جامعة الناصر
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# Seroprevalence of *Brucella abortus* and *Brucella melitensis* among Butchers in Thamar citry,Yemen.

Research project submitted

to the department of medical lab.

Faculty of Medicine and Health Sciences in

partial fulfillment of the requirement of Bachelor

degree in medical lab.

**By**


| | |
|---|---|
| Yaser Ahmed Mohammed | Moteea Mohammed AbdulKader |
| Ahmed Abdulkader Mansour | Gamil Ahmed Mohammed |
| Hussin Ahmed Ali | Hussiny Ali Obaid |
| Salem Gafar Salem | Abdullah Saleh Abdullah |
| Abdulhakeem Al-romi | Magdi Hiader Ibraheem |
| Magdi Mabrok Hadi | Magdi Mossa Farea |
| Mohammed Ahmed Mohammed | Mossa Mohamed Siaf |
| Nader Hussin Ahmed | Wadah Ahmed Abduh |


**Supervised by**

Dr: Ahmed Saif Moharram

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**AUTHORIZED BY AL-NASSER UNIVERSITY'S RESEARCH OFFICE**

جميع حقوق النشر محفوظة لمكتب البحوث والنشر بجامعة الناصر

بسم الله الرحمن الرحيم

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ{١} خَلَقَ الْإِنسَانَ مِنْ عَلَقٍ{٢} اقْرَأْ وَرَبُّكَ الْأَكْرَمُ{٣} الَّذِي عَلَّمَ بِالْقَلَمِ{٤} عَلَّمَ الْإِنسَانَ مَا لَمْ يَعْلَمْ{٥}

(العلق)

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# Abbreviations

**-EDTA:** Ethylene Di-amine tetra acetic acid.

**-RBT:** Rose Bengal test.

**-CFT:** Complement fixation test.

**-ELISA:** Enzyme –linked immunosorbent assay.

**-TAT:** Tube agglutination test.

**-SAT:** Slid agglutination test.

# Abstract

**B**rucellosis is a widespread zoonosis mainly transmitted from cattle, sheep, goats, pigs and camels through direct contact with blood, placenta, fetuses or uterine secretions, or through consumption of contaminated raw animal products (especially unpasteurized milk and soft cheese). In endemic areas, human brucellosis has serious public health consequences. Worldwide, with an estimated 500,000 new human cases each year. *Brucella melitens*is the most prevalent species causing human brucellosis, owing in part to difficulties in immunizing free-ranging goats and sheep.

Our research estimated the prevalence of brucellosis among butchers and determined the risk factor that increase the exposure to *B.abortus* and *B.melitensis* among this study group in Thamar city from May to December 2012 . The present study is Cross-sectional study target approximately all butchers that work in butchery and shops of meat selling (64 butchers). Samples were examined by rose Bengal test (SAT) in General Thamar Hospital lab.

The result of research as following : 2 samples were positive (as prevalence 3.1%). The other samples (62) were negative (96.9%).

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# 1.1 Introduction

Brucellosis is a systemic disease caused by bacteria of the genus *Brucella* that affects humans and numerous animal species. It has been a great concern for many countries, especially those in the Middle East and Mediterranean regions, in terms of public and animal health. Human brucellosis infections can be unpredictable, with periods of chronicity, re-infection, and relapse[9].

Brucellosis, also known as undulant fever or Mediterranean fever, is caused by bacteria of the genus Brucella. It is a highly transmissible zoonosis (human infection of animal origin) affecting a wide variety of mammals in which it predominantly causes abortion and epididymitis[12].

In a study performed in Basrah during the last 6 months of 2010.the result or prevalence in butchers was (12.5%) ($P<0.05$) [4].
In cas-control study performed in alexandria governorate (egypt) during the period 1988–2003. The result in butchers was 5.6% in cases and 1.4% in control [6].

In yemen Najy ,2000 found that the prevalence of brucellosis among the butchers was 25.5%[16].

## 1.2 Aims of study:

Thamar governorate is located in middle of yemen & it is agriculture governorate and famous in animal breeding so the present study designed to achieve the following objective:

1-To estimate the seroprevalence of *B. abortus* and *B. melitensis* among butchers in thamar city.

2-To determine the risk factor that increase the exposure to *B. abortus* and *B. melitensis* among this study group.

## 1.3 Classification of *Brucella*:

**Table 1** : classification of *brucella*[17].

| Scientific classification | | Species |
|---|---|---|
| Kingdom: | Bacteria | *B. abortus*<br>*B. canis*<br>*B. ceti*<br>*B. inopinata*<br>*B. melitensis*<br>*B. microti*<br>*B. neotomae*<br>*B. ovis*<br>*B. pinnipedialis*<br>*B. suis* |
| Phylum: | Proteobacteria | |
| Class: | Alphaproteobacteria | |
| Order: | Rhizobiales | |
| Family: | Brucellaceae | |
| Genus: | ***Brucella*** | |

But only four species that known to cause human diseas:

1- *Brucella abortus* in cattle.

2- *B. melitensis* in small ruminants.

3- *B. suis*in pigs .

4- *B. canis* in dogs[18].

## 1.4 Morphology:

*Brucellae* are small Gram negative non-capsulated coccobacilli or short rods. They do not show bipolar staining but may stain unevenly[7].

## 1.5 Epidemiology:

Worldwide, brucellosis remains a major source of disease in humans and domesticated animals. Although reported incidence and prevalence of the disease vary widely from country to country, still the most widespread. In humans, ovine/caprine brucellosis caused by *B.melitensis* by far the most important clinically apparent disease. The disease has a limited geographic distribution, but remains a major problem in the Mediterranean region, wes-tern Asia, and parts of Africa and Latin America. Recent reemergence in Malta and Oman indicates the difficulty of eradicating this infection. Sheep and goats and their products remain the main source of infection, but *B. melitensis* in cattle has emerged as an important problem in some southern European countries, Israel, Kuwait,and Saudi Arabia.

*B. melitensis* infection is particularly problematic because *B. abortus* vaccines do not protect effectively against *B. melitensis* infection; the *B. melitensis*Rev.1. vaccine has not been fully evaluated for use in cattle.Thus, bovine *B. melitensis* infection is emerging as an increasingly serious public health problem in some countries. A related problem has been noted in some South American countries, particularly Brazil and Colombia, where *B. suis* biovar 1 has become established in cattle . Insome areas, cattle are now more important than pigs as a source of human infection[3].Brucellosis is a chronic , life-long infection in animals.

Organisms localize in reproductive organs(male and female), and are shed in large numbers in milk, urine ,and other tissue discharged during delivery or spontaneous abortion [10].

## 1.6 Transmission:

❖ Animal to human : Brucellosis is spread to humans through contact with blood, body tissues, or body fluids of infected animals.

The most common method is consumption of unpasteurized milk and dairy products. Human infections may occur through breaks in the skin when handling infected animal tissues[19].

❖ Human to human transmission is rare, but has been reported after:

- blood transfusion.
- bone marrow transplantation.
- sexual intercourse.
- Rare congenital infections seem to result from transplacental transmission or the ingestion of breast milk.
- Congenital infections might also occur if the infant is exposed to organisms in the mother's blood, urine or feces during delivery[14].

❖ *Occupational exposure*

Certain occupations are associated with a high risk of infection with brucellosis.These include :

- People who work with farm animals, especially cattle, sheep, goats and pigs:
- Farmers.
- Farm laborers.
- Animal attendants.
- Stockmen.
- Shepherds.
- Sheep shearers.
- Goatherds.

- Pig keepers.
- Veterinarians and inseminators are at risk through direct contact with infected animals or through exposure to a heavily contaminated environment[13].

*Brucella* has a low infectious dose (10 organisms of *B. melitensis* are sufficient to cause infection in man), making infection a genuine risk to those occupationally exposed such as farmers, veterinarians and butchers and to the public through the consumptionof contaminated unprocessed milk, milk products and meats[5].

## 1.7 Pathogenesis:

### 1.7.1 Incubation period:

Incubation period is estimated at five days to three months. Most infections seem to become apparent within two weeks. Aerosolization of bacteria in biological weapons could result in a shorter incubation period[14].

### 1.7.2 Pathogenicity:

*Brucella* species are facultative intracellular bacteria that can multiply within phagocytic cells with human beings as end hosts. *Brucella* may enter the host via ingestion or inhalation, or through conjunctiva or skin abrasions. After infecting the host, the pathogen becomes sequestered within cells of the reticuloendothelial system[20].

### 1.7.3 Clinical Signs:

Typically, brucellosis begins as an acute febrile illness with nonspecific flu-like signs such as fever, headache, malaise, back pain, myalgia and generalized aches. Drenching sweats can occur, particularly at night. Splenomegaly, hepatomegaly, coughing and pleuritic chest pain are sometimes seen. Gastrointestinal signs including anorexia, nausea,

vomiting, diarrhea and constipation occur frequently in adults but less often in children.

### 1.7.4 Complications:

Complications are seen occasionally, particularly in the undulant and chronic forms. The most common complications are arthritis, spondylitis, epididymo-orchitis and chronic fatigue. Neurological signs occur in up to 5% of cases. They may include personality changes, meningitis, encephalitis and peripheral neuropathy. Uveitis, optic neuritis and papilledema have been reported. Endocarditis is one of the most serious complications, and is often the cause of death in fatal cases[14].

### 1.7.5 Stages of illness:

1. Acute stage: Fever, malaise, sweating, hepatosplenomegally, lymphadenopathy Associated with 80% spontaneous recovery.
2. Chronic stage: Generally associated with hypersensitivity manifestations like fever, chest pain, and arthritis.

## 1.8 Diagnosis:

### 1.8.1 Specimen:

Blood or bone marrow (iliac crestspecimen) for culture in the acute stage of infection[7].

### 1.8.2 Collection of blood :

Whenever possible blood should be collected before antimicrobial treatment has started. When the patient has recurring fever, collect the blood as the temperature begins *torise*. For other patients, collectthe blood as soon as possible after receiving therequest. To increase the chances of isolating a pathogen, it is usually recommended that at least two specimens (collected at different times) should be cultured.

A strict aseptic technique must be used to collect the blood inoculate culture media:

Using a pressure cuff, locate a suitable vein in the arm. Deflate the cuff while disinfecting the veinepuncture site. Wearing gloves, thoroughly disinfect the veinpuncture site as follows**.** Lift back the tape or remove the protective cover from the top of the culture bottle(s). Wipe the top of the bottle using an ethanol-ether swab. Using a sterile syringe and needle, withdraw about 20 ml of blood from an adult, or about 2 ml from a young child. Insert the needle through the rubber liner of the bottle cap and dispense 10–12 ml of blood into the diphasic culture medium bottle containing25 ml of broth. Using a fresh ethanol-ether swab, wipe the top of each culture bottle and replace the tape or protective cover(s). Without delay, mix the blood with the broth and mix the blood in the EDTA container. Clearly label each bottle with the name and number of the patient, and the date and time of collection. As soon as possible, incubate the inoculated media. Protect the cultures from direct sunlight until they are incubated[7].

## 1.8.3 Direct detection methods:

Direct stains of clinical specimens are not particularly useful for the diagnosis of brucellosis .Preliminary studies using conventional and real-time polymerase chain reaction assays indicate that these assays may prove to be reliable, sensitive, and specific means to directly detect *brucella spp* In clinical specimens[2].

## 1.8.4 Culture:

*Brucella* species are difficult to isolate, particularly *B. abortus*(rarely isolated from blood culture). The organisms are more likely to be isolated from the blood in acute brucellosis during times of fever. Isolation is extremely rare in chronic brucellosis.

Tryptone soya (tryptic soy) diphasic medium(Castaneda) is recommended for the isolation of *Brucella* species. Several commercially produced blood culture systems are also suitable and some provide rapid isolation. Brucellae are aerobic with *B. Abortus* requiring a carbon dioxide enriched atmosphere. They grow over a temperature range 20–40 ºC with an optimum of 37 ºC. Cultures should be kept for 4 weeks with subculturing every few- days. When subcultured on solid agar, colonies usually appear 2–3 days after incubation .A variety of colonial forms are produced by *Brucella* strains including smooth, mucoid, and rough colonies. They may be colour less or grey white .Subculturing on a slope of glucose tryptone agar with a lead acetate test strip in the neck of the tube is useful in testing for hydrogen sulphide (H2S)production. *B. melitensis* is H2S negative, most strains of *B. abortus* and *B. suis* are H2S positive[7].

## 1.8.5 Biochemical tests:

Only a few routine biochemical tests are helpful in differentiating *Brucella* species. All *Brucella* strains are catalase positive and usually oxidase positive (some strains of *B. abortus* are oxidase negative). They are indole negative and most strains hydrolyze urea[7].

## 1.8.6 Serological tests:

Serological diagnosis of brucellosis using rapid IgM/IgG immunochromatographic assay. A rapid and easy perform immunochromatographic test in cassette form has been developed recently by the Royal Tropical Institute (KIT) in Amsterdam to diagnose acute, subacute, chronic, and relapsing brucellosis. It can also be used to monitor treatment. The assay detects separately *Brucella* specific IgM and IgG antibodies. IgM antibodies develop early in brucellosis and remain present for several weeks to months following recovery.

IgG antibodies develop later and can persist for months to years after recovery. During and following treatment, antibody reactivity decreases. After 6 months, antibody levels are low or undetectable. The IgM/IgG immunochromatographic assay is performed by adding 5 _l patient's serum to the sample well of the test device followed by running fluid reagent. The result is read after 10–15 minutes. A positive test is indicated by the appearance of twopink lines, an upper line in the control area and a lower line in the test area. The intensity of colour reaction in the test area corresponds to the level of antibody, with a weak colour reaction being recorded as 1+ antibody reactivity, a moderatereaction as 2+, strong reaction as 3+ and a very strong reaction as 4+[7].

## A . Rose Bengal test:

This is a rapid slide agglutination test with a buffered stained antigen . it is widely used as a screening test in farm animals , but also give good results in human brucellosis . it is not affect by prozones or immunoglobulin switching.[4]

## B . Tube agglutination test :

This test is required to determine the antibody titre. It should also be performed when a patient with a negative slide testcontinues to show symptoms of brucellosis. The technique for the standard tube test will be provided by the manufacturer of the antigen reagents. Usually two-fold dilutions of serum from 1 in 20 to 1 in 640 in 0.4% phenol saline are tested. Most tube tests are read after 24–48 h incubation at 37 ºC. Control positive and negative *Brucella* sera must be included with each batch of tests.

## C . Complement fixation test :

As the disease progresses from the acute to the chronic phase and the organism become localized intracellularly in various parts of the

body, the IgM antibodies decrease; The agglutination titer falls and may become undetectable even while the patient is still ill.the absence of agglutination therefore dose not rule out the possibility of infection.IgM and IgA antibodies that remain present in the serum and are no longer capable of agglutinating may be detected by complement fixation or ELISA tests[4].

### D . Enzyme –linked immunosorbent assay

The ELISA for IgG and IgA antibodies shows a good correlation with active disease ,especially in long-standing infection. It has largely replaced the anti-human globulin (Coombs) test formerly used for detecting non-agglutinating (IgG) antibodies[4].

### 1.7.7 Intradermal tests:

The development of delayed hypersensitivity to intradermally administered *Brucella*-specific antigens is an indication of past exposure to infection but does not indicate its current significance. Although used in the past in some countries, the intradermal test is not recommended for diagnosis. The use of undefined and unstandardized antigen preparations may also provoke antibodies which interfere with subsequent serological tests[13].

### 1.7.8 Antimicrobial susceptibility test:

*Brucellae* are susceptible to many antimicrobials. Because the organisms are intracellular, they are difficult to eradicate. Tetracycline combined with streptomycin is the usual treatment. Antimicrobial resistant strains of *Brucella* have been reported[7].

## 1.8 Treatment:

The treatment recommended by the World Health Organization for acute brucellosis in adults is

1-rifampicin 600 to 900 mg .

2-doxycycline 200 mg daily for a minimum of 6 weeks[3].

## 1.9 Prevention:

Human brucellosis is usually prevented by controlling the infection in animals. Pasteurization of dairy products is an important safety measure where this disease is endemic. Unpasteurized dairy products and raw or undercooked animal products (including bone marrow) should not be consumed.

Good hygiene and protective clothing/equipment are very important in preventing occupational exposure. Precautions should be taken to avoid contamination of the skin, as well as inhalation or accidental ingestion of organisms when assisting at a birth, performing a necropsy, or butchering an animal for consumption. Particular care should be taken when handling an aborted fetus or its membranes and fluids. Risky agricultural practices such as crushing the umbilical cord of newborn livestock with the teeth or skinning aborted fetuses should be avoided.

The Strain 19 *B. abortus* vaccine and *B. melitensis*Rev-1 vaccine must be handled with caution to avoid accidental injection or exposure. Adverse events have also been reported with the *B. abortus*RB51 vaccine, although it is safer than Strain 19. Persistent infections with vaccine strains have occasionally beenreported in vaccinated animals. These strains can be shed in the milk or aborted fetuses and can infect humans. Obstetricians should also take precautions when assisting at human births, particularly in regions where brucellosis is common. Recently, an obstetrician became infected by ingesting amniotic fluid and secretions from a congenitally infected infant. In the laboratory, *Brucella spp*. should be handled under biosafety level 3 conditions or higher. Human vaccines are not available[14].

## 2.1 Study design:

This cross-sectional study done on 64 Yemeni butchers in Thamar city during the period from May to December 2012 , the central butchery and selling shop of meat selected for performing present study.

## 2.2 Study area:

This cross-sectional study was conducted in Thamar city among persons who deal with meat (selling and butchering ) .

## 2.3 Sample size:

Approximately our study selected all butchers that work in central butchery and selling shop of meat in Thamar city,Yemen (64 specimens).

## 2.4 Materials:

Disposible syringe(5ml),Gloves, cotton ,Alcohol,Test tubes(5ml) ,Rack, Centrifuge ,Microscope ,Rotator and Rose bengal reagent .

## 2.5 Sample collection:

- 3ml of fresh blood was collected from (64 butchers) as following :

1- Disinfected the site of blood collection with 70% alcohol. Drawen 3 2-cc of blood .

3- Labeled number of the sample on the tube .

4- Asked all persons selected with some question related to our study (Appendix).

5-Send the sample to General Thamar hospital .

## 2.6 Sample analysis:

### Serological examination (SAT)

- In Thamar hospital performed lab diagnosis of our study as following:

1- centrifuged the samples to obtained serum.

2- By pipette taken the serum from tube .

3-Placed tow drops on the new slide (one drop for *B . melitensis* and one for *B.abortus* ).

4- Added 1 drop of antigen reagent to each sample drop.

5-Placed the slide on a mechanical rotator at 80-100 r.p.m for 1 minute .

6- After 1 minute we examine the slid agglutination under microscope.

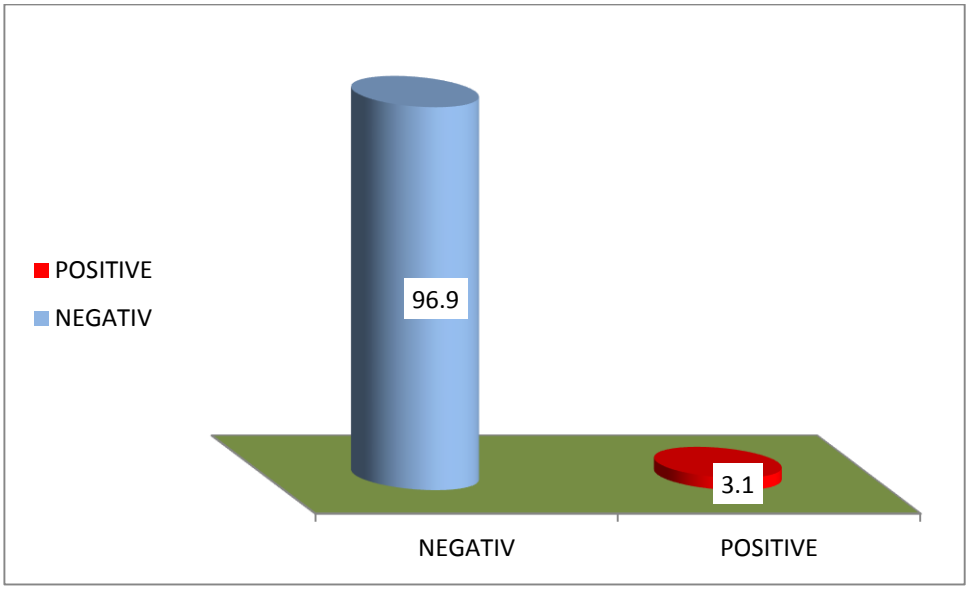
7- If seen agglutination the result is positive , and if we seen no agglutination the result is negative [7].

## 2.7 Result analysis

All results were registered, analyzed using SPSS14, Where the confidence Intervalue(CI) was 95%, and their significances by chi-square P-value less than or equal 0.05% was considered to indicate statistical significance documented.

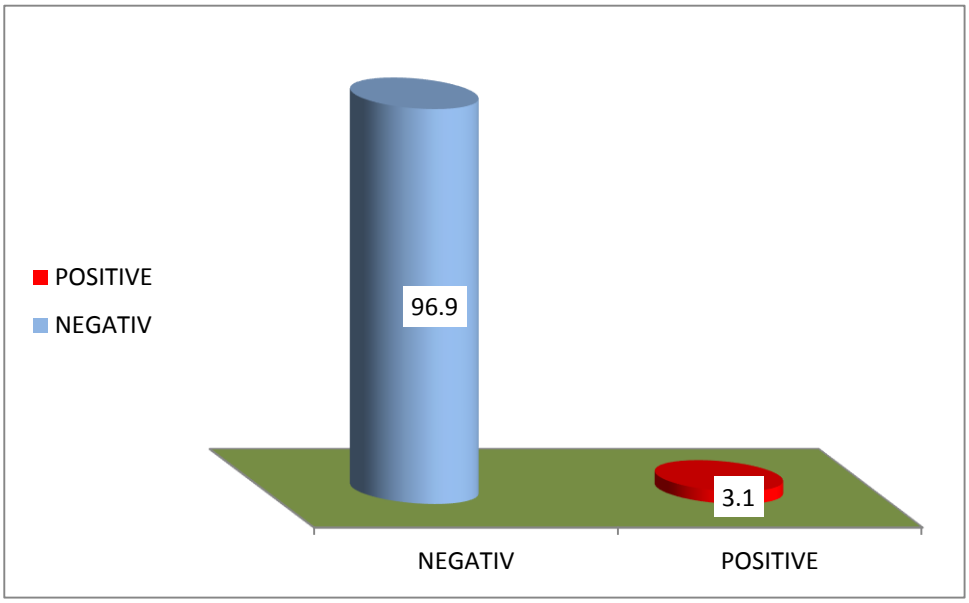
## 3.1 Result:

From a total of 64 specimens were analysis only two samples were positive for brucellosis and the prevalence was 3.1% which statistically significant (p.value <0.001) (figure1). the results presented in tables (2 to 7) and figures (1 to3).

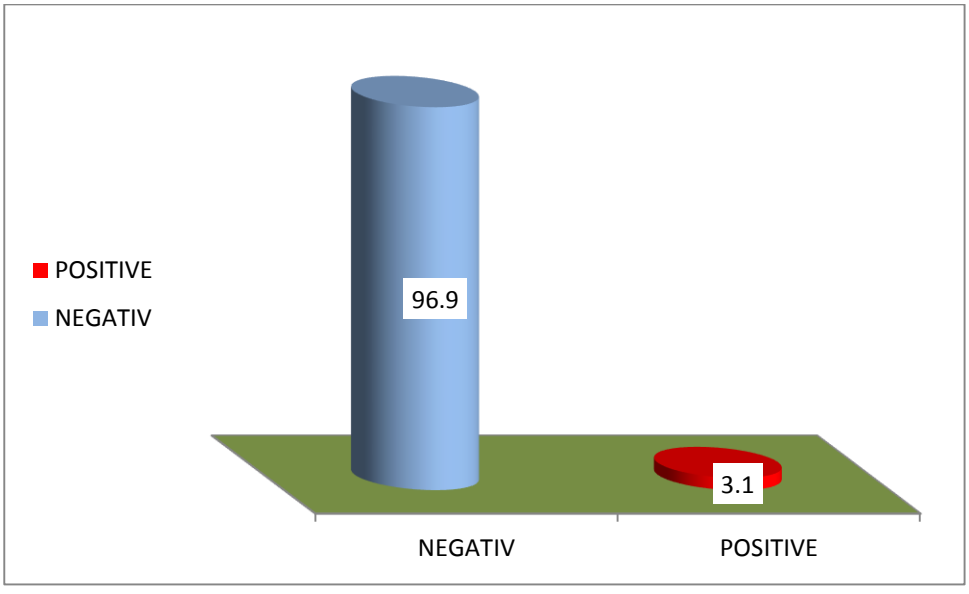



Fig(1): **prevalence of brucellosis among butchers in Thamar city**

The two species of *B. abortus* & *B. melitensis* were detected in both two positive samples (figures 2 &3).




Fig(2): **prevalence of *B.melitensis* among butchers in Thamar city**




Fig(3): **prevalence of *B. abortus* among butchers in Thamar city**

Our results showed that there are correlation between examination of cattle before butchering in the central butchery by veterinary and positive samples(table 2).

**Table 2:** correlation between examination of cattle before butchering and result

| Examination of cattle before butchering | Result | | | | Total(64) | | *p.value* |
|---|---|---|---|---|---|---|---|
| | Brucellosis | | Healthy | | | | |
| | No | % within examination of cattle before butchering | No | % within examination of cattle before butchering | No | % within total | 0.024 |
| Yes | 1 | 1.7% | 58 | 98.3% | 59 | 92.18% | |
| No | 1 | 20% | 4 | 80% | 5 | 7.82% | |
| Total | 2 | 3.1% | 62 | 96.9% | 64 | 100% | |

There are no correlation between others risk factors and positive samples (Table 3-7).

**Table 3 :**Correlation between type of meat the butchers dealing with and result.

| Type of meat | Result | | | | Total(64) | | *p.value* |
|---|---|---|---|---|---|---|---|
| | Brucellosis | | Healthy | | | | |
| | No | % within type of meat | No | % within type of meat | No | % within total | 0.45 |
| Cow | 0 | 0% | 14 | 100% | 14 | 21.78% | |
| Sheep | 0 | 0% | 0 | 0% | 0 | 0% | |
| Both | 2 | 4% | 48 | 96% | 50 | 78.12% | |
| Total | 2 | 3.1% | 62 | 96.9% | 64 | 100% | |

**Table 4:** Correlation between source of meat and result

| Source of meat | Result | | | | Total(64) | | *p.value* |
|---|---|---|---|---|---|---|---|
| | Brucellosis | | Healthy | | | | |
| | No | % within source of meat | No | % within source of meat | No | % within total | 0.85 |
| National | 1 | 2.8% | 35 | 97.2% | 36 | 56.25% | |
| Other countries | 0 | 0% | 0 | 0% | 0 | 0% | |
| Both | 1 | 3.6% | 27 | 96.4% | 28 | 43.75 | |
| Total | 2 | 3.1% | 62 | 96.9% | 64 | 100% | |

**Table 5 :** correlation between national source of meat and result.

| National source of meat | Result | | | | Total(64) | | *p.value* |
|---|---|---|---|---|---|---|---|
| | Brucellosis | | Healthy | | | | |
| | No | % within national source of meat | No | % within national source of meat | No | % within total | 0.36 |
| Thamar | 0 | 0% | 17 | 100% | 17 | 26.56% | |
| Other governorate | 0 | 0% | 2 | 100% | 2 | 3.12% | |
| Both | 2 | 4.4% | 43 | 95.6% | 45 | 70.32% | |
| Total | 2 | 3.1% | 62 | 96.9% | 64 | 100% | |

**Table 6 :** Correlation between type of dealing with meat and results.

| Type of dealing with meat | Result | | | | Total(64) | | p.value |
|---|---|---|---|---|---|---|---|
| | Brucellosis | | Healthy | | | | |
| | No | % within type of dealing with meat | No | % within type of dealing with meat | No | % within total | |
| Butchering | 1 | 7.7% | 12 | 92.3% | 13 | 20.32% | 0.61 |
| Selling | 0 | 0% | 20 | 100% | 20 | 31.25% | |
| Both | 1 | 3.2% | 30 | 96.8% | 31 | 48.43% | |
| Total | 2 | 3.1% | 62 | 96.9% | 64 | 100% | |

**Table 7 :** Correlation between site of butchering and result.

| Site of butchering | Result | | | | Total(64) | | p.value |
|---|---|---|---|---|---|---|---|
| | Brucellosis | | Healthy | | | | |
| | No | % within Site of butchering | No | % within Site of butchering | No | % within total | |
| Butchery | 1 | 1.7% | 57 | 98.3% | 58 | 90.62% | 0.12 |
| Shop | 1 | 20% | 4 | 80% | 5 | 3.12% | |
| Both | 0 | 0% | 1 | 100% | 1 | 1.56% | |
| Total | 2 | 3.1% | 62 | 96.9% | 64 | 100% | |

## 4.1Discussion

Humans get infected by direct contact with infected animal products, ingestion of contaminated food, and inhalation of contaminated aerosols. Brucellosis is an occupational disease, of those who come in contact with infected animals and their products such as abattoir workers, veterinarian, slaughterers, laboratory workers, shepherds and farmers.
Our study involve 64 butchers that works in butchery and shops of meat selling in Thamar city. The prevalence rate of brucellosis in butchers was (3.1%) which was statistically significant (p.value<0.0001) .

In compare with previous studies we found that our study approximately agree with study performed in Brazil, which revealed that the prevalence of brucellosis among butchers was found to be 4.1%[21].

While it is not agree with other studies performed in other countries such as one study from Pakistan published in 1992 ( less sensitive test used) reported that the prevalence of brucellosis was 8.33% , another studies from Saudi Arabia , Algeria , Pakistan and Yemen were reported that the prevalence was found to be 35%, 37.9%, 45.2% and 25.5% respectively[21].

The previous studies were tested using ELISA so that the results differ from our study may be due to the less sensitive test (serum agglutination) which used in our study.

Our study showed that there are a strong correlation between positive samples and examination of cattle before butchering in central butchery by veterinary (p. value :<o.o5) (table 2.)

The low prevalence of brucellosis in our study it may because the cattle and sheep are examined at central butchery before butchering.

In previous study performed on milk of cattle (goats ,sheep and cow ), in Thamar governorate in 2005, reported that the prevalence of brucellosis in Cow, Sheeps, and goats was found to be 0.8% , 2.6% and 2% respectively, since this study reveal low prevalence in animals that may reflect the reason of low prevalence of brucellosis among butchers in the present study [1].

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## seroprevalence of brucellosis among butchers in Thamer city

## Questionnaire :

Name: …… Lab No……..

| | | | |
|---|---|---|---|
| 1-what is the type of meat do you deal with | Cow | Sheep | Both |
| 2-what is the source of cattle | National | Others countries | Both |
| 3-what is the national source of cattle | Thamar | Others governorate | Both |
| 4-What is the type of dealing with meat | butchering | selling | both |
| 5- where is the site of butchering | Central butchery | Shop | both |
| 6- do you examine the cattle in central butchery by veterinary before butchering | Yes | No | |

| **RESULT** | Positive | | Negative | | Titer |
|---|---|---|---|---|---|
| | *B.abortus* | *B.melitensis* | *B.abortus* | *B.melitensis* | |
| | | | | | |

Signature ……..