

Ministry of Higher Education and Scientific Research  
University of Al-Qadisiyah  
College of Veterinary Medicine



# Ovine Brucellosis

A Research

Submitted to the Council of the college of Veterinary Medicine  
College/University of Al-Qadisiyah in partial fulfillment of the  
Requirements for the degree of Bachelor of Veterinary Medicine and surgery

By

Hussein Abbas Nagi Al-zubaidy

Supervised by

Dr. Saad H.G. Al-Husseiny

2016 A.D

1437A.D

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Certificate of supervisor

I certify that the research entitled (Ovine Brucellosis) was prepared under my supervision at the college of Veterinary Medicine/ University of Al-Qadisiyah.

 2/4/2016  
Supervisor

Dr. Saad H. G. Al-Husseiny

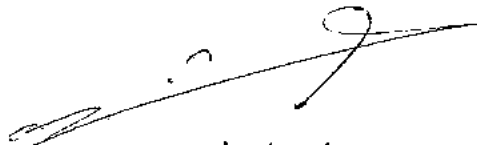
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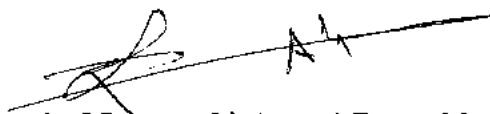
We are the head of Dept. of Int. & Prev. Med. Certify that Hussein Abbas Nagi is adequate for the Debate of Graduation project of Bachelor Degree of Bachelor of Veterinary Medicine and surgery



Instructor .

**Dr. Muthanna Hadie Hussein**

21. 4. 2016



**Head of Dept. of Int. and Prev. Med.**

**Dr. Asaad Jassim Abd**

21. 4. 2016

# Dedication

Firstly,I would like to introduce my best regards and special thanks to those people who are my real friends and most expensive

My mom dad brothers to those support in life to those guys my career my dear friends to whom they credited with lighting my way my teachers distinguished you my sincere thanks and gratitude for your kind efforts which contributed to take us out to be of help and a support order our beloved service

## Acknowledgement

I extend my sincere thanks and appreciation to the deanship of the faculty of Veterinary Medicine and a private dean of the faculty for guidance tireless efforts and constant encouragement of scientific research and normalize extend my sincere thanks and gratitude to the Branch of Internal and preventive Medicine for providing the requirements of scientific research was my teacher supervisor Dr.Saad Hashim role great in the design and delivery of search hath me all the love and appreciation for his effort will not never forget

## **Abstract**

Ovine Brucellosis is a highly infectious bacterial disease, which infect sheep.

The objective of this survey study was to investigate the ovine Brucellosis in different regions of Al-Diwaniyah province and its clinical effects on sheep flocks in this area.

Forty-three sheep were examined in this study, aged more than eight months, these Forty-three, were divided into 16 rams and 27 ewes, most of these flocks were have case history of abortion, retained placenta, placentitis, swelling of the testicles in males (orchitis) cases.

Moreover, these sheep were examined clinically and subjected into serological tests.

Sera was tested by Rose-Bengal plate test (RBPT), that revealed the percentage of infection was 23.25% in these samples.

Higher prevalence 6 (60%) was found in ewes on the other hand the lower prevalence 4(40%) was recorded in rams.

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*CHAPTER  
ONE*

*Introduction*

## Introduction:

Brucellosis is a highly infectious bacterial zoonotic disease that infect sheep, goats and cattle. Although it can affect other animals, its main threat is to cattle, sheep, goat & swine. (Alton, 1990).

It is widespread worldwide, it is caused by one of the three biovars of Brucella melitensis (Blasco, 1992).

Clinically the disease is characterized by one of the following signs: abortion, retained placenta, placentitis, swelling of the testicles in males (orchitis) and rarely it localizes in joints and causes arthritis (Terrestrial animal health).

In most circumstances the primary route of transmission is the placenta, fetal fluids and vaginal discharges expelled by the infected ewes or goats when they abort or have a full-term parturition (Nifelsen, and Gall, 2004).

Brucellosis has been considered one of the most serious infectious disease, where it causing many economical losses such as decreasing in milk production, infertility, loss of offspring through it causes abortion in ewes, weight loss and lameness (Muma, *et al.* 2007).

The world health organization (WHO) laboratory safety biosafety manual classifies Brucella particularly (Brucella melitensis) in risk group III (OIE, 2000).

Brucellosis is readily transmissible to human, causing acute febrile illness-Undulant fever Malta fever which may progress to a more chronic form & can also produce serious complications affecting the muscular-skeletal system, cardiovascular system & central nervous system, infection is often due to occupational exposure & is essentially acquired by the oral, respiratory or conjunctival routes (Al-Izzi, and Barhoom , 1988)

However, ingestion of dairy products constitutes the main risk to the general public; there is an occupational risk to veterinarians, abattoir workers who handle infected animals & aborted fetuses or placentas (Al-Thwyni, *et al* 2000).

As concerns Brucella ovis whose affects rams & ewes, the demonstration of genital lesions (unilateral or bilateral epididymitis & orchid-epididymitis) by palpating the testicles of rams may suggest the presence of this infection in a given flock, however, clinical diagnosis lacks sensitivity because not all rams infected with Brucella ovis (Blasco,1990).

Moreover, clinical diagnosis lacks specificity since other bacteria may cause genital lesions in rams; the most reported pathogens are Actinobacillus seminis, Haemophilus spp., Histophilus ovis (Garcia, 2009).



*CHAPTER  
TWO*

*Literatures of  
Review*

## 2-Literatures Review:

### 2-1-Definition:

Brucellosis is a highly & costly contagious bacterial zoonotic disease that infect many species of livestock such as sheep and goat (*Brucella melitensis*), cattle (*Brucella abortus*), sheep (*Brucella ovis* ) and swine (*Brucella suis*). (Dhahir, (2002).

Where it causing many clinical signs such that abortion, retained placenta, placentitis, epididymitis, orchi-epididymitis, decreased milk production, lameness, weight loss (OIE, 2000).

Terrestrial Animal Health code).It also affects human causing Malta fever or Undulant fever, although Brucellosis can affect other animals, its main threat is to cattle, sheep and goats. (Garrido, *et al.* 2001).

The disease is spread by infected rams and can be spread from one ram to the other one, when they are in close contact. In addition, it can be spread from an infected ram to a non-infected ram (Nielsen, 2002).

When both of them serve the same ewe, so that it considers a sexually transmitted disease of sheep (Trisha Esson, 2003).

On the other hand ram also can be exposed to infection via ewe, when serving or sniffing which has been recently aborted(OIE, 2000).

## **2-2-Etiology:**

1-*Brucella ovis*, which affects sheep.

2-*Brucella melitensis*, which affects sheep, goat and human.

3-*Brucella abortus*, which affects cattle.

4-*Brucella suis*, which affects swine.

## **2-3-Synonyms:**

1-Undulant Fever.

2-Malta Fever.

3-Mediterranean Fever.

4-Contagious Abortion.

## **2-4-Epidemiology:**

Brucella ovis clinical infection has been seen in rams (epididymitis) during the whole period.

### Brucella abortus

No accurate figures are available on the prevalence of brucellosis in cattle as most reports are based on non-representative laboratory results.

Cattle usually become infected after ingesting contaminated feed or water or licking an infected placenta, calf or fetus, or the genitalia of an infected cow soon after it has aborted or calved at which time very large

numbers of Brucella abortus are present, particularly in the placental lochia.(Silva et al. 2000).

Animals may also become infected by inhaling organisms or through the conjunctiva. Calves may acquire infections in utero or they may become infected after ingesting infected colostrum or milk. Although some will rid themselves of the infection within a few months, others may remain infected for life and may spread the disease at their first and subsequent parturitions. (Nielsen, 2004).

Although infected animals abort only once, subsequent calves are carried to full-term but may remain infected. Approximately 2,5 - 9% of heifers born of seropositive cows may be latently infected but serologically negative until the middle of their first gestation or even later, when, for the first time antibodies to B. abortus may be detectable or abortion may occur. (Songer and Post, 2005).

There appears to be no controlled studies showing that bulls are more resistant to B. abortus than heifers and cows. Bulls may become infected in utero or during early calf hood by the oral route and retain the infection into adult life. In bulls, the testes and accessory sex glands may be affected and reveal inflammatory changes. (Salem, *et al.* 1977).

Infected bulls may shed brucellae in their semen, seminal fluid and urine, and therefore in infected herds they should always be viewed with suspicion, particularly if artificial insemination using their semen is contemplated. (Popovis and Bragg, 2001).



*Brucella abortus* is sensitive to pasteurization temperatures and its survival outside the host is largely dependent on environmental conditions.

Large numbers of organisms are shed from the reproductive tract when infected cows abort. In those cows that lactate following abortion, milk, including colostrum is an important source of infection and bacteria may be excreted intermittently in milk throughout the lactation period.

Urine and feces of infected cattle are less important sources of the bacterium.

A contaminated environment or equipment used for milking or artificial insemination, are further sources of infection. Permanent calving camps and lush pastures, particularly if they are wet and muddy, may play a very important role in the spread of the disease.

Although *B. abortus* has been isolated from ixodid ticks and their eggs in Brazil, ticks probably do not play an important role in the transmission of the disease.

*Brucella abortus* infection in sheep and goats may occasionally cause them to abort, but the infection does not spread in these species and they are apparently not a real danger to cattle unless there is close association between the species.

### **Brucella ovis**

*Brucella ovis* produces a disease unique to sheep and is one of the most common causes of epididymitis in rams and a rare cause of abortion in ewes and neonatal mortality in lambs.

Low reproductive rates may occur in affected flocks. It is a non-zoonotic disease. The disease is spread primarily by infected rams.

*Brucella ovis* may be excreted in the semen of infected rams even before the development of lesions. Clinically or subclinically infected rams may excrete *B. ovis* in their semen for years. (Salem, 1977)

Infection may spread from ram-to-ram because of homosexual activity or venereally during coitus when non-infected rams mate with ewes, which passively harbor the bacteria. Ewes transfer organisms mechanically (Gall and Nielsen, 2004).

## **2-5-Mode of Transmission:**

Transmission requires close proximity (often by sexual contact), and is typically by direct ram to ram contact or from ram to ram by mating of the same ewe.

In animals, *B. melitensis* is usually transmitted by contact with the placenta, fetus, fetal fluids and vaginal discharges from infected animals. (Gall and Nielsen, 2004)

Small ruminants are infectious after either abortion or full-term parturition, goats usually shed *B. melitensis* in vaginal discharges for at least 2 to 3 months, but shedding usually ends within three weeks in sheep.

This organism can also be found in the milk and semen; shedding in milk and semen can be prolonged or lifelong, particularly in goats. Kids and lambs that nurse from infected dams may shed *B. melitensis* in the feces.

Most animals become infected by ingestion or through the mucous membranes of the oropharynx, upper respiratory tract and conjunctiva, but *Brucella* can also be transmitted through broken skin.

Although the mammary gland is usually colonized during the course of an infection, it can also be infected by direct contact, with subsequent shedding of the organisms in the milk (Al-Thwini, *et al.*, 2000)

Transmission during breeding is possible, but seems to be uncommon during natural mating. *B. melitensis* can be spread on fomites, and could be disseminated mechanically by carnivores that carry away infected material. In conditions of high humidity, low temperatures and no sunlight, *Brucella* can remain viable for several months in water, aborted fetuses, manure, wool, hay, equipment and clothes. *Brucella* species can withstand drying, particularly when organic material is present, and can survive in dust and soil. (Alton, *et al.*, 1988)

Survival is longer when the temperature is low, particularly when it is below freezing. Cattle and other species can be infected with *B. melitensis* after contact with infected sheep and goats. It has not been

established whether cattle can maintain this species indefinitely in the absence of contact with small ruminants. (Alton, *et al.*, 1988)

Cattle with infected udders can shed *B. melitensis* in the milk for months or years. Camels also shed this organism in milk.

Humans usually become infected by ingesting organisms (including contaminated, unpasteurized dairy products) or by the contamination of mucous membrane and abraded skin. (Al-Thwyni, *et al.*, 2000)

## **2-6-Distribution:**

*B. melitensis* is particularly common in the Mediterranean. It also occurs in the Middle East, Central Asia, around the Persian Gulf (also known as the Arabian Gulf), and in some countries of Central America (Garrido *et al.*, 2001)

This organism has been reported from Africa and India, but it does not seem to be endemic in northern Europe, North America (except Mexico), Southeast Asia, Australia, or New Zealand. .( Dhahir, *et al.*,2002)

Biovar 3 is the predominant biovar in the Mediterranean countries and the Middle East, and biovar 1 predominates in Central America. Sporadic cases or incursions are occasionally reported in *B. melitensis* - free countries. In the U.S., cases have mainly been reported in imported goats and rarely in cattle. (Al-Thwyni, *et al.*, 2000)

## **2-7-Clinical signs:**

The predominant symptoms in naturally infected sheep and goats are abortions, stillbirths and the birth of weak offspring. Animals that abort

may retain the placenta. Sheep and goats usually abort only once, but reinvasion of the uterus and shedding of organisms can occur during subsequent pregnancies ( Dhahir, *et al.*,2002)

Some infected animals carry the pregnancy to term, but shed the organism. Milk yield is significantly reduced in animals that abort, as well as in animals whose udder becomes infected after a normal birth. However, clinical signs of mastitis are uncommon. (Dhahir, *et al.*,2002)

Acute orchitis and epididymitis can occur in males, and may result in infertility. Arthritis is seen occasionally in both sexes. Many non-pregnant sheep and goats remain asymptomatic. ( Luchsinger and Anderson, 1979)

*B. melitensis* has also been associated with abortions in cattle, and abortions, orchitis and epididymitis in camels. In wild chamois, this organism has been linked to epididymoorchitis, polyarthritis, blindness and neurological signs. In dogs, infection with *B. melitensis* is often asymptomatic, and rapid elimination of this organism has been reported. (Muma, *et al.*, 2007)

## **2-8-Post mortem lesions:**

At necropsy, usually in ewes infected with *B.melitensis*, granulomatous inflammatory lesions may be present in the reproductive tract, udder, supramammary lymph nodes, other lymphoid tissues, and sometimes in the joints and synovial membranes.In rams,Necrotizing orchitis, epididymitis, seminal vesiculitis and prostatitis have been reported. (Munoz, *et al.*, 2005)

The fetus may be autolyzed, normal or have an excess of bloodstained fluid in the body cavities and an enlarged spleen and liver. Placentitis,

with edema and/or necrosis of the cotyledons and a thickened and leathery intercotyledonary region can be seen. (Nielsen , *et al.*, 2004)

The interval between the infection and the development of lesions in rams varies considerably, being anything from 50 to 250 days. In rams, the first detectable abnormality may be a marked deterioration in semen quality associated with the presence of inflammatory cells and organisms ( Salem, *et al.*, 1977)

Hot, swollen and oedematous testicle in a ram with ovine brucellosis  
The most consistent clinical sign is enlargement, particularly of the tail of the epididymis, which may be barely perceptible or up to a four- or fivefold increase in size. The head, body or the entire epididymis are less often affected. ( Songer and Post 2005)

The lesions often occur unilaterally, but bilateral involvement is relatively common. Rams suffering from acute epididymitis are not usually systemically affected. The entire testis on the affected side may be hot, swollen and oedematous but only a localized swelling of the epididymis is detectable in animals that are less severely affected.

Clinically detectable lesions may be acute to chronic. Although chronic lesions may follow an acute epididymitis, they more commonly develop insidiously without clinical evidence of the acute phase. (Popovis and Bragg 2001)

Enlargement of the entire epididymus in a ram with ovine brucellosis  
Chronic epididymitis is clinically characterized by enlargement and an increased consistency of the affected parts. As a result of fibrous adhesions, the mobility of the affected testis in the scrotum is often reduced. The marked increase in scrotal circumference caused by the

epididymal and testicular lesions can be seen from a distance. (Songer and Post 2005)

The testis is seldom primarily affected. In some cases the testis on the affected side may be slightly atrophied and have a somewhat softer consistency than normal, while in others with a severe, chronic epididymitis, the testis may be severely atrophied and firm. ( Songer and Post 2005)

Affected rams may be sterile, or have reduced fertility. The degree of impairment depends on whether the lesions are uni- or bilateral, and on the course and severity of the lesions. .(Popovis, and Bragg, 2001).



*CHAPTER  
THREE*

*Materials and  
Methods*



### **Study Area and Study Animals:**

A total 43 of Sheep blood samples (17 from Rams and 26 from Ewes) were collected randomly from unvaccinated sheep flocks suffering from abortion , with different ages for the period from December /2015 to March /2016.

These samples collected from different districts in Al-Diwaniyah province.

### **Blood Sample Collection**

About 10 mL of blood was collected form the jugular vein of each selected animal using plain vacutainer tubes and allowed to clot overnight at room temperature. The serum samples were centrifuged, separated and transported in iceboxes to Laboratory of clinical pathology of the veterinary college / Qadisiyah University, and stored at -20°C until testing.

### **Serological Tests**

The Rose Bengal plate test (RBPT) was performed according to the standard procedure.(linear chemicals® ) The (RBPT) or buffered Brucella antigen test (BBA) is a rapid slide agglutination procedure developed for the direct detection of Brucella antibodies in the sheep sera.

The bacterial suspension of kit is reactive with both immunoglobulin G and immunoglobulin M antibodies being the former detected earlier (sub-clinical) infections and over a large period during the disease (chronic stage) than the conventional tube agglutination test.

The assay is performed by testing the buffered suspension (pH 3.6) of B. abortus strain colored with Rose Bengal against unknown sera and contain 0.95 g\L of sodium azide.

The presence or absence of a visible agglutination indicates the presence or absence of antibodies in the sample tested.

**Test procedure:**

- 1- Transported the test and samples to room temperature.
- 2- Resuspended the antigen vial gently. Aspirated dropper several times to obtain thorough mixing.
- 3- Placed 1 drop (50  $\mu$ L) of the serum under test into one of the circle on the card.
- 4- Dispensed 1 drop of positive control serum and 1 drop of negative control serum into 2 additional circles.
- 5- Added 1 drop of Rose Bengal antigen to each circle next to the sample to be tested.
- 6- Mixed the contents of each circle with a disposable stirrer while spreading over the entire area enclosed by the ring. Used separated stirrers for each mixture.
- 7- Rotated the slide slowly either by hand or by means of a mechanical rotator (100 r.p.m) for a period of 4 minutes.
- 8- Observed immediately under a suitable light source for any degree of agglutination.



*CHAPTER  
FOUR*

*Results  
And Discussion*

## Results and Discussion

Rate of infection of sheep brucellosis by using rose Bengal plate test (RBPT) according to areas of study, 43 sheep sera samples tested, 10 (23.25%) were sero-positive with (RBPT) in different regions in Al-Qadisiyah province.

While 33 (76.74%) were sero-negative with (RBPT). Higher prevalence 6 (60%) was found in ewes besides the lower prevalence 4(40%) recorded in rams.

Table (1), figure (1), and figure (2) below.

Animals	Seropositive No.	Percentage %	Seronegative No.	Percentage %	Total No.
Ewes	6	60	21	63.63	27
Rams	4	40	12	36.36	16
Total	10	23.25	33	76.74	

**Table (1)**

**The serology and percentages of rams and ewes, which affected with Brucellosis**

**Figure (1)**

**The sero-negative of Rose Bangel plate test (RBPT)**



**Figure (2)**

**The sero-positive of Rose Bangel plate test (RBPT)**



Even though the study revealed a low sero-prevalence of ovine brucellosis, the economic significance of the disease is high.

Therefore, screening of animals to *Brucella* antibodies during arrival at the farm should be performed and test and slaughter policy may be applied for the control of brucellosis at sheep farms.

Results of this study revealed differences in the infection rates of sheep brucellosis according to gender

Our results disagreed with most other studies (Al-lzzi *et al* 1985; Al-lzzi and Barhoom 1988; and Selaisel, 1998). in Baghdad; in addition to Dhahir (2002) in Baghdad, Salah-Eldin and Dyala provinces; Saleem, *et al* (2004); Al-Hangawe (2006); Al-Farwachi (2010) in Ninevah province and in a percentage of 23.3%; 15%; 53.5%; 13.3%; 6.74%; 11.8%; and 66.7% respectively when comparison with the previous studies.

In present results; the percent of infection are differ from previous studies due to the number of animals tested and geographical variabilities



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