal reactions given by animals in the herds. However, it should be supported by a reliable serological test. The allergic test should be used as a flock or herd test rather than as a test for individual animals (Alton *et al.,* 1988).

2.8. Brucella vaccines

Both killed and live attenuated vaccines have been examined for their potential role in the control and eradication of Brucellosis in cattle, sheep, goats, and swine. Live attenuated vaccines carry several advantages above their killed counterparts. First, immunity derived from their use tends to be cell-mediated and long lasting. As they are administered live, the organism is allowed to replicate within the host, thus making them less expensive. However, some live and attenuated vaccines may cause abortion in pregnant females and therefore their use is often relegated to males and non-gravid females.

2.8.1. Brucella vaccines for cattle

B. abortus vaccines have been used as live and attenuated (Cassidy, 2010). *B. abortus* strain 19 is attenuated and unable to grow in the presence of erythritol (Schurig *et al*., 2002). However, the presence of the O-side chain caused vaccinated animals to become seropositive thus confounding sero-diagnosis since vaccinated animals could not be differentiated from field-infected animals. The production of antibody against the O-side chain due to vaccination with strain 19 makes this strategy unfavorable for using as a vaccine considering the current test and slaughter practices used to control Brucellosis in cattle (Cassidy, 2010).

B. abortus strain 45/20 was utilized in the first attempt to use a rough, live, attenuated vaccine to protect against bovine Brucellosis (Schurig *et al*., 2002). Attenuation was achieved through serial passage of the *B. abortus* 45/20 field isolate in a guinea pig 20 times. The resulting strain 45/20 was able to protect guinea pig and cattle from Brucellosis. However, the strain would revert to a smooth phenotype *in vivo*, and offset the purpose of developing rough strain.

The *B. abortus* strain RB51 was developed at Virginia Polytechnic Institute and State University during the 1980's and conditionally approved by the United States Department of Agriculture for using in cattle in 1996 (Cassidy, 2010). Multiple passages of *B. abortus* strain 2308 on rifampicin containing medium created the rifampicin resistant, rough strain. Strain RB51 produces little to no O-side chain in its LPS. The vaccine is a living, attenuated vaccine and has never been licensed for human use (Schurig *et al*., 2002). Other rough Brucella vaccine strains have been created (*B. melitensis* VTRM1 and *B. suis* VTRS1) by deleting a large segment of the wboA gene encoding a glycosyltransferase involved in O-side chain biosynthesis in the parent of each strain. Strains VTRM1 and VTRS1 did protect against challenge but did not have the same degree of attenuation as strain RB51. This indicates that wboA encodes a potential virulence factor, but other genes are also affected in strain RB51 (Cassidy, 2010).

2.8.2. Brucella vaccines for sheep and goats

The Rev-1 vaccine is a non-dependent inverse mutant of a streptomycin-dependent strain of *B. melitensis*. Even though it is used as an attenuated vaccine, it retains a residual pathogenicity that may cause abortion if inoculated into pregnant ewes, as well as excretion of the organisms in milk if inoculated during lactation. Apart from these inconveniences, Rev-1 has advantages, as it is stable, is not transmissible to other sheep and provides long immunity. Vaccinated animals produce antibodies that can be demonstrated by serological tests. Complement-fixing antibodies disappear 6–8 weeks after vaccination, in most cases. For this reason, Rev-1 is employed only in young animals before they reach their reproductive age. However, the vaccine is also administered to adult ewes, in which reduce the likelihood of abortions and excretion in the milk. Reduced doses of the vaccine and subconjunctival administration have also been used (Castrucci, 2007). Considering the fact, subcutaneous administration confers a longer persistence of antibodies and the obvious difficulty in performing two separate vaccinations. It has been suggested that the animals should be vaccinated only once, intraconjunctivally, with a 10^9 dose of vaccine. Following the Rev-1 vaccination, bacteria are disseminated widely followed by their localization in the prescapular lymph nodes on the side of inoculation. In most cases, the organism disappears after 3 months. Rev-1 induces a very efficient immunity, lasting for more than 2.5 years. A deletion mutant of strain Rev-1 was recently obtained. According to the results of tests conducted on mice, it would allow serological differentiation between infected and vaccinated sheep (Cloeckaert *et al*., 2004). The deleted gene encodes the periplasmic protein BP26, the immunodominant antigen in the serological response of *B. melitensis* in sheep. The authors suggested that the use of this Rev-1 bp26 deletion mutant as a vaccine is valuable for the eradication of *B. melitensis* infections in sheep. Several other prospective candidate vaccines are also under investigation (Blasco, 2006).

The H-38 vaccine is produced by a virulent strain of *B. melitensis* biotype 1, inactivated with formaldehyde and suspended in adjuvant oil (Arlacel A). One dose containing 3×10^{11} bacteria induces good protection that lasts for 15 months. As it is an inactivated vaccine, it may be used in pregnant and lactating animals. Unfortunately, two disadvantages have been reported: 1) the antibody response develops more slowly compared with Rev-1, and 2) H-38 frequently causes a local reaction at the inoculation

site, which can be severe. Moreover, the vaccine may change from one batch to the other (Castrucci, 2007).

2.9. Treatment, Prevention and Control

Brucellosis is one of the drug-neglected diseases and the treatment in domestic animals is not indicated. However, human are usually treated with doxicycline and rifampicine antibiotics. Single agent therapy for Brucellosis has now been abandoned because of the high rates of failure and relapse and the potential development of antibiotic resistance. Relatively short treatment periods (less than 8 weeks) with antibiotic combinations have been associated with high rates of relapse (Luzzi *et al*., 1993). The combination of doxycycline and an aminoglycoside (gentamicin, streptomycin, or netlimicin) for 4 weeks followed by the combination of doxycycline and rifampin for 4 to 8 weeks is the most effective regimen. The doxycycline /aminoglycoside combination is more effective than the doxycycline/rifampin combination in that rifampin reduces levels of doxcycline in plasma (Corbel, 1997).

Within 4 to 14 days after the initiation of therapy, patients become a febrile and constitutional symptoms disappear. The enlarged liver and spleen return to their normal size within 2 to 4 weeks. An acute, intense flare-up of symptoms may follow the start of treatment, especially with that of tetracyclines. This reaction is transient and does not necessitate the discontinuation of therapy. The strategies for preventing Brucellosis have to be modified to different animal production systems. Failures of disease control are mostly due to the absence of efficient scheme for which the veterinary infrastructure exists, the low socioeconomic status of the animal holder, and the lack of reliable serological laboratories adopted to develop good control and eradication programs (Saravi *et al.,* 1995). There are two alternatives:

1. Test and slaughter (t+s) i.e., recognition of all animals which have responded to a Brucella infection and subsequent culling of the reactors. Part of the scheme has to be a careful control of all animals, which will be newly added to the herds as well as a production system, which prevents contact with infected neighboring farms and/or contaminated feed or pastures (Radostitis, *et al*., 1994).

2. Vaccination of exposed herds with inactivated or live vaccines. Workers in the meat and dairy industries in the former Soviet Union, China, and France have been vaccinated. The vaccine (two injections given 2 weeks apart, each containing 1 mg of an

insoluble fraction of phenol-extracted bacteria) markedly reduces the rate of infection. However, the vaccine induces fever in 6% of recipients and severe pain at the injection site by 16%. Moreover, immunity is short-lived, and vaccination should be repeated every 2 years. This vaccine is not used in the United States (Hellmann, *et al.,* 1984).

Prevention of human Brucellosis is primarily dependent on control of animal sources of infection. Modifications in processing of milk and dairy milk products, as well as animal surveillance and animal immunization, have greatly reduced the dangers of this disease within the United States. The population at risk consists of persons in contact with animals or their contaminated products. Available vaccines are suitable only for animals. The disease remains one of economic importance in many countries of the world, where the control of infected animals has not been readily accomplished (Alton *et al.,* 1967).

3- MATERIALS AND METHODS

3.1 Study Area

The present study was conducted at the laboratory of Veterinary Medicine, Veterinary Director in Duhok–Iraq. The samples were collected from Duhok and Kahramanmaraş.

3.2 Sample Collection and Handling

One-hundred eighty serum and milk samples were collected from different flocks of sheep and goat. Samples were equally representative from Duhok (90) and Kahramanmaraş (90) during the lambing and kidding season from March to December 2013. The specimens, blood and milk were packed in polyethylene cool box with ice packs and kept cool during transportation to the laboratory (OIE, 2000). The sampling list is illustrated in Table3.1

Table 3.1: The samples collected from Sheep and Goat

Animals	No of serum samples		No of milk samples	
	Duhok	Kahramanmaraş	Duhok	Kahramanmaraş
Sheep	70	70	70	70
Goat	20	20	20	20
Total	90	90	90	90

3.2.1 Blood samples

Five ml of blood was collected aseptically from the Jagular vein of dams in sterile plain vacuum tubes without anticoagulant (vacutainer). The tubes were placed vertically at room temperature for 1 h and then centrifuged at 3000 rpm for 5 min. After the serum was separated, it was put into a screw capped plastic vials and stored at -20°C for serological tests (Alton *et al*., 1988).

3.2.2 Milk samples

The udder was thoroughly washed and cleaned with 70% ethyl alcohol and dried with clean cloth. After discarding few drops of milk, approximately 5 ml of milk from each quarter was collected in sterile screw capped plastic vilas and transported to the laboratory. Milk samples were stored at -20°C for ELISA (Alton *et al*. 1988).

3.3 Methods Used for Detection of Brucella Antibody and *Brucella Spp.*

3.3.1 Rose Bengal Plate test (RBPT)

The Rose Bengal antigen kit was obtained from the Salucea Ltd (Denmark). According to the kit's manufacturer, 25 µl of serum and antigen were added to the wells. The antigen and serum were mixed thoroughly with the speeder and the plate was rotated for 4 minutes. Any degree of agglutination was recorded as positive, from a clear rimming to full agglutination with clear supernatant fluid.

3.3.2 Indirect enzyme-linked immunoabsorbent assay (i-ELISA)

The i-ELISA antigen kit was obtained from the Ingenasa Ltd (Spain). According to the kit's manufacturer, all reagent and serum were brought to room temperature (18-25°C) before starting the test. The serum samples were diluted one- hundred fold (1:100) with sample diluents in tubes in order to standardize incubation times, and then transferred to the antigen coated plates. Positive and negative controls (100 µl) were dispensed respectively into wells A1 and A2, and wells A3 and A4. Diluted serum (100 µl) was dispensed into the remaining wells and incubated at 20-25°C for 60 min. Liquid contents of all wells were discarded in a waste reservoir. Then plates were washed 3 times with 300-µl wash solution using ELISA washer and discarded the liquid content after each wash. Plates were firmly tapped onto absorbent material for removing residual fluid. One-hundred µl of conjugate was added into each well and plates were incubated at 25°C for 30 min. The washing process was repeated 5 times and followed by 100 µl substrate addition into each well. The incubation was then performed at 20-25°C for 5 min. The stop solution (sulfuric acid) was added (100 µl) to each well and the optical density (OD) was measured at 450 nm wavelength using an ELISA reader (Bioteck ELISA).

3.3.3 Indirect ELISA for detection of antibody in milk

The i-ELISA antigen kit was obtained from the ID Screen®, Ltd (France). Briefly, all reagent and milk were brought to room temperature (18-25°C) before starting the test. Milk samples were diluted one- hundred fold (1:100) with sample diluents for standardizing the incubation times, and then transferred into the antigen-coated plates. Positive and negative controls (50 µl) were dispensed respectively into wells A1 and A2, and wells A3 and A4. Diluted milk (50 µl) was dispensed into the remaining wells of plates were incubated at 21°C for 45 min. Liquid contents of all wells were discarded. The plates were then washed 3 times with 300 µl solution by ELISA washer and firmly tapped onto absorbent material. One-hundred µl of conjugate was dispensed into each well and plates were incubated at 21°C for 30 min. The washing process was repeated 3 times and followed by 100 µl of substrate addition into each well. The plates were incubated at 21°C for 15 min. Then the stop solution (100 µl) was added into each well to stop the reaction. The absorbance was measured at 450 nm using a spectrophotometer.

3.3.4 Statistical analysis

The sensitivity, specificity and overall agreement between the various tests were performed with the statistical formula described by Bowers, (2008).

Table 3.2. Gold standard test

Gold standard test				
		Positive	Negative	Total
The test to be compared	Positive	a	b	a+b
	Negative	c	d	c+d
	Total	a+c	b+d	a+b+c+d=N

The notations used above are defined as follows;

a = Number of samples positive to both conventional and the gold standard tests

b = Number of samples positive to conventional but negative to the gold standard test

c = Number of samples negative to conventional but positive to the gold standard test

d = Number of samples negative to both conventional and the gold standard tests

a + b + c + d = Total number of samples (N)

Definitions and formula of the indices used for comparing the different assays are described as follows;

Sensitivity: It is the capacity to test disease-detected animals compared with gold standard test (a/a+c x 100).

Specificity: It is the capacity to test non-disease detected animals compared with gold standard test (d/b+d x 100).

Overall agreement: It is the proportional similarity of results for both tests (a+d/N x 100).

4. RESULTS AND DISCUSSION

4.1 Serological Results

4.1.1 Rose Bengal plate test (RBPT)

Out of 180 serum samples from sheep (140) and goat (40), 17 samples were found to be positive (9.4%).

One-hundred forty serum samples were tested by RBPT in sheep. Among those, 13 (9.2%) samples were found to be positive from Duhok (9; 12.85%) and Kahramanmaraş (4; 5.71%).

Forty serum samples were tested by RBPT in goat. There were 4 (10%) samples being positive from Duhok (3; 15%) and Kahramanmaraş (1; 5 %). The results are presented in Table 4.1.

Table 4.1 Serodetection of *Brucella* spp. in sheep and goats by RBPT

Animals	No. Serum samples		Serum RBPT		Total
	Duhok	Kahramanmaraş	Duhok	Kahramanmaraş	
			Positive (%)	Positive (%)	
Sheep	70	70	9 (12.85%)	4 (5.71%)	9.2 %
Goat	20	20	3 (15%)	1 (5%)	10 %
Total	90	90	12 (13.33%)	5 (5.55%)	9.4 %

4.1.2 Serum ELISA

Among 180 serum samples from sheep (140) and goat (40), 30 samples were found to be positive (16.66%).

One-hundred forty serum samples were tested by i-ELISA in sheep. Among those, 25 (17.8%) samples were found be positive from Duhok (19; 27.14%) and Kahramanmaraş (6; 8.57%).

Forty serum samples were tested with i-ELISA in goats. There were 5 (12.5%) positive samples from Duhok (3; 15%) and Kahramanmaraş (2; 10%). The results are presented in Table 4.2.

Table 4.2. Serodetection of Brucella antibody in sheep and goats serum by i-ELISA

Animals	Number of serum samples		Serum ELISA		Total
	Duhok	Kahramanmaraş	Duhok	Kahramanmaraş	
			Positive (%)	Positive (%)	
Sheep	70	70	19 (27.14%)	6 (8.57%)	17.8%
Goat	20	20	3 (15 %)	2 (10%)	12.5%
Total	90	90	22 (24.44%)	8 (8.88)	16.6%

4.1.3 Milk ELISA

Among 180 milk samples from sheep (140) and goat (40), there were 29 samples found to be positive (16.1%).

One-hundred forty milk samples were tested by milk-ELISA in sheep. There were 24 (17.1%) positive samples from Duhok (18; 25.71%) and Kahramanmaraş (6; 8.57%).

Forty milk samples were tested with milk-ELISA in goat. There were 5 (12.5%) positive samples from Duhok (3; 15%) and Kahramanmaraş (2; 10 %). Brucella antibody results in milk are presented in Table 4.3.

Table 4.3. Serodetection of Brucella antibody in milk of sheep and goats by i-ELISA

Animals	Number of Milk samples		Milk ELISA		Total
	Duhok	Kahramanmaraş	Duhok	Kahramanmaraş	
			Positive (%)	Positive (%)	
Sheep	70	70	18 (25.71%)	6 (8.57%)	17.1%
Goat	20	20	3 (15%)	2 (10%)	12.5%
Total	90	90	21 (23.33%)	8 (8.88%)	16.1%

The relative sensitivity and specificity of RBPT were compared, with the results of serum ELISA considering the serum ELISA as a gold standard test. The results are presented in Table 4.4.

Table 4.4. Sensitivity and specificity of RBPT compared to serological serum ELISA for detection of Brucella antibody

Tests		Serum ELISA		Total	Sensitivity	Specificity	Overall Agreement
		Positive	Negative				
RBPT	Positive	14	3	17	46.66%	98%	89.44%
	Negative	16	147	163			
	Total	30	150	180			

Fourteen samples were positive in both RBPT and serum ELISA. There were 16 samples found to be negative in RBPT but positive in serum ELISA. There were 3 positive samples in RBPT but negative in serum ELISA. In addition, there were 147 positive samples in both RBPT and serum ELISA.

The relative sensitivity and specificity of milk ELISA were compared, with the results of serum ELISA considering the serum ELISA as a gold standard test. The results are presented in Table 4.5.

Table 4.5. Sensitivity and specificity of m-ELISA compared to serological serum ELISA for detection of Brucella antibody

Tests		Serum ELISA		Total	Sensitivity	Specifity	Overall Agreement
		Positive	Negative				
Milk ELISA	Positive	28	1	29	93.33%	99.33%	98.33%
	Negative	2	149	151			
	Total	30	150	180			

In the present study, there were 28 positive samples in both m-ELISA and serum ELISA, while two samples were negative in m-ELISA but positive in serum ELISA. There was one positive sample in m-ELISA but negative in serum ELISA, while there were 149 positive samples in both m-ELISA and serum ELISA.

The higher positivity by milk ELISA than RBPT in the present study might be due to false positives in milk. This could be explained by recent parturition, end of lactation and sub-clinic mastitis (Alton *et al.,* 1988).

The sensitivity of RBPT and milk-ELISA were 46.66 and 93.33%, respectively. Considering with serum ELISA as a gold standard test, specificity was 98 and 99.33%, respectively. The overall agreements for RBPT and milk-ELISA with the serum ELISA were 89.44 and 98.33% . Thus, milk-ELISA was more sensitive than RBPT.

Gupta *et al.* (2012) found sensitivity of milk-ELISA and RBPT as 95.45 and 68.18%, respectively. The specificities of milk-ELISA and RBPT were 95.5 and 81.81%, respectively.

Biancifiori *et al.* (1996) found that the specificity of milk-ELISA was 65% compared to RBPT. Whereas it was 100% when applied to Brucella free herds. They found that milk ELISA was less sensitive than the tests detected antibodies in serum. They also concluded that the concentrations of immunoglobulins in colostrum and milk were tended to decrease sharply soon after parturition, while they remained constantly high in serum. They also concluded that milk-ELISA for Brucella antibodies in ewe milk could be regarded as a complementary diagnostic tool for individual testing. However, it would be unsuitable using as a screening test applied to pooled flock milks. Thus, variable results were obtained by different tests for detecting the antibodies in serum and milk in our study. However, we detected more samples that are positive using serum ELISA.

4.2 Comparative efficacy of the serological tests

In the present study, the serodetection of ovine samples was assessed by RBPT and i-ELISA. The overall RBPT seropositivity was in 17 samples (9.4%) with 12 samples (9.2%) in sheep and 5 samples (10%) in goat. A lower seroprevalence was found in sheep (1.1%) and goat (4.4%) from northern Iraq and Mosul province using RBPT (Mathur *et al*., 1974). Whereas, Karim *et al*. (1979) reported much lower seroprevalence of infection rate in sheep and goat (0.93 and 2.55%). Montasser et al. (2011) in Egypt also observed the lower seroprevalence (5.2%) in sheep.

The RBPT test results in our study were similar to Arslan *et al.* (2011 a, b). They found 8.77 and 6.8% infection rates in sheep and goat, respectively. Mansur (2000) and Al-Abdali (2005) found that the Brucella sinfection rates were 7.1 and 6.64% in sheep, respectively. Al-Izzi *et al.* (1985) also found the disease prevalence 7.9% in Baghdad.

While the results in our study were lower than the results of Al-Naqshabandy (2012) having 21.27 and 17.14% in sheep and goat, Saleem *et al.,* (2004) found much lower infection rate in sheep (13.3%).

Although brucellosis prevalence in small ruminants by i-ELISA in our study was positive, Isabel (2013) in Tajikistan found higher value for sheep (28.73%) and goats (13.57%).

The result of i-ELISA test showed that the incidence is similar to Teshale *et al.,* (2006) in Ethiopia who observed the incidences 14.6% in sheep and 16.45% in goats. Rahman *et al.,* (2011) observed lower incidences in sheep (1.25%) and goats (2.50%) in Bangladesh.

The serodetection of Brucellosis from other studies in Turkey revealed that the prevalence of Brucellosis was 37.1% in sheep (Otlu *et al.,* 2007). Karaman *et al.* (2000) also showed that 15.6% of the aborted sheep were positive for Brucellosis. Güler *et al.* (1998) reported that 17.6% of the Brucellosis in aborted sheep rises in the same region between 1987 and 1998. Two studies carried out in Eastern Turkey showed that the seroprevalence of Brucellosis were 11.8% in Elazığ and surrounding areas (Muz *et al.,* 1999) and 23.1% in Van (Gürtürk *et al.,* 2000).

The brucella seroprevalence in another study from Trakya region of Turkey was 16.1% (Erdoğan *et al.,* 1993). The prevalence of Brucellosis was higher especially in the Northeastern Turkey, including Kars, than in other parts of Turkey (Şahin *et al.,* 2004). In another serological study carried out in Kars, 17.95% of the aborted sheep were positive for ELISA (Baz *et al.,* 2000).

In the present study, the was assessed by i-ELISA, a total of 90 sera comprising of 70 sheep and 20 goat were processed for antibody detection in Duhok found 22 (27.14%) positive in sheep and 3 (12.5%) in goat. The higher brucellosis serodetection rates were also observed in another study carried out in Duhok where 36.17 and 31.42% of the positive samples were from aborted sheep and goats detected by ELISA (Al-Naqshabandy, 2012).

The differences between the results of previous and current studies are expected. High incidences in the previous studies conducted long time ago could have been due to many factors, such as lack of integrated programs to control the disease, the absence of health strategies to get rid of aborted fetuses, poor nutrition and the methods of

management (Kabagambe *et al.,* 2001). In addition, role of wild animals may act as a reservoir and spread the disease (Godfroid, 2002).

The differences between the results may be due to number of samples taken from different geographical regions as well as the samples taken from aborted animals compared to samples taken randomly.

Although the highest seroprevalence of Brucella antibody was detected in sheep milk (17.1%) in our study, Ibrahim *et al.* (2012) in Egypt observed higher Brucella antibody in milk-ELISA of sheep (46.6%) and goats (35.3%). In addition, Kaltungo *et al.* (2013) observed higher Brucella antibody in milk-ELISA of sheep (18.1%) and goats (25.2%). The review of milk-ELISA results showed an agreement that with Chand *et al.* (2005) who observed the incidence of 14.63% in sheep.

The results of our study may differ slightly. There may be many reasons including number of sample, geographic region, age and sex of the animal etc. (Charanjeet *et al.,* 2004).

The RBPT results should be confirmed by other highly efficient tests (Al-Dahouk *et al.*, 2003). The serological tests could give false positive results (1-3%), as well as they do not distinguish between vaccinated and infected animals (Radostits *et al*., 2007).

5. CONCLUSION

The present study was conducted to detect Brucella antibody in serum and milk with ELISA and RBPT for specifying *Brucella spp*. One-hundred eighty samples from sheep and goats were screened for presence of Brucella antibody. Within these, 30 serum samples were found positive for Brucella antibody by i-ELISA yielding an overall seropositivity of 16.6% with higher rate in sheep (17.8%) than goats (12.5%). Comparing two serological tests for antibody detection in serum, the highest positive results were obtained by i-ELISA (16.6%) followed by RBPT (9.4%). Considering the ELISA as a gold standard test, the sensitivity and specificity of RBPT were found to be 46.6 and 98.6% respectively. Between two locations, detection of Brucella infection in Duhok resulted higher (24.44%) positivity by ELISA than Kahramanmaraş (8.88%). Comparing serum and milk ELISA antibodies, the ELISA detected antibodies had an equal number between samples. The sensitivity and specificity of milk-ELISA were found to be 93.1 and 99.3%, respectively.

For controlling brucellosis in farm animals, all infected ones must be slaughtered separately in abattoirs with much care to discontinue the existence of the pathogen. Routine screening for brucellosis is very important for detecting positive cases as early as possible so that reduce the risk of contamination to other animals within the herd and take proper measures on time. It is also important to develop a comprehensive plan and cooperating with health and veterinary centers for eradication of the disease in farm animals. Compulsory strain Rev-1 vaccination between day 1 to 6 months of age needs to be practiced in animals under intensive and semi intensive management systems.

REFERENCES

Abbas, B. A., Aldeewen, A. B., 2009. Occurrence and Epidemiology of *Brucella* spp. in raw milk samples at Basrah province, Iraq. *Bulgarian Journal of Veterinary Medicine*, 12: 136-142.

Al-Abdali, E.B., 2005. Brucellosis infection and some biochemical tests in Ninevah Province, Ph.D. thesis in veterinary international and Preventive Medicine. College of Veterinary Medicine, Mosul University.

Alavi, S. M., Rafiei, A., Nikkhooi. A., 2007. The effect of lifestyle on Brucellosis among nomads in Khuzestan province, Iran. *Pakitan Journal of Medical Sciences*, 23: 358-360.

Al-Dahouk, S., Tomaso, H., Nockler, K., Neubauer, H., Frangoulidis, D., 2003. Laboratory based diagnosis of Brucellosis: overview of the literature, Part II: Serological tests for Brucellosis. *Clinical Laboratory*, 49: 577-589.

Al-Eissa, Y. A., 1999. Brucellosis in Saudi Arabia: past, present, and future. *Annals of Saudi Medicine*, 19: 403-405.

Al-lzzi, S.A., Al-Bassam, L.S., Al-Delaimi, A.K., 1985. A study on ovine Brucellosis in Baghdad. *Iraqi Journal of Veterinary Medicine*, 9:19-27.

Al-Majali, A. M., Al-Qudah, K. M., Al-Tarazi, Y. H., Al-Rawaashdeh, O. F., 2008. Risk factors associated with camel Brucellosis in Jordan. *Tropical Animal Health and Production*, 40: 193-200.

Al-Naqshabandy, A. A., 2012. The diagnosis of Brucellosis in aborted small ruminant using conventional methods and PCR technique, Ph. D. thesis in Veterinary clinical pathology, Faculty of Veterinary Medicine. Duhok University.

Al-Talafhah, A. H., Lafi, S. Q., Al-Tarazi, Y., 2003. Epidemiology of Bovine Brucellosis in Awassi sheep in Northern Jordan. *Preventive Veterinary Medicine*, 60: 297-306.

Alton, G.G., Jones, L.M., Buchanan, R.E., Gibbons, N.E., 1967. Laboratory techniques in Brucellosis. *World Health Organization Monogram Series*, Geneva. 55.

Alton, G. G., Jones, L. M., Angus, R. D., Verger, J. M., 1988. Techniques for the Brucellosis laboratory. *Institute National Dela Recherche Agronomique*, 81-134.

Arslan, S.H., Hassan, M.M., Mohammad, B.A., Al-Hussary, N.A., Al-Obaidi, Q.T., 2011a. Changes in some biochemical parameter accombined with Brucellosis in sheep. *Iraqi Journal of Veterinary Medicine*, 25(2): 107-110.

Arslan, S.H., Mohammad, B.A., Al-Hussary, N.A., Al-Obaidi, Q.T., Hassan, M.M., 2011b. Changes in some biochemical parameter accombined with Brucellosis in native goats. *Iraqi Journal of Veterinary Medicine,* 25(1): 23-27.

Baum, M., Zamir, O., Bergman-Rios, R., Katz, E., Beider, Z., Cohen, A., Banai, M., 1995. Comparative evaluation of micro agglutination test and serum agglutination test as supplementary diagnostic test for Brucellosis. *Clinical Microbial,* 33(8):2166-2170.

Baz, E., 2000. The detection of antibodies against *Chlamydia psittaci* using complement fixation test (CFT) and enzyme-linked immunosorbent assay (ELISA) in aborted sheep in Kars district. Ph.D. thesis, University of Kafkas, Institute of Health Science, Kafkas University.

Bercovich, Z., Muskens, J. A., 1999. The efficacy of the skin delayed-type hypersensitivity using a Brucella in prepared from a mucoid strain of *Brucella abortus* to detect Brucellosis. *Veterinary Journal,* 157(1): 61-67.

Biancifiori, F., Nannini, D., Matteo, A., Belfiore, P., 1996. Assessment of an Indirect ELISA in milk for the diagnosis of ovine Brucellosis, *Comparative Immunology. Microbiology and Infectious Diseases*, 19: 17-24.

Bishop, G. C., Bosman, P. P., Herr, S., 1994. Bovine Brucellosis. (Editors: Coetzer, J.A., Thomson, G. R., Tustin, R. C.,) Infectious diseases of livestock with Special Reference to Southern Africa. Oxford University Press. Cape Town.1053-1066.

Blasco, J.M., 2006. Existing and future vaccines against Brucellosis in small ruminants. *Small Ruminant Research,* 62: 33-37.

Bowers, D., 2008. Medical statistics from scratch (Editors: Wiley, J., Sons Ltd Southern gate, United Kingdom. 178-201.

Bricker, B. J., 2002 PCR as a diagnostic tool for Brucellosis. *Veterinary Microbiology.* 90: 435-446.

Cassidy, C.C., 2010. *Brucella abortus* strain RB51 outer membrane vesicles as a vaccine against Brucellosis in a murine model, M.Sc. thesis in biomedical and veterinary Sciences. Faculty of the Virginia Polytechnic Institute and State University.

Castrucci, G., 2007. *Brucella melitensis* infection. In: Disease of sheep. (Editors: Aitken, I.D.,) *Moredun Research Institute*, Edinburgh. 137-142.

Celli, J., Gorvel, J.P., 2004. Organelle robbery: Brucella interactions with the endoplasmic reticulum. *Current Opinion in Microbiology,* 7:93-97.

Chand, P., Rajpurohit, B.S., Malhotra, A.K., Poonia, J.S., 2005. Comparison of milk-ELISA and serum-ELISA for the diagnosis of *Brucella melitensis* infection in sheep. *Veterinary Microbiology*, 108: 305-311.

Charanjeet, M. S., Katoch, R. C., Prasenjeet, D., Rajinder, K., 2004. Application of RBPT, SAT and Avidin-Biotin serum ELISA for detecting Brucellosis among livestock in himachal Pradesh. *Indian Journal Comparative Microbiol Immunol Infectious Disease*, 24(1): 15-18.

Cherwonogrodsky, J.W., Perry, M.B., Bundle, D. R., 1987. Identification of the A and M antigens of Brucella as the O-polysaccharide of smooth lipopolysaccharides. *Canadian Journal of Microbiology*, 33: 979-981.

Cloeckaert, A., 2001. Classification of Brucella species isolated from marine mammals by DNA polymorphism at the Omp2 locus. *Microbes and Infectious Journal*, 3:729-739.

Cloeckaert, A., Jacques, I., Grilló, M.J., Marín, C.M., Grayon, M., Blasco, J.M., Verger J.M., 2004. Development and evaluation as vaccines in mice of *Brucella melitensis* Rev.1 single and double deletion mutants of the bp26 and omp31 genes coding for antigens of diagnostic significance in ovine Brucellosis. *Vaccine*, 22(21-22): 2827-2835.

Corbel, M. J., 1997. Brucellosis: an overview. *1st International Conference on Emerging Zoonoses - Emerging infectious disease,* 3(2): 213-221.

Corbel, M. J., Stuart, F. A., Brewer, R. A., 1983. Observations on serological cross-reactions between smooth *Brucella* species and organisms of other genera, *3rd International Symposium on Brucellosis; in Develop Biology Standard*, United Kingdom. 341-348.

Crosby, E., Liosa, L., Miro, Q. M., Carillo, C., Gotuzzo, E., 1984. Haemolitic Changes in Brucellosis. *Infectious Diseases*, 150:419-424.

Dajani, Y., Masoud, A., Barakat, H., 1989. Epidemiology and diagnosis of human Brucellosis. *Tropical Medicine and Hygiene,* .92: 209-214.

Davis, D.S., Nielsen, K., Duncan, J.R., 1990. Brucellosis in wildlife in animal Brucellosis, Florida, 321-334.

Dohoo, I. R, Wright, P. F., Ruckerbauer, G. M., Samagh, B. S., Robertson, F. J., Forbes, L. B., 1986. A comparison of five serological tests for bovine Brucellosis. *Canadian Journal of Veterinary Research,* 50(4): 485-493.

Elberg, S.S., 1981. A guide to diagnosis, treatment and prevention of human Brucellosis. World Health Organization, 71.

Erdoğan, İ., Gürel, A., Tekin, C., Uyanık, F., Bitgel, A., 1993. Detection and distribution of bacterial abortion in sheep, goats and cattle in the Thrace Region. *Pendik Veterinary Microbiology*, 24(1): 23-35.

FAO. 2009. *Brucella melitensis* in Eurasia and the Middle East, FAO technical meeting in collaboration with *World Health Organization* and *World Organization for Animal Health*. Rome.

Fekete, A., Bantle, A. J., Halling, S. M., Sanborn, M., 1990. Preliminary development of a diagnostic test for *Brucella* using polymerase chain reaction. *Journal of Applied Bacteriology,* 69(2): 216-227.

Gallien, P., Dorn, C., Alban, G., Staak, C., Protz, D., 1998. Detection of Brucella species in organs of naturally infected cattle by polymerase chain reaction. *Veterinary Record,* 142 (19): 512-514.

Godfroid, J., Bosman, P. P., Herr, S., Bishop, G. C., Coetzer, J. A., Tustin, R. C., 2004a. Bovine Brucellosis. In *Infectious Disease of Livestock,* 2nd Edition, Oxford University Press, 1510-1527.

Godfroid, J., Garin-Bastuji, B., Blasco, J. M., Thomson, J., Thoen, C. O., Coetzer, J. A., Tustin, R. C., 2004b. *Brucella melitensis* infection, In *Infectious Disease of Livestock,* 2nd Edition, Oxford University Press: 1535-1541.

Godfroid, J., Saegerman, C., Wellemans, V., Walravens, K., Letesson, J.J., Tibor, A., Mcmillan, A., Spencer, S., Sanna, M., Bakker, D., Pouillot, R., Garin-Bastuji, B., 2002. How to substantiate eradication of Brucellosis when a specific serological reaction occurs in the course of Brucellosis testing. *Veterinary Microbiology*, 90:461-471.

Grimont, F., Verger, J. M., Cornelis, P., Limet, J., Lefèvre, M., Grayon, M., Régnault, B., Van-Broeck, J., Grimont, P. A., 1992. Molecular typing of *Brucella* with cloned DNA. *Research Microbiology,* 143:55-65.

Güler, L., Gündüz, K., Baysal, T., 1998. Bacteriological and serological examination of material from sheep that aborted at the Konya Veterinary Control and Resarch Institute in 1998. *Veterinarium*, 9: 3-10.

Gupta, V. K., Gupta, G., Pathak, M., Kumar, A., Vihan, V. S., 2012. Milk-enzyme linked immunosorbent assay for serological diagnosis of Brucella melitensis infection in goats. *Indian Journal of Animal Sciences,* 82: 17-19.

Gürtürk, K., Solmaz, H., Ekin, İ. H., Aksakal, A., Gülhan, T., 2000. Bacteriological and serological examinations of aborting sheep in Van Region. Journal Faculty of Veterinary Medicine University of Yüzüncü Yıl, 11:19-22.

Haghdoost, A. A., Kawaguchi, L., Mirzadeh, A., Rashidi, H., Sarafinejad, A. A., Baniasadi, R., Davies, C., 2007. Using GIS in explaining spatial distribution of Brucellosis in an endemic district in Iran. *Iranian Journal Public Health*, 36: 27-34.

Hellmann, E., Staak, C.H., Baumann, M., 1984. Bovine Brucellosis among two different cattle population in Bahrel Ghazal province of Southern Sudan. *Tropical Medicine Parasitology,* 35:123-126.

Herman, L., De Ridder, H., 1992. Identification of *Brucella* spp. by using the polymerase chain reaction. *Applied and Environmental Microbiology,* 58(6): 2099-2101

Ibrahim, A.K., Abeer. A., Abdelall, A., Amin, A.S., 2012. Long-term diagnostic studies for detection of *Brucella spp.* in milk samples. *Global Veterinaria,* 8: 54-61.

Ilhan, F., Yener, Y., 2008. Immunohistochemical detection of *Brucella melitensis* antigens in cases of naturally occurring abortions in sheep. *Veterinary Diagnostic Investigation,* 20: 803-806.

Isabel, L., 2013. Brucellosis in small ruminants – a seroprevalence study in peri-urban farming around the region of Dushanbe, Faculty of Veterinary Medicine. Swedish University.

Kabagambe, E.K., Elzer, P.H., Geaghan, J.P., Opuda-Asibo, J., Scholl, D.T., Miller, J.E., 2001. Risk factors for Brucella seropositivity in goat herds in eastern and western Uganda. *Preventive Veterinary Medicine*, 52: 91-108.

Kaltungo, B.Y., Saidu, S.N., Sackey, A.K., Hazeem, H.M., 2013. Seroprevalence in milk of sheep and goats in Kaduna North sentorial District of Kaduna state Nigeria. *International Journal of dairy science,* 8(2): 56-64.

Karaman, Z., Küçükayan, U., 2000. The serological and microbiologic examination results for the materials consisted of aborted sheep sera that had been sent to Etlik Central Veterinary Control and Research Institute between 1993-1997. *Etlik Veterinary Microbiology*, 11: 23-30.

Karim, M.A., Penjouian, E.K., Dessouky, F.I., 1979. The prevalence of brucellosis among sheep and goats in northern Iraq. *Tropical Animal Health and production*, 11: l86-l88.

Ko, J., Splitter, G.A., 2003. Molecular host-pathogen interaction in Brucellosis: current understanding and future approaches to vaccine development for mice and humans. *Clinical Microbiology Reviews,* 16: 65-78.

Kohler, S.S., Michaux-Charachon, F., Porte, M., Ramuz, J., Liautard, J.P., 2003. What is the nature if the replicative niche of a stealthy bug named Brucella. *Trends in Microbiology,* 11:215-219.

Luzzi, G. A., Brindle, R., Sockett, P.N., Solera, J., Klenerman, P., Warrel, D.A., 1993. Brucellosis imported and laboratory acquired cases, an over view of treatment trials. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 87:138-141.

Madkour, M. M., Kasper, D.L., 2001. Brucellosis. (Editors: Braunawald, E., Fauci, A.S., Kasper, D.L., Hauser, S.L., Longo, D.L., Jameson, J.L.,) *Harrison's Principles of Internal Medicine,* 1: 917-925.

Mansur, R.S., 2000. Epidemiological and diagnostic study of Brucellosis in Ninevah province, M.Sc. thesis in Microbiology, College of Veterinary Medicine Mosul University.

Mantur, B.G., Amarnath, S.K., 2008. Brucellosis in India-a review. *Biological Science*, 33: 539-547.

Mathur, P.B., El- Dessouky, F.I., Karim, M.A., Mohamad, A.K., Ayoub, M.A., 1974. Preliminary survey of Brucellosis in cattle, sheep and goat in northern Iraq, Technical report. 6. FAQ.

Mehdi, J.M., Mohammadreza, H.R., Soleiman, M., Ali, B., 2012. How is total antioxidant status in plasma of patients with Brucellosis. *Caspian Journal International Medicine,* 1(1): 363-367.

Memish, Z. A., Mah. M. W., 2001. Brucellosis in laboratory workers at a Saudi Arabian hospital. *American Journal of Infection Control***,** 29: 48-52.

Montasser, A.M., Affi, M.M., El-Bayoumy, E.M., Abdul-Raouf, U.M., Mohamad, H.A., 2011. Efficiency of serological test for detection of Brucellosis in ruminant as south provinces of Egypt. *Global Veterinary*, 6 (2): 156-161.

Mosallanejad, B., Najafabadi, G. M., Avizeh, R., Mohammadian, N., 2009. A serological survey on *Brucella canis* in companion dogs in Ahvaz. *Iranian Journal Veterinary Research*, 10: 383-386.

Muñoz, P. M., Marín, C. M., Monreal. D., Garin-Bastuji, B., Díaz, R., Mainar-Jaime, R. C., Moriyón, I., Blasco, J. M., 2005. Efficacy of several serological tests and antigens for diagnosis of bovine brucel osis in the presence of false-positive serological results due to *Yersinia enterocolitica* O:9. *Clinical and Diagnostic Laboratory Immunology*, 12(1): 141-151.

Muz, A., Ertaş, H.B., Öngör, H., Gülcü, H.B., Özer, H., Eröksüz, H., Dabak, M., Başbuğ, O., Kalender, H, 1999. Bacteriologic, Serologic and Pathologic Studies on Abortus Cases of Goats and Sheep in Elazığ and it's Vicinity. *Turkish Journal of Veterinary and Animal Sciences*, 23(1):177-188.

Nicolettie, P. (1980). The epidemiology of bovine brucellosis. (Editors: Brady, C. A., Conelius, C. A.,). Advance in veterinary Science and Comparative Medicine, Academic Press, New York, USA. 69-98.

Nielsen, K., Duncan, J. R., 1990. Animal Brucellosis. Boca Raton, Florida, USA. 1-453.

Nielsen, K., 2002. Diagnosis of Brucellosis by serology. *Veterinary Microbiology,* 90: 447-459.

Nielsen, K., Smith, P., Yu, W., Nicoletti, P., Jungersen, G., Stack, J., Godfroid, J., 2006. Serological discrimination by indirect enzyme immunoassay between the antibody response to *Brucella* spp. and *Yersinia enterocolitica* O:9 in cattle and pigs. *Veterinary Immunology and Immunopathology,* 109: 69-78.

OIE (Office International des Epizooties)., 2007. Brucellosis. The center for food security and public health. College of Veterinary Medicine Iowa state university. 1-13.

OIE (Office International des Epizooties)., 2004. Bovine Brucellosis. Manual of Standards for Diagnostic Tests and Vaccines for Terrestrial Animals (5th edition) *World Organization for Animal Health,* Chapter 2.3.1.

OIE (Office International des Epizooties)., 2000. Sampling Methods. Manual of Standards for Diagnostic Tests and Vaccines (3rd edition) *World Organization for Animal Health,* Chapter 1.1.1.

Otlu, S., Şahin, M., Atabay, H. I., Unver, A., 2008. Serological investigations of brucellosis in cattle, farmers, and veterinarians in the Kar district of Turkey. *Acta Veterinaria Brno,* 77: 117-121.

Otlu, S., Şahin, M., Unver, A., Çelebi, Ö., 2007. Detection of *Brucella melitensis* and Chlamydophila Abortus Antibodies in aborting Sheep in the Kars Province of Turkey. *Bulletin Veterinary Institute in Pulawy,* 51: 493-495.

Ouahrani-Bettache, S., Soubrier, M. P., Liautard, J. P., 1996. PCR for the detection and identification of *Brucella* species and strains. *Applied Bacteriology*, 81:154-160.

Pei, J., Ficht, T. A., 2004. *Brucella abortus* rough muntants are cytopathic for macrophages in culture. *Infection and immunity,* 72: 440-450.

Porte, F., Naroeni, A., Ouahrani-Bettache, S., Liautard, J.P., 2003. Role of the *Brucella Suis* lipopolysaccharide O antigen in phagosomal genesis and in inhibition of

phagosome-lysosome fusion in murine macrophages. *Infection and Immunity,* 71(3):1481-1490.

Quinn, P. J., Carter, M. E., Markey, B., Carter, G, H., 1999. *Brucella* species. In Clinical Veterinary Microbiology. *Elsevier Health Sciences*, 261-267.

Quinn, P.J, Markey, B.K., Carter, M.E., Donnelly, W.J., Leonard, F.C., 2002. Veterinary Microbiology and microbial disease, *1st Blackwell Science LTD. London*.163-167.

Radostitis, O.M., Blood, D.C., Gay, C.C., 1994a. Brucellosis caused by *Brucella abortus* (Bangś disease) In: *Veterinary Medicine a textbook of the diseases* of *cattle, sheep, pigs, goats and horses. 8th Edition, the Bath Press*, 787-803.

Radostitis, O.M., Blood, D.C., Gay, C.C., 1994b Brucellosis caused by *Brucella melitensis* In: *Veterinary Medicine a textbook of the diseases of cattle, sheep, pigs, goats and horses. 8th Edition, the Bath Press,* 810-813.

Radostits, O. M., Gay, C. C., Hinchcliffe, K. W., Constable, P. O., 2007. Veterinary medicine. A textbook of the diseases of cattle, sheep, pigs and horses. *10th ed. Saunders Elsevier,* 971-972.

Radwan, A. I., Bekairi, S. I., Mukayei, A. A., Al-Bokmy, A. M., Prasad, P. V., Azar, F. N., Coloyan, E. R., 1995. Control of *B. melitensis* infection in large camel herd in Saudi Arabia using antibiotherapy and vaccination with Rev-1 vaccine. *Review Scientific and Technical,* 14: 719-732.

Ratushna, V. G., Sturgill, D. M., Ramamoorthy, S., Reichow, S. A., He, Y., Lathigra, R., Sriranganathan, N., Halling, S. M., Boyle, S. M., 2006**.** Molecular targets for rapid identification of *Brucella* spp. *BMC Microbiol,* 6, 13.

Saegerman, C., Vo, T. O., De, W. L., Gilson, D., Bastin, A., Dubray, G., Flanagan, P., Limet, J. N., Letesson, J.J., Godfroid, J. 1999. Diagnosis of bovine Brucellosis by skin test: conditions for the test and evaluation of its performance. *Veterinary Record*, 145(8): 214-218.

Şahin, M., Atabay, H. İ., Otlu, S., Unever, A., Celebi. O., 2004. Serological and cultural investigation of the prevalence of Brucellosis in cattle, sheep and humans in Kars and its surrounding area. VI. *National Congress of Veterinary Microbiology Proceedings.* 133.

Saleem, A.N., Rhaymah, M.S., Shamoon, G.N., 2004. Isolation and seroprevalence of ovine Brucellosis. *Iraqi Journal Veterinary Science*, 18: 31-38.

Saravi, M., Wright, P., Gregoret, R., Gall, D., 1995. Comparative performance of Enzyme linked immunosorbent assay (ELISA) and conventional assays in the diagnosis of bovine Brucellosis in Argentina. *Veterinary Immunol Immunopathol*ogy, 47: 93-99.

Schurig, G., Sriranganathan N., Corbel, M., 2002. Brucellosis vaccines: past, present and future. *Veterinary Microbiology*, 90(1-4): 479-496.

Shareef, J. M., 2006. A review of serological investigations of Brucellosis among farm animals and humans in Northern Province of Iraq (1974-2004). *Journal Veterinary Medicine*, 53: 38-40.

Rahman, S., Hahsin, F. A., Ahasan, S., Her, M., Kim, J. Y., Kang, S., Jung, S. C., 2011. Brucellosis in sheep and goat of Bogra and Mymensingh districts of Bangladesh. *Korean Journal Veterinary Research*, 51(4): 277-280.

Teshale, S., Muhie, Y., Dagne, A., Kidanemariam, A., 2006. Seroprevalence of small ruminant Brucellosis in selected districts of Afar and Somali pastoral areas of Eastern Ethiopia : the impact of husbandry practice. *Review Medicine Veterinary*, 157 (11): 557-563.

Thimm, B.M., 1982. Brucellosis: Distribution in Man, Domestic and Wild Animals. Springer Verlag, New York, 245.

Tizard, I., 1992. Veterinary Immunology: an introduction. 4th, WB Saunders Company. London. 522.

Tun, T.N., 2007. Prevalence survey of bovine Brucellosis (*Brucella abortus*) in dairy cattle in Yangon Myanmar. M.Sc. thesis of veterinary public health. Chiang Mai University and Freie University.

Walker, R.L., 1999. *Brucella*. In: veterinary Microbiology. (Editors: By Hirsh, D.C., Zee, Y.C.,). Oxford University press, 196-203.

Weidmann, H., 1991. Survey of means now available for combating Brucellosis in cattle production in the tropics. *Animal research and Development*, 33: 100-111.

WHO 1971. Fifth report, Joint FAO/WHO Expert committee on Brucellosis. *WHO Technical Report Series*, Geneva, 464.

William, T.H., 1975. Disease transmitted from animals to man. (Editors: Charles C. Thomas) Publisher *Springfield Illions*, 1206.

Wise, R.I., 1980. Brucellosis in the United States: Past, present and future. *American Medical Association*, 244: 2318-2325.

Yacoub, A. A., Bakr, S., Hameed, A. M., Al-Thamery, A. A., Fartoci, M. J., 2006. Seroepidemiology of selected zoonotic infections in Basra region of Iraq. *La Revue de Sante de la Mediterranean Orientale*, 12: 112-118.

Yetkin, M. A., Erdinç, F. S., Bulut, C., Tülek, N., 2005. Epididymoorchitis due to Brucellosis in central Anatolia, Turkey. *Urology International*, 75: 235-238.

Young, E.J., 1983. Human Brucellosis. *Review of Infectious Disease*. 5: 821-842.

Young, E.J., 1995. An Overview of Human Brucellosis. *Clinical Infectious Disease,* 21(8)283-289.

Za, M. O., Ga, R., Bannatyne, M., Khan, Y., 1999. Diagnosis of Brucellosis in a Saudi Arabian hospital: the correlation of serologic titer with blood culture results. *Antimicrobial Agents and Chemotherapy*, 39: 238.

Zhan, Y., Cheers, C., 1995. Differential activation of Brucella reactive CD4+ cells by Brucella infection or immunization with antigenic extracts. *Infection Immunization,* 63: 969-995.

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