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Assessment of Brucellosis among Butchers and non-Butchers in Sana'a city, Yemen

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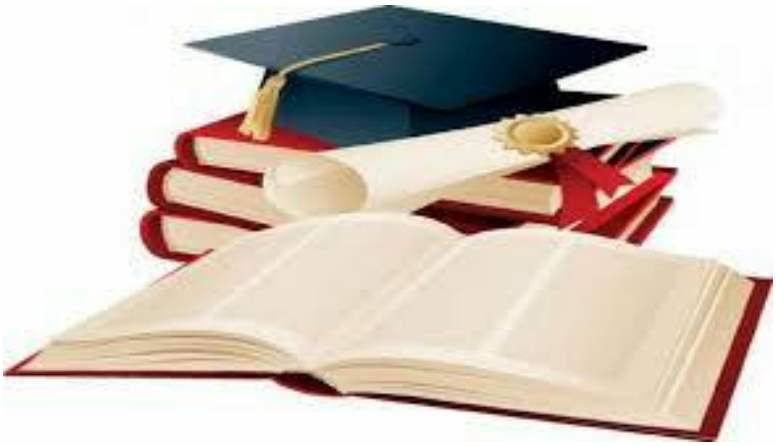

﴿وَما أُوتِيْتُمْ مِنَ العِلْمِ إلا قَلِيلًا﴾

صدق الله العظيم (الإسراء ٨٥)

Abstract

Background: Brucellosis is one of the major zoonosis globally with great veterinary and public health importance, particularly in developing countries where people are having frequent contact with livestock and animal products.

Objectives: To assessment the seroprevalence of brucellosis among butchers and non-butchers in Sana'a city, Yemen, to identify the risk factors associated with brucellosis, to compare the seroprevalence of brucellosis among butchers and non butchers, and to assess people knowledge and practice about brucellosis.

Methods: This cross-sectional study was conducted in Sana'a city from June 2021 to November 2021toassessment of seroprevalence of brucellosis among one hundred participants of butchers and one hundred participants of non-butchers from all 10 districts of Sana'a city, Yemen, whole blood samples were collected from each participant and separating by centrifugation, then sera were examined by using antibody agglutination test.

Also, the questionnaire was filled for each participant which include the age, knowledge about brucellosis, practice for brucellosis and risk factors as times of skin injuries and consumption of raw milk.

Results: The prevalence of *Brucella* antibodies results among participants out of 200 samples there were 52(26%) samples positive, the seroprevalence among butchers(33%) was higher than non butchers(19%). The difference was statistically significant with p. value = 0.024.

Conclusion: It can be concluded from this study, that the evidence of human brucellosis in Sana'a city, Yemen, seroprevalence of brucellosis was more common in butchers people.

Key words: *Brucella*, Brucellosis, Butchers, sera, Sana'a, Yemen.

Dedication

From ***Abubakr Al-mahmody***, head of the research group "Assessment of Brucellosis among butchers and non-butchers in Sana'a city " and all members of this group

To

- Research supervisor ***Dr -Muaath Al-safani,*** master degree in medical microbiology lecturer in microbiology department 21 September University.
- ***Pro. Dr-Ghamdan Al-Tahish***

Assistant professor of microbiology and molecular department.

- ***Dr/ Mohammed Helbob***

And we thank the supporting laboratories **''Al-Ain specialist**" and all the butchers and non-butchers who participated in our research.

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Symbol	Interpretation
CDC	Center for Disease Control
CFT	Complement fixation test
DNA	Deoxyribonucleic acid
ELISA	Enzyme-link immune sorbent assay
FM	Farrell's medium
FPA	Fluorescence polarization assay
IDA	Indirect antihuman globulin
IFN	Interferon
IL	Interleukin
IRC	International Research Conference
LPS	Lipopolysaccharide
MET	Mercap-to ethanol test
MRT	Milk ring test
PCR	Polymerase chain reaction
RT-PCR	Real-time polymerase chain reaction
RES	Reticuloendothelial system
RBT	Rose Bengal plate test
R	Rough
SAT	Serum agglutination test
STA	Standard tube agglutination
SDA	Serum Dextrose Agar
SAM	Slide agglutination method
S	Smooth

S-LPS	Smooth lipopolysaccharide
SAT	Standard agglutination test
TAT	Thematic Apperception Test
TMP-SMX	Trimethoprimsulfamethoxazole
TSA	Trypton soya Agar
TNF	Tumor necrosis factor
WHO	World Health Organization

Chapter one

Introduction and Literature Review

1 Introduction and Literature Review

1.1 introduction

Brucella is an aerobic, Gram-negative, non-fermenting, facultative intracellular, non-motile, non-spore-forming, cocci, coccobacilli or short rods based on DNA homology and represent a single species (Grimon *et al.*,1992 /Moreno *et al*,1990).Of six pathogenic Brucella species, humans are mainly affected by B. *abortus*, B. *melitensis*, B. *suis* and B. *canis* (Moreno, E.; Cloeckaert,*et al.*, 2002).

Brucellosis, commonly known as "undulant fever", "Mediterranean fever" or "Malta fever" is a leading cause of zoonosis worldwide caused by the bacterial genus *Brucella* (Corbel M *etal* 2006). The disease is known colloquially as undulant fever because of its remittent character. Brucellosis is still one of the most neglected zoonosis to economic and human health impact (WHO, 2006). It is an infectious disease caused by a bacteria belonging to the *Brucella* genus, capable of infecting humans as well as cattle, sheep, goats, pigs and other animals such as rats, dogs and whales (Cunha *et al*., 2003;Pessegueiro *et al*., 2003; OIE, 2009).

Man to man transmission of the disease is rare (Omer MK *et al*,2002). It is transmitted to humans by direct or indirect contact with infected animals predominantly domesticated ruminants and swine or their products (Corbel *et al*, 2006).

Stockpersons, other livestock workers handling cattle or carcasses, and consumers of raw milk and non-pasteurized milk products' are the main risk groups (WHO, 2006; Young 1995; FAO, 2002; Office of Public Health, 2008; Karadzinska-Bislimovska *et al*., 2010). Transmission of infection to humans occurs through breaks in the skin, following direct contact with tissues, blood, urine, vaginal discharges, aborted fetuses or placentas. Food-borne infection occurs following ingestion of raw milk and other dairy products. Occupational airborne infection in laboratories and abattoirs has also been documented. Accidental inoculation of live vaccines (such as *Brucella abortus* Strain 19 and *Brucella melitensis* Rev.1) can also occur, resulting in human infections (Robinson,

2003; Sayour *et al*., 2015). These routes of infection are more important in veterinarians, lab technicians, butchers and farmers who have direct contact with animals and their products such as blood, meat, placenta and fetus (S Beheshti,*et al*,2005).

The entry of the organism is through the conjunctiva, respiratory mucosa, digestive tract and damaged skin (Mesner *et al* 2007). The most common reported symptoms and signs were fever, headaches, fatigue, malaise, chills, sweats, myalgia, lack of appetite, weight loss and arthralgia(Ali *et al*,2013/ Young EJ.1989). Although brucellosis commonly presents as an acute febrile illness, its clinical manifestations vary widely, and definitive signs indicative of the diagnosis may be lacking.Thus the clinical diagnosis usually must be supported by the results of bacteriologic and/or serologic tests (Al-Shamahy and Wright, 2001; Edwards and Jawad, 2006).

Brucellosis affects about 500,000 individuals worldwide each year (WHO, 2006). Brucellosis is an endemic disease in the South and Central Asia, Middle East, North and East Africa, Mediterranean countries of Europe, and South America. The reported incidence of human brucellosis in endemic disease areas varies widely, from <0.01 to >200 per 100,000 population (Boschiroli et al., 2001). Nevertheless, in most countries the incidence of human brucellosis is unknown and it has been estimated that the incidence may be 25 times higher than the reported incidence due to under-reporting or misdiagnosis (WHO, 2006). The incidence of reported human brucellosis in countries of Arabian Peninsula including Yemen is very high ranged from 86 to 100 cases/10000/annum (Cooper, 1992; Al-Shamahy et al, 2002). Also brucellosis in animals well recognized in Arabian Peninsula. The seroprevalence in animals is ranged from 1% to 15% as reported from Kuwait, Saudi Arabia, Oman, and Yemen (Cooper, 1992; Ismali et al, 1995; Al-Shamahy et al 2001). In Yemen the seroprevalence of human brucellosis was ranging from 0.3 to 32.3% (Al-Arnoot S,*et al* 2017). which remains a major endemic health problem(Qais Y,*et al*,2018).

Transmissions of human brucellosis can be intervened through some specific measures such as vaccination of animals against brucellosis, provision of personal safety

measures, and intervention related to food safety (Khamassi Khbou,*et al* 2017). However, such specific measures depend on knowledge and awareness about the specific risk factors and status of infection (Seleem, M.N.; *et al* 2010). Its distribution is worldwide apart from the few countries where it has been eradicated from the animal reservoir (Al-Shamahy and Wright, 2001; Edwards and Jawad, 2006).

1.2 Literature review

1.2.1 Historical View

According to Hughes (1897), Brucellosis was first described by Hippocrates. An accurate description of the disease was made in 1861 by Marston, who was an assistant surgeon in the British Army stationed in Malta, and named it Malta fever (Edwards and Jawad, 2006). In 1886 David Bruce (1855-1931), a British army surgeon, isolated a *cocco-bacillus* that he named "*Micrococcus melitensis*" from the spleen of a man who had died of "Malta Fever (Plumb *et al*., 2013). After ten years of *Micrococcus melitensis* discovery, the Danish scientist Bernhard Bang identified "*Bacillus abortus*" (Brucella abortus) in bovine aborted fetuses (Bang, 1897). In 1914 another organism similar to Micrococcus melitensis which was finally designated as *Brucella suis* and isolated from aborted pigs in United States, (Traum, 1914).

Brucellosis caused by B. *suis* was first described swine herds in India. It was initially thought to be a pathogenic B. *abortus* but was later named B. *suis* (Alton, 1990). In 1918 this disease was further studied by many American Microbiologists and based on findings pasteurization of milk was proposed as a preventive measure for infection (Evans, 1918). Since 1920, in addition to B. *melitensis*, B. *abortus* and B. *suis*, at least 7 new species have been identified as belonging to the Brucella genus (Oslen and Palmer, 2014). Initially, *Brucella abortu*s, was named as Bacillus abortus by Bang in 1897 and eventually renamed in 1920(Longo *et al*., 2009). Finally, in 1920 Louis Meyer and

Wilbur Shaw honored David by proposing a Bruce single new genus named Brucella to group these pathogenic bacteria (Meyer and Shaw, 1920).

The first human blood culture of B. *abortus* (rather than B. *melitensis*) was obtained in 1924 (Keefer, 1924). In 1926, 46 human cases were reported in the USA (Spink, 1948). The first cases to be recognized in Iowa or Minnesota, where Brucellosis became a major endemic problem, occurred in 1927 (Hench *et al*., 1990). Many species of brucella, infections with B. *abortus*, B. *melitensis* and B *suis* are the most pathogenic to humans, considered as pathogenic, and priority pathogens by the USA Center for Disease Control (CDC) (CDC, 1997). In 1930 the greatest reliance was placed on various therapeutic vaccines (Dalrymple-Champneys, 1935).Nevertheless, there were small trials of numerous drugs (Neumann, 1936). However, 1938 the first drug to be tried widely that appeared to hold promise for a rapid effect was sulfanilamide (Blumgart, 1938; Bynum, 1939).

Trying for discovering a vaccine was between 1947 and 1951 also antibiotic therapy became established, although without complete reliability (Pulaski and Amspacher, 1947). The latter report was the first to find streptomycin combined with oral sulfadiazine to possibly be effective (Eisele and McCullough, 1947). Next, in 1948, the trial of chlortetracycline (aureomycin) was made in Mexico, initially combined with sulfadiazine. This drug had the practical advantage over streptomycin of oral administration and lack of ototoxicity (Spink *et al*., 1948).

In 1950 cases in which aureomycin failed were reported, while chloromycein and tetramycin are equally effective and better tolerated than aureomycin (Green and Stein, 1950; Harris, 1950; Knight *et al*., 1951; Spink *et al*., 1951). In 1960 concluded that the tetracycline drugs constitute the treatment of choice and are recommended in an oral dose of 500 mg every six hours for a minimum of 21 days. If there is a relapse this course should be repeated combined with 14 days of streptomycin (Spink, 1960).

1.2.2 The Family of *Brucellaceae*

The *Brucellaceae* are a family of the gram-negative Rhizobiales. They are named after Sir David Bruce, a Scottish microbiologist. They are aerobic chemoorganotrophes. The family comprises pathogen and soil bacteria *Brucella*, the type-genus and brucellosis, a disease caused by *Brucella* (Plumb *et al*., 2013). A family of bacteria containing small, coccoid to rod-shaped, gram-negative cells that occur singly, in pairs, in short chains, or in groups. The cells may not show bipolar staining. Motile and no motile species occur. These organisms are parasites and pathogens that affect warm-blooded animals, including humans. The type genus is *Brucella*. The *Brucellaceae* family of bacteria is also widespread in marine mammals and causes brucellosis (Anwar and Choi, 2014).

1.2.3 The Genus of *Brucella*

Brucella are coccobacilli or short rods 0.6 to1.5 μm long by 0.5 to 0.7 μm in width. They are arranged singly and less frequently in pairs or small groups. The morphology of Brucella is fairly constant except in old cultures, where pleomorphic forms may be evident. *Brucella* are nonmotile. They do not form spores, flagella, or pili. True capsules are not produced (Ahmad *et al*., 2014).

Brucella are Gram-negative and usually do not show bipolar staining.They are not truly acid-fast but resist deceleration by weak acids, thus stain red by the Stamp's modification of Ziehl-Neelsen method, which is sometimes used for the microscopic diagnosis of brucellosis from smears of solid or liquid specimens (Shome *et al*., 2014).

1.2.4 The Species of *Brucella*

Ten *Brucella* species are currently recognized comprise Six Classical out of ten species, and four novel species of *Brucella*. *Brucella pinnipediae* and *Brucella cetacea* (*Brucella ceti*) which infect marine mammals but are potential human pathogens , as well as *Brucella microti* which isolated from common voles (Microtus arvalis) , and

Brucella inopinata which isolated from wound of a patient with clinical signs of brucellosis (Ancora *et al*., 2014; Poester *et al*., 2010).

1.2.4.1 Brucella melitensis

Gram–negative coccobacillus or short rod. This organism is a facultative intracellular pathogen. *Brucella melitensis* contains three biovars (biovar 1, 2 and 3). All three biovars cause disease in small ruminants, but their geographic distribution varies (Shome *et al*., 2014). *Brucella melitensis* is the type most frequently reported as a cause of human disease and the most frequently isolated from cases. It is the most virulent type and associated with severe acute disease. It is recorded as endemic in several countries and accounts for a disproportionate amount of human brucellosis. The organism is normally associated with infection in Sheep and Goats, but other species, including Dogs, Cattle and Camels can be infected.

In some countries, particularly in the Middle East, *Brucella melitensis* infection of Cattle has emerged as an important problem. Contrary to some traditional views, *Brucella melitensis* remains fully virulent for man after infecting Cattle. The bovine infection presents a particularly serious problem because of the large volume of infected milk that can be produced by an individual animal and becauseof the extensive environmental contamination that even single abortions or infected births can produce (Corbel, 2006).

1.2.4.2 Brucella abortus

Is the main causative agent of brucellosis in bovines and is reported to cause severe economic loss in farm animals in the form of various reproductive disorders like abortion, retained placenta in female animals and orchitis in male animals (Prajapati *et al*., 2014).

The infected animals excrete *Brucella* in milk that is the main source of infection in humans especially when it is consumed in raw form (Ali et al., 2013). *Brucella abortus* is the most widespread cause of infection, but associated with much less human disease.

Infection in man is often sub clinical and, where disease does occur, it is usually less severe than that caused by *Brucella melitensis* or *Brucella suis*. Cattle are by far the most common source of *Brucella abortus* but bison, buffalo, camels, dogs and yaks are important in some areas. (Ali et al., 2013; Robinson, 2003).

1.2.4.3 Brucella suis

Brucella suis is a Gram-negative, facultative, intracellular coccobacillus, capable of growing and reproducing inside of host cells, specifically phagocytic cells. They are also not spore-forming, capsulated, or motile. Flagellar genes, however, are present in the *Brucella suis* genome, but are thought to be cryptic remnants because some were truncated and others were missing crucial components of the flagellar apparatus. Interestingly, in mouse models, the flagellum is essential for a normal infectious cycle, where the inability to assemble a complete flagellum leads to severe attenuation of the bacteria (Taleski, 2010).

1.2.4.4 Brucella neotomae

Brucella neotomae is an economically important cause of epididymitis, orchitis and impaired fertility in rams. Similar symptoms have been reported in male red deer in New Zealand. Brucella neotomae is occasionally associated with abortion in ewes, and can cause increased perinatal mortality in lambs (CFSPH, 2009).

1.2.4.5 Brucella canis

Brucella canis face an abortion of their pre-developed fetus. Males face the chance of infertility; the reason is that they develop an antibody against the sperm. This may be followed by inflammation of the testes which will generally settle down a little while after. Symptoms do not only include testicular inflammation, infertility in males, and abortion in females. Another symptom is the infection of the spinal plates or vertebrae, which is called diskospondylitis (Brower *et al*., 2007).

1.2.4.6 Brucella ovis

Brucella ovis causes a genital infection of ovine livestock manifested by epididymitis, infrequent abortions, and increased lamb mortality. Passive venereal transmission via the ewe appears to be a frequent route of infection, but ram-to-ram transmission is also common. Infected ewes may excrete *Brucella ovis* in vaginal discharges and milk and, accordingly, ewe-to-ram and lactating ewe-to-lamb transmission could also be determinant mechanisms of infection. Accordingly, the ewes are as relevant as rams in the epidemiology of infection, and control or eradication of *Brucella ovis* is feasible only if females are included in the corresponding programme (OIE, 2009a).

1.2.4.7 Brucella pinnipedialis

Brucella pinnipedialis is a species of Gram-negative, non-motile, non-spore-forming coccoid bacterium, first isolated from a breast implant infection site (Scholz *et al*., 2009).

1.2.4.8 Brucella ceti

Brucella ceti is a species of *Brucella*. It causes infections and related diseases in cetaceans (Foster *et al*., 2007).

1.2.4.9 Brucella microti

Brucella microti is a species of bacteria, first isolated from the common vole, Microtus arvalis. Its genome has been sequenced. It is Gram-negative, non-motile, non-spore-forming, coccoid, with the type strain. It is pathogenic (Audic et al., 2009).

1.2.4.10 Brucella inopinata

Is a species of Gram-negative, non-motile, non-spore-forming coccoid bacterium, first isolated from a breast implant infection site. It is a potential pathogen for Brucellosis (Scholz *et al*., 2009).

Table(1.1):-DifferentialCharacteristicsofspeciesoftheGenus*Brucella*

Species	Colony morphology	Serum Requirement	Oxidase	Urease	Preference host
Brucellamelitensis	**S**	**–**	**+**	**+j**	**Sheep,Goat**
Brucellaabortus	**S**	**–**	**+e**	**+f**	**Cattleandother Bovid**
Brucellasuis	**S**	**–**	**+**	**+h**	**Biovar1:Swine, Hare** **Biovar2: Swine** **Biovar3: Swine** **Bovar4:wild Reindeer** **Biovar5:wildrodents**
Brucellaneotomae	**S**	**–**	**-**	**+h**	**Desert Wood rat**
Brucellacanis	**R**	**–**	**+**	**+h**	**Dogs**
Brucellaovis	**R**	**+**	**-**	**-**	**Rams**
Brucellapinnipedialis	**S**	**–**	**+**	**+**	**Pinnipeds**
Brucellaceti	**S**	**–**	**+**	**+**	**Cetaceans**
Brucellamicroti	**S**	**–**	**+**	**+**	**Vole(Microtus arvalis)**
Brucellainopinata	**S**	**–**	**+**	**+**	**?**

(Banaiand Corbel,2010).

S: smooth, R: rough

e-Some African isolates of *B.abortus* biovar3 are negative

f- Intermediate rate, except strain 544 and some field strains that are negative. h- Rapid rate.
j- Slow rate, except some strains that are rapid.

1.2.5 Morphology, culture and biochemical characteristics

Morphology, Culture and Biochemical Characteristics. It is generally accepted that, members of the genus *Brucella* are small, nonmotile, non-spore forming, gram-negative, cocci, coccobacilli or short rods 0.5-0.7μm in diameter and 0.5-1.5 μm in length. They occur singly, in pairs short chains or small groups (Hassan, 2009).

Brucella is aerobic, many strains require supplementary carbon dioxide10% for growth on primary isolation, the optimal temperature for growth is 37ºCand pH 6.6-7.4 (Garrity *et al*., 2005).

culture of *brucella* improved by addition of equine or bovine serum Culture basal media. Blood agar, Trypton soya Agar (TSA), Columbia Agar, Serum Dextrose Agar (SDA) and potato agar are used for isolation. There are some selective media made by addition of different antibiotics supplement to suppress the growth of organisms other than *Brucella* such as Farrell's medium and thayer-Martin's medium (OIE, 2009).

Biochemical characteristics of Brucella is catalase positive, oxidase positive but negative strains occurs, produce H2S, hydrolyze urea, acidB. *neotomae*, indole negative, methyl red negative, non- hemolytic and do not liquefy gelatin (Garrity *et al*., 2005).

1.2.6 Clinical features

Broucellosis almost invariably causes fever, which may be associated with profuse sweats especially at night. In endemic areas, brucellosis may be difficult distinguish from the many other causes of fever. However, two features recognized to distinguish brucellosis from other tropical fevers, such as typhoid and malaria. Left untreated of the fever of brucellosis shows an undulating pattern that persists for weeks before the

commencement of an afebrile period the may be followed by relapse (Gerberding *et al*, 2008).

The fever of brucellosis is associated with musculoskeletal symptoms and signs the clinical syndromes caused by the different no men species are similar, although B. *melitensis* tends to be associated more acute and aggressive presentation and B.*suis*with focal abscess induction. B. *abortus* infection may be more insidious in onset and more likely to become chronic (Mantur *et al*, 2007).

The incubation period varies from 1 w eek to several months, and the onset of fever and other symptoms may be abrupt or insidious. In addition to experiencing fever and sweats ،patients become increasingly apathetic and fatigued; lose appetite and weight; and have nonspecific myalgia, headache, and chills. Overall, the presentation of brucellosis often fitsone of three patterns: febrile illness that resembles typhoid but is less severe; fever and acute monoarthritis, typically of the hip or knee, in a young child; and long-lasting fever, misery ،and low-back or hip pain in an older man. In an endemic area (e.g., much of the Middle East). a patient with fever and difficulty walking into the clinic would be regarded as having brucellosis until it was proved otherwise (Memish *et al*, 2000; Al-Shamahy and Wright, 2001).

There may be gastrointestinal and nervous symptoms. Lymph nodes enlarge, and the spleen becomes palpable. Hepatitis may be accompanied by jaundice. Deep pain and disturbances of motion, particularly in vertebral bodies, suggest osteomyelitis. These symptoms of generalized Brucella infection generally subside in weeks or months, although localized lesions and symptoms may continue. After the initial infection, a chronic stage may develop, characterized by weakness, aches and pains, low-grade fever, nervousness, and other nonspecific manifestations compatible with psychoneurotic symptoms. Brucellae cannot be isolated from the patient at this stage, but the agglutinin titer may be high. The diagnosis of "chronic brucellosis" is difficult to establish with certainty unless local lesions are present. (Jawetz, Melnick, & Adelberg's Medical Microbiology Twenty-Seventh Edition, Pappas G, Akritidis N, *et al* 2005).

1.2.7 Reservoirs of infection

Reservoirs of infection Brucellosis is a zoonotic disease; hence the ultimate sources of infection are infected animals. The key species are the major food-producing animals: cattle, sheep, goats, pigs. Others, including bison, buffalo, camels, dogs, horses, reindeer and yaks are less important, but they can be very significant local sources of infection in some regions. Recently, the infection has also been identified in marine mammals, including dolphins, porpoises and seals, and these may present an emerging hazard to persons occupationally exposed to infected tissues from them. The risk of disease and its severity is to a significant extent determined by the type of Brucella to which an individual is exposed. This will be influenced by the species of host animal acting as source of infection (Abu Shaqra, 2000).

B.*melitensis* is the type most frequently reported as a cause of human disease and the most frequently isolated from cases. It is the most virulent type and associated with severe acute disease. It is recorded as endemic in several countries and accounts for a disproportionate amount of human brucellosis. The organism is normally associated with infection in sheep and goats, but other species, including dogs, cattle and camels can be infected. In some countries, particularly in the Middle East, B. *melitensis* infection of cattle has emerged as an important problem (Arroyo-Carrera et al, 2006). Contrary to some traditional views, B. *melitensis* remains fully virulent for man after infecting cattle. The bovine infection presents a particularly serious problem because of the large volume of infected milk that can be produced by an individual animal and because of the extensive environmental contamination that even single abortions or infected births can produce (Cooper, 1992).

B. *abortus* is the most widespread cause of infection, but associated with much less human disease. Infection in man is often sub-clinical and, where disease does occur, it is usually less severe than that caused by B. *melitensis* or B.*suis*. Cattle are by far the most common source of B. *abortus* but bison, buffalo, camels, dogs and yaks are important in some areas. B. *suis* has a much more restricted occurrence than B. *melitensis* and B.

abortus. It is locally important as a source of human infection which can be as severe as that produced by B. *melitensis* (Cirl *et al*, 2003).

1.2.8 Transmission of *Brucella*

The possible means of acquisition of brucellosis include: person-to-person transmission, infection from a contaminated environment, occupational exposure usually resulting from direct contact with infected animals, and food borne transmission.

1.2.8.1 Person-to-person transmission

This is extremely rare. Occasional cases have been reported in which circumstantial evidence suggests close personal or sexual contact as the route of transmission. Of more potential significance is transmission through blood donation or tissue transplantation. Bone marrow transfer in particular carries a significant risk. It is advisable that blood and tissue donors be screened for evidence of brucellosis and positive reactors with a history of recent infection be excluded. Transmission to attendants of brucellosis patients is most unlikely but basic precautions should be taken. Laboratory workers processing samples from patients run a much greater risk (Lulu *et al*, 1988; Khan, 2001; Kato *et al*, 2007).

1.2.8.2 Infection from a contaminated environment

This is difficult to document but probably occurs more frequently than is recognized. Infected animals passing through populated areas or kept in close proximity to housing may produce heavy contamination of streets, yards and market places, Inhalation brucellosis may then result from exposure to contaminated dust, dried dung etc. Contact infection may also result from contamination of skin or conjunctivae from soiled surfaces.

Water sources, such as wells, may also be contaminated by recently aborted animals or by run-off of rain water from contaminated areas. Brucella spp. can survive for long periods in dust, dung, water, slurry, aborted fetuses, soil, meat and dairy products. The

precise duration of survival is dependent on many variables such as the nature of the substrate, number of organisms, temperature, pH, sunlight, the presence of other microbial contaminants (Madkour, 2001).

1.2.8.3 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 labourers, 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.

Infection may occur by inhalation, conjunctival contamination, accidental ingestion, skin contamination especially via cuts or abrasions, and accidental self-inoculation with live vaccines. The families of farmers and animal breeders may also be at risk as domestic exposure may be inseparable from occupational exposure when animals are kept in close proximity to living accommodation. In some areas, the animals are kept in the yards of houses and may even be brought inside, especially in severe weather. In the case of recently aborted animals, this has resulted in infection of entire households. The use of dried dung as a fuel may also import infection into households. It should be noted that brucellosis often presents as clusters of cases in a family or tribal group, usually relating to a common infected food source, and often follows an outbreak in animals. Children can be particularly at risk as they may adopt newborn or sick animals as pets. In some areas they may be the only group presenting with acute symptoms, as older members of the community are likely to be immune or chronically infected (Mantur *et al*, 2007).

Persons involved in the processing of animal products may be at high risk of exposure to brucellosis. These include slaughter men, butchers, meat packers, collectors of fetal calf serum, processors of ides, skins and wool, renderers and dairy workers. Direct and environmental contamination may present hazards through inhalation, ingestion, mucous contamination and skin contact or penetration. Staff employed in the

maintenance of farm premises, factories or plants used for processing animal products are often overlooked as occupationally exposed groups but may be at considerable risk from environmental contamination. Laboratory staff involved in culturing *Brucella* are at particular risk.

In some countries in which brucellosis is no longer endemic, this potential hazard may be overlooked or considered no longer relevant. Nevertheless, the performance of diagnostic procedures on patients with unsuspected imported disease may lead to culture of organisms which are not correctly identified until laboratory-acquired infection raises the level of suspicion (Maley et al, 2006). For example: Rapid identification gallery test systems has caused *Brucella* strains to be misidentified as *Moraxella spp*, with serious consequences for the staff. Inhalation of aerosols generated by manipulation of cultures presents the greatest hazard, especially if breakage of containers occurs during such processes as centrifugation.

Also the preparation and use of live vaccines is hazardous as strains such as B. *abortus* S19 and B. *melitensis* Rev 1 are not completely avirulent for humans. The rough vaccine strain B. *abortus* RB 51 appears to be of low pathogenicity but still presents a potential hazard through accidental injection and is rifampicin-resistant. The use of virulent strains to prepare diagnostic antigens should also be avoided where possible (Memish and Mah, 2001; Robichaud *et al*, 2004; Maley *et al*, 2006; Mantur *et al*, 2007;).

1.2.8.4 Foodborne transmission

This is usually the main source of brucellosis for urban populations. Ingestion of fresh milk or dairy products prepared from unheated milk is the main source of infection for most populations. Cow, sheep, goat or camel milk contaminated with B. *melitensis* is particularly hazardous as it is drunk in fairly large volume and may contain large numbers of organisms. Butter, cream or ice-cream prepared from such milk also presents a high risk. Soft cheeses prepared from sheep or goats milk by addition of rennet are a particularly common source of infection in Mediterranean and Middle Eastern countries (Al-Shamahy *et al*, 2001; Murray and Corbel, 2006). The cheese-making process may

actually concentrate the Brucella organisms, which can survive for up to several months in this type of product. Hard cheeses prepared by lactic and propionic fermentation present a much smaller risk. Similarly, yoghurt and sour milk are less hazardous.

Brucella dies off fairly rapidly when the acidity drops below pH 4, and very rapidly below pH 3.5. Equipment used in the transport or processing of infected milk or other raw material may contaminate uninfected products unless good hygienic practice is observed (Wallach *et al*, 1994). Meat products are less frequently associated with infection, mainly because they are not usually eaten raw. However, this is a not unknown practice among butchers and abattoir workers.

Muscle tissue usually contains low concentrations of *Brucella* organisms but liver, kidney, spleen, udder and testis may contain much higher concentrations. In some countries, dishes prepared from these organs may be eaten raw or undercooked. Fresh blood, either alone or mixed with fresh milk, may also be drunk and presents an obvious potential hazard (Pappas, 2006). In many countries, the consumption of "health foods" has become fashionable. These often include unpasteurized milk or milk products and may pose a particular risk. There is often considerable resistance to accepting that such "healthy" products can be dangerous.

Raw vegetables may be contaminated by infected animals and present a hazard. In endemic areas, tourists consuming "ethnic" food products may be particularly at risk. Persons with achlorhydria resulting from disease or through consumption of antacids or H2 antagonists may have an increased risk of acquiring brucellosis through ingestion of contaminated foods. Individuals with immunodeficiency states resulting from disease or treatment with immunosuppressive agents may also be at increased risk of severe brucellosis, although this is difficult to quantify (Al-Dubooni *et al*, 1986; Alballa, 1995; Al-Shamahy *et al*, 2000; Al-Shamahy *et al*, 2001).

1.2.9 Entry into the host and evading the immune system

The most common portals of entry for brucella in animals and humans are mucous membranes of the respiratory (aerosol) (Franz *et al.,* 2001), and digestive tracts, and in the natural host, also the conjunctiva and membranes covering the sexual organs.

Bacteria are eventually, taken up by phagocytic cells (macrophages, dendritic cells,) and reach the regional lymph nodes, leading to subsequent systemic dissemination (Ackermann *et al.,* 1988; Salcedo *et al.,* 2008). As brucella cannot multiply outside their mammalian hosts, the most important aspect of brucella ecology is their ability to establish an intracellular replicative niche and remain protected from the host immune responses (Bargen *et al.*, 2012).

Brucella lack classic virulence factors like toxins, fimbriae and capsules which raises the possibility that they might have unique and subtle mechanisms to penetrate host cells, elude host defenses, alter intracellular trafficking to avoid degradation and killing in lysosomes and modulate the intracellular environment to allow long-term intracellular survival and replication (Delrue *et al.,* 2004; Debagues *et al.,* 2004; Lapaque *et al.*, 2005).

Brucella has developed mechanisms to avoid innate immunity by minimizing stimulation of pattern recognition receptors (PRRs) of the host. The brucella cell envelope has high hydrophobicity and its LPS has a non canonical structure that elicits a reduced and delayed inflammatory response compared with other gram-negative bacteria (Rittig *et al.,* 2001; Rittig *et al.,* 2003).

1.2.10 Pathogenicity and immunity

Brucella spp. are facultative intracellular organisms, surviving and multiplying within cells of reticuloendothelial system (RES) and their disease spectrum is partially explained by the ability of the organism to evade host defense mechanisms by virtue of intracellular existence. Survival and multiplication of Brucella organisms in phagocytic cells are features essential to establishment, development, and chronicity of the disease (Till, 2014). The infection spreads hematogeneously to tissues rich in elements of RES including the liver, bone marrow, lymph nodes and spleen. Organisms may also localize in other tissues, including joints, the central nervous system, the heart and the kidneys (Slack, 2004).

Brucella form granulomas made up of epitheloid cells, polymorphonuclear leukocytes, lymphocytes, and giant cells in tissues and organs. Granulomas are known to be more frequent in B. a*bortus* infections (Gul and Erdem, 2015). Release of bacteria into the peripheral circulation results in hematogenous seeding of other organs and tissues, thereby leading to the protean clinical manifestations of human brucellosis (Winn *etal*., 2006).

The *Brucellae* that infect humans have apparent differences in pathogenicity. B. *abortus* usually causes mild disease without suppurative complications; noncaseating granulomas of the reticuloendothelial system are found.B. *canis* also causes mild disease. B. *suis* infection tends to be chronic with suppurative lesions; caseating granulomas may be present. B. *melitensis* infection is more acute and severe. Persons with active brucellosis react more markedly (fever, myalgia) than normal persons to injected *Brucella* endotoxin. Sensitivity to endotoxin thus may play a role in pathogenesis. Placentas and of cattle, swine, sheep, fetal membranes and goats contain erythritol, a growth factor for brucellae. The proliferation of organisms in pregnant animals leads to placentitis and abortion in these species. There is no erythritol in human placentas, and abortion is not part of Brucella infection of humans. (Jawetz, Melnick, & Adelberg's Medical Microbiology Twenty-Seventh Edition, Pappas G, Akritidis N,et all 2005).

The response to infection and its outcome are influenced by the virulence, phase, and species of the infecting strain. Differences have been reported between B. *abortus* and B. *suis* in the modes of cellular entry and subsequent compartmentalization and processing.

The mechanisms of protective immunity against human brucellosis are presumed to be similar to those documented in laboratory animals. Exposure to brucellosis elicits both humoral and cell-mediated immune responses. Antibodies promote clearance of extracellular brucellae by bactericidal action and by facilitation of phagocytosis by

polymorphonuclear and mononuclear phagocytes; however, antibodies alone cannot eradicate infection (Arellano- Reynoso *et al*, 2005).

Furthermore, IgG levels decrease faster than IgM levels with treatment. Even after eradication of active infection, IgM antibodies can remain positive in low titers for months or even years. A high level of IgG and IgA antibodies for longer than 6 months is a sign of chronic infection or relapse (Gul and Erdem 2015).

Organisms taken up by macrophages and other cells can establish persistent intracellular infections. The key target cell is the macrophage, and bacterial mechanisms for suppressing intracellular killing and apoptosis result in very large intracellular populations. Opsonized bacteria are actively phagocytosed by neutrophilic granulocytes and by monocytes. In these and other cells, initial attachment takes place via specific receptors, including Fc, C3, fibronectin, and mannose-binding proteins. Opsonized but not unopsonized—bacteria trigger an oxidative burst inside phagocytes. Unopsonized bacteria are internalized via similar receptors but at much lower efficiency. Smooth strains enter host cells via lipid rafts. Smooth lipopolysaccharide (LPS), cyclic glucan, and possibly an invasion attachment protein (IalB) are involved in this process. Tumor necrosis factor (TNF-) produced early in the course of infection stimulates cytotoxic lymphocytes and activates macrophages, which can kill intracellular brucellae (probably mainly through production of reactive oxygen and nitrogen intermediates) and may clear infection (Corbel, 2006). However, virulent Brucella cells can suppress the TNF- response, and control of infection in this situation depends on macrophage activation and interferon (IFN) responses. Cytokines such as interleukin (IL) 12 promote production of IFN, which drives TH1-type responses and stimulates macrophage activation. Inflammatory cytokines, including IL-4, IL-6, and IL-10, downregulate the protective response. As in other types of intracellular infection, it is assumed that initial replication of brucellae takes place within cells of the lymph nodes draining the point of entry. Subsequent hematogenous spread may result in chronic localizing infection at almost any site, although the reticuloendothelial system, musculoskeletal tissues, and genitourinary system are most frequently targeted. Both acute and chronic inflammatory

responses develop in brucellosis, and the local tissue response may include granuloma formation with or without necrosis and caseation. Abscesses may also develop, especially in chronic localized infection (Almuneef and Memish, 2003; Roux et al, 2007).

1.2.11 Risk factors

The most important species of Brucella responsible for human disease in order of their significance are B. *melitensis*, B. *abortus*, and B. *suis* (Acha and Szyfres, 2003). Transmission of brucellosis occurs from ingesting (foodborne), directly contacting (penetration through breaks in the epidermis), and inhaling the organism (occupationally exposed). The most common means of exposure is eating contaminated animal products in endemic areas (Olsen and Palmer, 2014). The less common means of infection include person to person (Meltzer *et al.,* 2010; Wyatt, 2010), accidental infection with live vaccines (Strausbaugh and Berkelman, 2003; Ashford *et al.,* 2004) and through blood donation and tissue transplantation (WHO, 2006).

Brucellosis is also one of the most common laboratory-acquired infections. Laboratory workers while handling specimens containing Brucella species may generate aerosol and may results in infection (Noviello *et al.,* 2004). In countries where milk and dairy products are always pasteurized before consumption, brucellosis principally affect persons who are in close contact with animals and animal products (occupationally exposed) (Seleem *et al.,* 2010).

The occupationally exposed vulnerable groups include veterinary doctors, butchers, veterinary technicians, insemination service employees, zoo technicians, farmers working on multi-herd farms, employees of meat and milk processing enterprises (Galin ska and Zagorski, 2013).

Brucellosis may also occur through international travel to endemic countries. Tourists or business travelers to endemic areas may acquire brucellosis by consumption of unpasteurized milk or dairy products. They may also import contaminated cheese or

other dairy products into their countries and infect their families (Godfroid *et al.,* 2005; FAO, 2006).

Epidemiologic studies have identified the following risk factors for Brucella infection in general population which conducted in different localities in Yemen. The results of the studies on human brucellosis show significant risk factors for infection were found to related to occupation as a farmer, shepherd and microbiologist (Al- Shamahy *et al.*, 2000).

The highly significant risk factors for infection were related to be associated with ownership of livestock animals (Al-Haddad *et al.,* 2013), contact with placental membrane, clearing viscera of animals (Nasher, 2006) and direct contact with animals execration or products (Milking, Handling new born, Animal slaughter) and indirect contact with livestock (drinking unpasteurized milk (Al- Shamahy *et al.,* 2000; Nasher, 2006; Al-Haddad *et al.,* 2013), drinking laban (Al- Shamahy *et al.,* 2000; Al-Haddad *et al.,* 2013).

1.2.12 Epidemiology

The endemic of this disease is especially in countries of the Mediterranean basin, the Middle East, the Indian subcontinent and parts of Mexico and Central and South America. Human brucellosis is found to have significant presence in rural/nomadic communities where people live in close association with animals. Worldwide, reported incidence of human brucellosis in endemic disease areas varies widely, from <0.01 to >200 per 100 000 population (Nassaji *et al.,* 2008).

The Brucellosis 2003 International Research Conference (IRC) estimated that 500,000 human infections occur per year worldwide, with incidences ranging from less than one case per 100,000 population in UK, USA and Australia, through 20 to 30 cases per 100,000 in southern European countries such as Greece and Spain. B. *melitensis* is the type most frequently reported as a cause of human disease and the most frequently

isolated from cases. It is the most virulent type and associated with severe acute disease (Corbel *et al.*, 2006).

1.2.12.1 Prevalence of brucellosis in Global countries

Brucellosis in humans almost always originates from an animal reservoir (Godfroid et al., 2013). The highest prevalence of human disease is currently found in areas of Africa, Asia, Latin America, and the Middle East. Despite being endemic in many developing countries, brucellosis remains under diagnosed and underreported (Godfroid *et al.*, 2005).

The range of reported incidence of human brucellosis cases per 100,000 people per year are 0.28-268.81 in North Africa and Middle East, 34.86 in Sub-Saharan Africa, 0.03-32.49 in Western Europe, 88.0 in Central Asia, 12.8425.69 in Centraland Southern Latin America and 0.02-0.09 in North America (Dean *et al.*, 2012b).

Table(1.2): Summary of reports prevalence of human brucellosis in Global countries.

Country	Population	Sample size	Duration of study	Prevalence (%)
Brazil	Human brucellosis	455	2014	1. 98%
Iran	Human brucellosis	1698	2001 – 2009	24. 56%
Turkey	Hunan brucellosis	2913	2010	18%

1.2.12.2 Prevalence of brucellosis in Arab countries

Brucellosis in some Arab countries reported more than 70 cases per 100,000 such as Kuwait and Saudi Arabia (Yohannes *et al.*, 2013).

Humans brucellosis in the countries with the highest incidence of brucellosis include Saudi Arabia, and Palestinian Authority held areas, Syria, Jordan and Oman. But Bahrain is reported to have no incidence (Refai, 2002; Pappas *et al.*, 2006).

Brucellosis, a zoonotic disease of global importance, is endemic in Saudi Arabia (national seroprevalence, 15%) (Matyas and Fujikura, 1984).

Table (1.3) The prevalence of human brucellosis among some Arab countries

Country	Sample Size	Type of Study	Method	Duration of Study	Prevalence%
Egypt	480	Observational	STAT	2012	6.26%
Saudi Arabia	159	Cross Sectional	TAT	1995-2001	12.6%
KSA	159	S.C	Culture	1995-2001	25.9%

(Hassanain and Ahmed, 2012 -Fallatah *et al.,* 2005 , Assad & Alqahtani, 2012).

1.2.12.3 Prevalence of brucellosis in Yemen

In Yemen, there is a little information of animal and human brucellosis. The serological investigations and bacteriological isolations of brucella carried on the country are very scarce. In spite the disease is reported in all domestic ruminants of the country, Yemeni people lack awareness about the zoonotic potential of the disease with their existing habit of raw milk consumption and close contact with domestic animals. Destruction of human and animal brucellosis by test-and-slaughter is impracticable in developing countries including Yemen because of limited resources to compensate farmers whose animals are slaughtered during such screening programs (Al-Haddad *et al.,* 2013).

Also, the national program is not available for prevention and control of brucellosis in the country. Only, there is one veterinary lab for animals which located in Sana'a. The

main obstacles limiting the control of the disease are: security of the country, shortage of funds, laboratory facilities and trained manpower. Prevalence of brucella infection reported in humans in Yemen. Until now, no attempts have been made of brucella isolation, identification of genotypes and estimation of disease and the prevalence studies done in human are summarized below (Table 1.4).

Human brucellosis in Yemen: The seroprevalence of human brucellosis in the pervious studies was ranging from 0.3 to 32.3%. The first report was conducted a survey of *Brucella* antibodies among Yemeni blood donors. It investigated 1405 human serum samples using serum agglutination test (SAT) and revealed 0.4% positive. The prevalence founded from this study was in different localities which are Sana'a 0.7%, Taiz 0.8% and Hajja 0.35% (Al- Shamahy, 1997). Saleh, (2000) used STA to tested 385 human serum samples from slaughterhouse workers in different areas in Yemen. He reported that a high prevalence of *Brucella* antibodies were 27%. 32.3%, 25.5%, 25.7%, 26.2% and 22.2% positive in Sana'a, Aden, Taiz, AL- Hodeidah, Ibb and Hajjah respectively. Another study conducted in Sana'a used STA to tested 215 of serum samples and it showed 10.1% and 24.4% positive for males and females brucellosis respectively (Nasher, 2006).

The lower than prevalence of human brucellosis was conducted in Shabwah city with prevalence 2.2% in males and 1.6% in females (Agrah, 2011). In Al- Dala'a city the crude sero-prevalence of brucellosis among tested individuals was 6.7% for male was 5.5%, lower than that of female 7.2%. among 749 asymptomatic individuals from 3 randomly selected areas were included in the study (Al-Haddad *et al.,* 2013).

1.2.13 Brucellosis among slaughterhouse workers

Brucellosis is a disease which can be accidentally transmitted to men. It is widely distributed, having high morbidity and low mortality.

In slaughterhouse workers, contact with infection sources can be represented by carcasses and viscera of slaughtered animals and by formation of aerosols present in the slaughtering area.

Various *Brucella* species are well known causes of brucellosis in slaughterhouse workers by cattle, sheep, and goats.

Table (1.4) prevalence of human brucellosis in Yemen during 1993- 2011

City	Population	Sample Size	Type of Study	Duration of Study	Prevalence (%)
Hodeidah	Slaughterhouse	70	Cross Sectional	1999 to 2000	25. 7%
Sana'a	Slaughterhouse	75	Cross Sectional	1999 2000	27%
Taiz	Slaughterhouse	90	Cross Sectional	1999-2000	25%
Sana'a	Male	169	Cross Sectional	2005-2006	10%

(Saleh, 2000, Nasher, 2006, Al-Haddad *et al.,* 2013). C.S= cross sectional

1.2.14 Diagnosis of human brucellosis

The diagnosis of human brucellosis is usually based on the isolation of *Brucella spp*. from blood, tissue specimens, body fluids and bone marrow, the serological tests for the detection of anti-*Brucellaspp*. antibodies and the molecular methods for the detection of *Brucella* spp. (Sakran *et al.,* 2006). In countries where brucellosis is zoonotic (present in animal reservoirs), human confirmed cases are based on clinical symptoms associated with positive serology without attempts to isolate *Brucella* spp (CDC, 1997).

1.2.14.1 Isolation of brucella from blood and tissue

The confirmatory diagnosis of brucellosis necessitates the isolation of the pathogen from blood, bone marrow or other tissues and body fluids. The isolation of Brucella depends on the stage of disease (acute versus chronic), antibiotic pre-treatment, the existence of an appropriate clinical specimen and the culturing methods used. Isolation is much higher during the first two weeks of symptomatic disease and in blood cultures taken during the pyrexial phase (Memish *et al.,* 2000).

However, isolation of Brucella spp. has the risk of laboratory-acquired infections and is time consuming, and the sensitivity of culture method is often low (30 -70% in chronic cases and 80-90% in acute cases), depending on the culture medium, Brucella species, disease stage and quantity of circulating bacteria (Franco *et al.,* 2007; Espinosa *et al.,* 2009).

Brucella selective media such as Farrell's medium (FM) is most commonly used for the isolation due to the presence of nalidixic acid and bacitracin in FM, growth of B. *suis* and several B. *melitensis* and B. *abortus* strains can be significantly inhibited (Farrell, 1974). So, a new selective medium containing vancomycin, colistin, nystatin, nitrofurantoin and amphotericin B has been developed for veterinary samples (De Miguel *et al.,* 2011).

Bone marrow cultures have proven to be more sensitive (15-20% more than peripheral blood culture) than blood cultures for the detection of Brucella spp. at any stage of disease, and the mean time to detection is significantly reduced (Gotuzzo *et al.,* 1986; Mantur *et al.,* 2008).

This method has also proven its usefulness in patients treated with antibiotics. As bone marrow aspiration and biopsy is painful, the procedure should be restricted to seronegative patients in whom there is a strong clinical suspicion of brucellosis (Gotuzzo *et al.,* 1986).

1.2.14.2 Identification and Typing

Usually, the colonies of Brucella spp are recovered from clinical specimens on FM or other media, within 24–48 h of aerobic incubation or under 5–10% CO2 incubation at 37°C. The colonies of smooth Brucella strains are raised, convex, circular, translucent, and 0.5 mm to 1 mm in diameter. Colony morphology, as well as virulence, antigenic properties and phage sensitivity of the bacteria, is subject to changes after subcultivation or prolonged culture (more than four days) (Cardoso *et al.,* 2006).

In the clinical microbiology laboratory a few tests can be done for the presumptive identification of the pathogen, namely showing Gram-negative coccobacilli, positive oxidase and catalase tests, and positive slide agglutination reaction with Brucella-specific antisera (Alton GG *et al.,* 1988; Corbel MJ 1997).

The identification of brucella species and biovars is based on CO2 requirement, H2S production, urease activity, agglutination with monospecific sera, selective inhibition of growth on media containing dyes such as thionin or basic fuchsin, and phage typing. Thus, smooth brucellae dissociate to rough forms, which grow in less convex and more opaque colonies with a dull, dry, yellowish-white granular appearance (Alton *et al.*, 1988).

Developed a powerful tool for bacterial identification termed as matrix-assisted laser desorption ionization time of flight mass spectrometry. In comparison with conventional phenotypic techniques or molecular methods, it is rapid, precise and cost effective. Using this technology, brucella can be identified and differentiated up to the species and biovar level within 48 hours (Ferreira *et al.,* 2010).

1.2.14.3 Serological diagnosis

Serological testing is fast, non-hazardous and more sensitive than culture and therefore preferred in routine clinical practice. However, serological tests can only indirectly prove brucella infections by high or rising titers of specific antibodies. Agglutination titers ≥1: 160 or a fourfold rise of titers in follow-up sera are considered to be indicative of active infection. Diagnostic titers, however, can be detected months or

even years after acute infection despite therapeutic success and negative blood cultures (Ariza *et al.,* 1992).

In addition, a high proportion of the population in endemic regions may have persistent antibody titers due to ongoing exposure to Brucella. Validation of serological tests and setting cut-off in the context of its use are crucial for the diagnosis of human brucellosis (Kiel and Khan, 1987; Memish *et al.,* 2002).

Serological test also allows the monitoring of treatment response. In the first week of infection, immunoglobulin IgM isotype antibodies predominate, followed by a shift to IgG in the second week (Al Dahouk *et al.,* 2002).

Just detection of antibody without clinical signs and symptoms or a history of potential exposure does not indicate brucellosis (Alton *et al.,* 1988; Ariza *et al.,* 1992; Al Dahouk and Nockler, 2011). In this study, the SAT was used for the diagnosis of brucellosis in slaughterhouse workers and general population. The principle and procedures are similar as described in next section.

The test is performed in tubes by reacting a known standardized volume and concentration of whole *Brucella* cell suspension with a standardized volume of doubling serum dilutions, usually ranging from 1:20 to 1:1280. The suspension mixture is incubated in a water bath at 37°C for 24 h, and agglutination at the bottom of the tubes is examined visually. Most authors considered a STAT titer of 1:160 as diagnostic in conjunction with a compatible clinical presentation (Konstantinidis *et al.,* 2007; Gomez *et al.,* 2008).

WHO and OIE provide guidelines for STAT and RBT standardization, but not for the ELISA. In general a new cut-off should be determined under local conditions to avoid false positives (Rahman, 2015).

There are many other serological tests for demonstrating *Brucella* antibodies in serum, milk, whey, vaginal mucus, semen, and muscle juice. The commonly used tests are (MRT), (SAT), (coombos') test, (2-ME), (RBPT), and (ELISA) (Morgan *et al.,* 1969).

1.2.14.4 Rose Bengal Plate Test (RBPT)

RBPT is often used to screen entire herds for evidence of infection with brucellosis (Alton *et al.,* 1975; Sutherland, 1980; Alton *et al.,* 1988; MacMillan, 1990).

The sensitivity of RBPT is very high (>99%) but the specificity can be disappointingly low. As a result, positive test results require confirmation by a more specific test (Barroso-Garcia *et al.,* 2002).

The principle of the test depends on an antigen-antibody reaction resulting in agglutination smooth brucella culture stained with Rose Bengal dye is mixed in a buffered acidic suspension and mixed with an equal volume (drops) of serum (Sutherland, 1980; Alton *et al.,* 1988). The acidic buffer is used to decrease problems associated with non-specific agglutination (Corbel, 1972).

Primarily, the RBPT detects both (IgG) and IgM (Allan *et al.,* 1976). A high sensitivity of the test is one of its key features with only 0.4 to 1.8% of RBPT seronegative animals testing positive on the CFT (Myers, 1972; Brinley-Morgan *et al.,* 1978).

1.2.14.5 Compliment Fixation Test (CFT)

CFT is the most widely used test for the serological confirmation of brucellosis in animals (Alton, 1990). This test detects predominately IgG antibodies as most of IgM antibodies will be destroyed during serum deactivation. Although, the CFT is as sensitive as the RBPT and ELISA, but under field conditions, the sensitivity of the test have been reported to be sometimes lower (88.6%) than those of RBPT (92.1%) and ELISA (100%) for diagnosis of infection in sheep (Blasco *et al.,* 1994a).

Technically, CFT is challenging because a large number of reagents must be titrated daily and a large number of controls of all the reagents is required (Poester *et al.,* 2010).

1.2.14.6 Serum Agglutination test (SAT)

This test is based on the reactivity of antibodies against the smooth lipopolysaccharide of brucella. Excess of antibodies resulting in false negative reaction due to prozone effect can be overcome by applying a serial dilution of 1:2 through 1:64

of the serum samples thus increasing the test specificity. The test is performed at a near neutral pH, which makes it more efficient in detecting IgM antibody. Hence, it is best used to detect acute infections. It is less effective for IgG, resulting in low assay specificity (Kaltungo *et al.,* 2014).

1.2.14.7 Fluorescence polarization assay (FPA)

FPA is a simple technique for measuring antigen/ antibody interaction and may be performed in a laboratory setting or in the field. It is homogeneous assays, requiring no washing steps or removal of unreacted components. It can be performed in a 96-well format or in a tube format. The tube format can be used in the field for rapid diagnosis.

The serum or milk incubation time is a minimum of 2 minutes while the whole blood assay requires only 15 seconds of incubation. Because only 2 reagents, antigen and diluents buffer are required. this test is technically simple and relatively inexpensive. It does require a fluorescence polarization analyzer of which several are available at various costs (Poester *et al.*, 2010).

Thus, the fluorescence polarization assay is very accurate and the sensitivity: specificity can be manipulated by altering the cut-off value between positive and negative reactions to provide a very sensitive screening test as well as a highly specific confirmatory test (Poester *et al*., 2010).

1.2.14.8 Indirect Coombs (antihuman globulin) test

The Coombs test is an extension of SAT, used for the detection of incomplete, blocking or non-agglutinating IgG (Alton GG *et al* 1988). Those SAT tubes containing serum dilutions and whole B. *abortus* or B. *melitensis* cells (as antigens) that were negative after incubation for 24 h are centrifuged, the supernatant decanted and the cell pellet resuspended and washed with phosphate-buffered saline, repeated three times. Standardized antihuman globulin reagent (anti-IgG) is added to the last pelleting in each test tube. The pellet is resuspended and incubated in a water bath at 37 ◦C for 24 h. Agglutination can be determined visually, as for SAT, by using an agglutinoscope or a drop on a slide examined under the microscope. The test is good for complicated and

chronic cases and misses around 7% of cases compared with ELISA (Araj GF *et al*.,1997).

1.2.14.9 Brucellacapt (Vircell, Granada, Spain)

This test is based on an immunocapture agglutination technique and in a single step detects non-agglutinating IgG and IgA antibodies, as well as agglutinating antibodies. The test is performed according to the manufacturer's instructions: a specified volume of each serum dilution is added to a microplate with U-shaped wells pre-coated with antihuman immunoglobulin. Then a whole-cell, formaldehyde-killed, coloured B. *melitensis* antigen suspension is added. The plate is incubated for 18–24 h at 37 ◦C before reading visually. Positive reactions show agglutination over the bottom of the well. Negative reactions present a pellet in the centre of the bottom of the well. Brucellacapt offers a valuable alternative to the Coombs test, since it shows similar sensitivity and specificity, similar performance (especially in diagnosing complicated and chronic cases), and most importantly is more rapid and less cumbersome to carry out (Orduna A *et al*., 2000).

1.2.14.10 Enzyme-linked immunosorbent assay (ELISA)

ELISA is the test of choice for complicated, focal and chronic cases, especially when other tests are negative while the case is under high clinical suspicion. It can reveal total and individual specific immunoglobulins (IgG, IgM, IgA) rapidly (4–6 h) with high sensitivity and specificity (Araj GF *et al*.,1986). Briefly, the test is carried out in 96-well microtitre plates that are pre-coated/fixed with predetermined Brucella antigen (whole cells or sonicate, purified LPS, protein extracts or other standardized antigen). A standardized volume and dilutions of serum are added to react with the antigen in the wells, and the plates are incubated and washed. An enzyme-conjugated (e.g. alkaline phosphatase, horseradish peroxidase) antihuman IgG, IgM or IgA is added to designated wells. After addition of the enzyme substrate and incubation, the optical density of the wells can be read at the appropriate enzyme wavelength. Cut-off values for seropositive samples can be extrapolated from incorporated negative and positive controls and/or a

standardized assay. In addition to the detection of immunoglobulin classes, ELISA can also detect Brucella-specific IgG subclasses and other Brucella immunoglobulins, such as IgE (Araj GF *et al*.,1988; 1990).

1.2.14.11 Immunochromatographic lateral flow assay

This assay is run using a composite strip in a plastic device consisting of a nitrocellulose detection strip and a reagent pad. The former contains Brucella LPS antigen, as a Brucella-specific capture probe, and a reagent control applied in distinct lines. The reagent pad contains dried and stabilised detection reagent consisting of a colloidal gold-conjugated antihuman IgG or IgM. The serum sample is added to a sample well, followed by test liquid. The result is read based on positive or negative staining after 10–15 min by visual inspection of the antigen and control lines in the test window. These Brucella-specific IgG and IgM lateral flow assays have been advocated for screening/surveillance of patients with brucellosis in endemic areas and as outbreak and field tests. They are simple, rapid, easy to perform and read, with high (>90%) sensitivity and specificity (Smits HL 2003; Mizanbayeva S, *et al*,.2009).

1.2.14.12 Molecular assays

Advances in molecular-based technology have been utilised for the laboratory diagnosis of human brucellosis. In-house developed conventional polymerase chain reaction (PCR) and real-time PCR (RT-PCR) assays have been attempted for the direct detection of *Brucella* from clinical specimens, to monitor treatment response, and for the identification, speciation and differentiation of recovered *Brucella* spp. The sensitivities of these assays in the direct detection of *Brucella* from clinical specimens have been quite variable, ranging from 50% to 100%, and specificity has varied between 60% and 98%. This variation might be related to different DNA extraction methods, detection formats and limits, and to different types of specimens used (Kattar MM2007; Mayefield JE 1988; Quipo-Ortuno MI *et al*.,2008). The ribosomal 16S–23S internal transcribed spacer region seems to constitute a suitable target in clinical specimens and formalin-

fixed paraffin-embedded archived tissue, as well as for the speciation of isolates from culture (Kattar MM *et al*., 2007).

Overall, molecular assays have good potential in the investigation of patients with brucellosis. So far, however, further standardisation and optimisation are necessary to achieve consistency and reliability of results before they are incorporated in routine laboratory investigations (Quipo-Ortuno MI *et al*.,2008).

1.2.15 Brucellosis treatment

The broad aims of antimicrobial therapy are to treat current infection and relieve its symptoms and to prevent relapse. Focal disease presentations may require specific intervention in addition to more prolonged and tailored antibiotic therapy. In addition، tuberculosis must always be excluded, or to prevent the emergence of resistance therapy must be tailored to specifically exclude drugs active against tuberculosis (e.g., rifampin used alone) or to include a full antituberculous regimen (Saltoglu *et al*, 2002).

Early experience with streptomycin monotherapy showed that relapse was common; thus dual therapy with tetracyclines became the norm. This is still the most effective combination, but alternatives may be used, with the options depending on local or national policy about the use of rifampin for the treatment of nonmycobacterial infection. Antimicrobial efficacy can usually be predicted by in vitro testing; however, the use of fluoroquinolones remains controversial despite the good in vitro activity and white cell penetration of most agents of this class. Low intravacuolar pH is probably a factor in the poor performance of these drugs (Montejo *et al*, 1993).

For adults with acute nonfocal brucellosis (duration, <1 month), a 6 weeks course of therapy incorporating at least two antimicrobial agents is required. Complex or focal disease necessitates 3 months of therapy. Adherence to the therapeutic regimen is very important, and poor compliance underlies almost all cases of apparent treatment failure; such failure is rarely due to the emergence of drug resistance, although increasing resistance to trimethoprimsulfamethoxazole (TMP-SMX) has been reported at one

center. There is good retrospective evidence that a 3 weeks course of two agents is as effective as a 6 weeks course for treatment and prevention of relapse in children, but this point has not yet been proven in prospective studies (Pappas et al, 2005, Hasanjani *et al*, 2006).

The "gold standard" for the treatment of brucellosis in adults is IM streptomycin (0.75– 1 g daily for 14–21 days) together with doxycycline (100 mg twice daily for 6 weeks). In both clinical trials and observational studies, relapse follows such treatment in 5–10% of patients. The usual alternative regimen (and the current World Health Organization recommendation) is rifampin (600–900 mg/d) plus doxycycline (100 mg twice daily) for 6weeks. The relapse/failure rate is ~10% in trial conditions but rises to >20% in many nontrial situations, possibly because doxycycline levels are reduced and clearance rates increased by concomitant rifampin administration. Patients who cannot tolerate or receive tetracyclines (children, pregnant women) can be given high-dose TMP-SMX instead (2 or 3 standard strength tablets twice daily for adults, depending on weight) (Montejo et al, 1993).

Evidence is beginning to accumulate that other aminoglycosides can be substituted for streptomycin eg. netilmicin or gentamicin given at a dosage of 5–6 mg/kg per day for at least 2 weeks. (Shorter courses have been associated with high failure rates in adults). A 5 -7 days course of therapy with gentamicin (and 3 weeks course of TMP-SMX) is probably adequate for children with uncomplicated disease. Early experience with fluoroquinolone monotherapy was disappointing, but high-dose ofloxacin (400 mg twice daily) or ciprofloxacin (500 mg twice daily), given together with rifampin for 6 weeks, may become accepted as an alternative to the other 6 week regimens for adults (Murray and Corbel, 2005).

Significant neurologic disease due to Brucella requires prolonged treatment (i.e., for 3–6 months), usually with ceftriaxone supplementation of a standard regimen. *Brucella* endocarditis is treated with at least three drugs (an aminoglycoside, a tetracycline, and rifampin), and many experts add ceftriaxone and/or a fluoroquinolone

to reduce the need for valve replacement. Treatment is usually given for at least 6 months, and clinical endpoints for its discontinuation are often difficult to define. Surgery is still required for the majority of cases of infection of prosthetic heart valves and prosthetic joints (Pappas et al, 2006).

There is no evidence base to guide prophylaxis after exposure to *Brucellae* (e.g., in the laboratory), inadvertent immunization with live vaccine intended for use in animals, or deliberately released *Brucellae*. Most authorities recommend the administration of rifampin plus doxycycline for 3 weeks after a low risk exposure (e.g., a nonspecific laboratory accident) and for 6 weeks after a major exposure to aerosol or injected material. However, such regimens are poorly tolerated, and doxycycline monotherapy of the same duration may be substituted (Bouza et al, 2005).

1.2.16 Prevention

Vaccines based on live attenuated *Brucella* strains, such as B. *abortus* strain 19BA or 104M, have been used in some countries to protect high-risk populations but have displayed only short-term efficacy and high reactogenicity. Subunit vaccines have been developed but are of uncertain value and cannot be recommended at present. Research in this area has been stimulated by interest in biodefense and may eventually yield new products, some of which may be based on the live attenuated WR 201 variant of B. *melitensis* strain 16M. The mainstay of veterinary prevention is a national commitment to testing and slaughter of infected herds/flocks (with compensation for owners), control of animal movement, and active immunization of animals. These measures are usually sufficient to control human disease as well. In their absence, pasteurization of all milk products before consumption is sufficient to prevent nonoccupational animal-to-human transmission. All cases of brucellosis in animals and humans should be reported to the appropriate public health authorities (Ashford *et al*, 2004; Rolan et al, 2007).

1.2.16.1 Example for prevention and control programs in Arabic countries

a. Palestine

The Palestinian Brucellosis Control Programme started in 1998, adopting the strategy of mass vaccination of sheep and goats (all ages and both sexes) via the conjunctival route using full dose Rev.1 vaccine. A survey estimated the overall initial animal prevalence at 18.6 percent, with 72.9 percent of the flocks infected. The total sheep population was estimated at 530 822 and the goat population at 341 017. The vaccination campaign started with mass vaccinations in 1999 and was repeated every two years until 2008, with vaccination coverage ranging between 25 to 95 percent depending on the year. From 2000 to 2005, vaccination of replacement animals only was carried out every two years with coverage at around 35 percent. Infrastructure weakness, limited resources, political and security crises, poor vaccine quality, deficient farmer awareness and uncontrolled animal movements were the major limitations. Despite these problems, the sheep and goat prevalence was reduced to 4.8 percent and the flock/herd prevalence to 46.3 percent as of 2005. There was also a significant decrease in human infections over this period (S. Alfuqaha).

b. Egypt

Brucellosis, particularly B.*melitensis*, is endemic in Egypt, presumably affecting large numbers of animals as well as humans. It appears to be of particular risk in rural communities, especially in Upper Egypt. Several attempts have been made to control the disease by the national veterinary services, with assistance from development agencies and international organizations. A new Spanish-Egyptian cooperation project for the control of ruminant brucellosis in the Upper Egypt area was funded by a Spanish Cooperation project from 2005-2009. This project included seven governorates, and involved primarily smallholders. The project also sought to strengthen the Egyptian veterinary services' capabilities to control brucellosis by improving surveillance at both field and laboratory levels and by implementing a massive vaccination campaign,

training veterinary personnel, implementing brucellosis public awareness campaigns and enforcing brucellosis control legislation) (B. Molina-Flores).

Chapter Tow

Aims of the study

2 Aims of the Study

2.1 General objective

To assess the seroprevalence of brucellosis, among butchers and non-butchers in Sana'a city, Yemen.

2.2 Specific objectives

1- To identify the risk factors associated with brucellosis.

2- To compare the seroprevalence of brucellosis among butchers and non butchers.

3- To assess people knowledge and practice about brucellosis.

Chapter Three

Materials and Methods

3 Materials and Methods

3.1 Study population

Butchers and others from general population (non butchers) from Sana'a city were enrolled in this study

3.2 Study design

A cross-sectional study was designed to assessment the Seroprevalence of brucellosis among butchers and non-butchers in Sana'a city, Yemen.

3.3 Study period and setting

This study was carried out from June 2021 to November 2021 during a period of 5 months, the samples of this study were collected from butchers and non butchers people in all 10 districts of Sana'a city (Al-thaorah, Az'zal, Al-tahreer, old Sana'a, Shu'aub, Ma'ain, Alsafiyah, Alsabeen, Al-wehdah and Bani Harith).

3.4 Sample size

Two hundred participates enrolled in this study. One hundred participates were selected from butchers and one hundred participates were from general population(non butchers).Also questionnaire for data collection was filled for each participant, questionnaire in appendix.

3.5 Inclusion criteria

Butchers and non-butchers people with any age .

3.6 Exclusion criteria

Female

3.7 Data collection

3.7.1 Questionnaire design: A full history was taken from each studied individual and the findings were recorded in a predesigned questionnaire, data collected included name, age, sex, occupation and risk factors of brucellosis and laboratory results.

3.7.2 Specimen collection: Principally, serum was used for detection of *Brucella* antibodies.3-5 ml of whole blood in gel tube was collected aseptically by vein-puncture from butchers and slaughterhouse workers, and from general population who did not contact with animals. Serum was separated in Al-Ain specialist laboratory in Sana'a city by centrifugation at 3000 rpm for 5 minutes after clotting, then the sera transported to microbiology lab in 21september university of Medical and Applied Sciences in Sana'a city to keeping the serum at -20C° until testing of *brucella* antibodies against B.*melitensis* and B.*abortus.*

3.8 Laboratory tests

3.8.1 Slide agglutination (qualitative test, SPINREACT)

-After we got of the samples out of the freezer and reagents out of the refrigerator, we waited until they had room temperature.

- Reagent vials were mixed vigorously before using

-50 µof the serum to be tested and 1 drop of each reagent (*Brucella.aborts* reagent and *Brucella.melitensis* reagent) were placed into two separate circles on the slide test.

- The drops on the slide were mixed with a stirrer to separating them over the entire surface of the circle, different stirrers were used for each circle.

- The slide placed on a mechanical rotator or shaker for 2 minutes.

Reading and interpretation (SPINREACT)

The results examined macroscopically for the presence or absence of visible agglutination immediately within 1 minute after removing the slide from the rotator.

-The presence of agglutination indicates an antibody anti-*Brucella* concentration equal or greater than 25 IU/ml.

-The positive result was confirmed under microscope.

3.9 Statistical analysis

Personal and clinical data were obtained from each participate and recorded into a predesigned questionnaire, and then data were statistically analyzed using statistical package for social science (SPSS 21).

Chapter Four

Results and discussion

4 Results and discussion

4.1 Results

The studied samples consisted of 200 males of butchers and non-butchers in Sana'a city, Yemen during a period of six months, starting in June 2021 and ending in December 2021. All the samples of the participates were randomly collected.

Table (4.1): Results of seroprevalence of brucellosis among all participates

Brucella antibodies tests results		
	Frequency	Percent
Negative	148	74
Positive	52	26
Total	200	100

Table 4.1: shows distribution of results of *Brucella* antibodies among the participants as the following; out of 200 samples, 148(74%) samples were negative and 52(26%) samples were positive.

Table (4.2): Shows results of the seroprevalence of *brucella* among participants in relation to occupation as the butchers and non-butchers

Occupational status with results		Results		Total	p. value
		Positive	negative		
Butchers	Count	**33**	67	100	**0.024**
	%	**33.0%**	67.0%	100.0%	
Non-butchers	Count	19	81	100	
	%	19.0%	81.0%	100.0%	
	Total count	52	148	200	
	Total %	26.0%	74.0%	100.0%	

Table (4.2): Shows the seroprevalence of *brucella* results among participants in relation to occupational status as the butchers and non-butchers. Out of 100 samples of butchers , 33 samples were positive while 67 samples were negative with percentage 33% and 67% respectively, and out of 100 samples of non-butchers there were 19 samples positive and 81 samples negative with percent 19% and 81% respectively, these results were statistically significant (p. value = 0.024).

Table (4.3): shows distribution of *Brucella* antibodies among participants in relation to *Brucella* spp.

Brucella spp		
Brucella species	Frequency	Percent
B. abortus	18	34.6%
B.abortus + B.melitensis	24	46.2%
B.melitensis	10	19.2%
Total	52	100%

Table 4.3: shows distribution of *Brucella* antibodies among participants in relation to *Brucella* spp. Of 52 positive samples, 18(34.6%) were positive for B.abortus, 24(46.2%) were positive for *B.* abortus and *B.melitensis*, and 10(19.2%) were positive for *B.melitensis*.

Table (4.4): shows results of seroprevalence of brucellosis according to ages groups

Age group	Brucella test result		Total	Value	p. value
	Positive	Negative			
<20	5	19	24	2.269	0.811
	20.80%	79.20%	100.00%		
20-24	13	32	**45**		
	28.90%	71.10%	100.00%		
25-29	10	28	38		
	26.30%	73.70%	100.00%		
30-34	7	29	36		
	19.40%	80.60%	100.00%		
35-39	6	18	24		
	25.00%	75.00%	100.00%		
40 and more	11	22	33		
	33.30%	66.70%	100.00%		
Total	52	148	200		
	26.00%	74.00%	100.00%		

Table 4.4: shows results of seroprevalence of brucellosis according to ages group, the ages of participates were ranged from 15 to 60 years, and divided to six ages groups, the main age group was 20-24 years which includes 45 participates. The prevalence of brucellosis increased with age reach the peak at the age group 40 and more, followed by (28.90%) at the age group of 20-24 years old, (26.30%) at the age group of 25-29 years old, (25.00%) at the age group of 35-39 years old (20.80%) at the age group of <20 years old, and finally (19.40%) at the age group of 30-34 years old.

Table (4.5): shows distribution of seroprevalence of brucellosis in relation to accidental skin injuries during animal slaughter

Accidental skin injuries during animal slaughter	Brucella test result		Total	Value	p. value
	positive	negative			
>5	21	36	57	6.370	**0.044**
	36.8%	63.2%	100.00%		
1-5	7	15	22		
	31.8%	68.2%	100.0%		
0	24	97	121		
	19.8%	80.2%	100.0%		
Total	52	148	200		
	26.0%	74.0%	100.0%		

Table 4.5 shows Results of seroprevalence of brucellosis in relation to risk factors; when accidental skin injuries considered as a risk factor, the highest prevalence rate was occurred with>5 times accidental skin injuries (36.8 %), this risk factor was statistically significant (p. value= 0.044).

Table 4.6: shows distribution of seroprevalence of brucellosis in relation to consumption the raw milk

Doyou consume raw milk	*Brucella* test result		Total	p. value
	Positive	Negative		
Yes	19	44	63	0.363
	30.20%	69.80%	100.00%	
No	33	104	137	
	24.10%	75.90%	100.00%	
Total	52	148	200	
	26.00%	74.00%	100.00%	

Table 4.6: shows distribution of seroprevalence of brucellosis in relation to consumption the raw milk, when we considered the consumption the raw milk as a risk factor of brucellosis, the prevalence for those who consumed raw milk was positive 19% while

69.80% negative, and for those who did not consumed raw milk was 24.10% positive while 75.90% negative and these results considered not statistically significant.

Table (4.7) shows results of seroprevalence of brucellosis in relation to cattle at home of participates

Have cattle at home	Brucella test result		Total	OR	95% Confidence Interval		p. value
	Positive	negative			Lower	Upper	
Yes	16	51	67	0.845	0.428	1.668	0.628
	23.90%	76.10%	100.00%				
No	36	97	133				
	27.10%	72.90%	100.00%				
Total	52	148	200				
	26.00%	74.00%	100.00%				

Table 4.7 shows seroprevalence of brucellosis in relation to cattle at home, there is no statistical significant.

Table (4.8) shows knowledge about brucellosis

Have you ever heard of brucellosis		
	Frequency	Percent
No	133	66.6%
Yes	67	33.5%
Total	200	100%

Table 4.8 shows result of having known of brucellosis or heard of someone who had suffered from brucellosis was referred to as knowledge of the disease. The majority of the respondents were had not any knowledge about brucellosis as showed in table 3.8

Table (4.9) shows practice of brucellosis

Have you ever tested for brucellosis		
	Frequency	Percent
No	192	96%
Yes	8	4%
Total	200	100%

Table 4.9 shows having practice about brucellosis this means do the participates had tested of brucellosis, and the most of them were not tested for brucellosis.

4.2 Discussion

This study documented the seroprevalence of brucellosis from participants of Butchers and non-Butchers in Sana'a City, Yemen, using cross-sectional study.

We found that26% of all participants had positive results of *Brucella* antibodies by using the SAT (slide agglutination test).

The results of seroprevalence of brucellosis among participants in relation to occupation as the butchers and non-butchers, among 100 samples of butchers there were 33 samples positive and 67 samples negative with percentage 33% and 67% respectively, non-butchers samples from 100 samples there were 19 samples positive and 81 samples negative with percentage 19% and 81% respectively, these results were statistically significant (*p*.value= **0.024**). In a study by Saleh(2000) in Yemen, his study was to determine seroprevalence of brucellosis among slaughterhouse workers (Butchers, veterinarians, laboures, abattoir, administrative) in six governments, among 120 butchers he reported 30(25%) positive results 7(24.1%) out of 29 in Sana'a, 2 (20%) out of 5 in Aden, 8(26.6%) out of 30 in Taiz, 5(22.7) out of 22 in Hodidah, 6(23%) out of 26 in Ibb, 2(25%) out of 8 in Hajjah by using standard tube agglutination.

However, other studies performed in other countries presented differing proportions, of human brucellosis sero-prevalence among slaughterhouse workers for instance, a Nigerian study found that the brucellosis sero-prevalence was among butchers 59.3% (Aworh, M. K,2013) it was higher than our study.

While an Ethiopian study found that the sero-prevalence in butchers as determine by the Rose Bengal Plate test, was 4.7% (Tsegay, A.; Tuli, G,2017).

In Ethiopia, very few studies have been conducted to determine prevalence of brucellosis in human, particularly the workers. The few similar studies in the literature, particularly the work done in association with occupational groups as in the present study, reported prevalence of 16 (4.8%) and 3 (1.2%) brucellosis.

In Addis Ababa and Western Ti gray slaughterhouse, respectively *(*Kassahun *J*.2006*).* And Mukhtar and Kokab, reported prevalence of 97 (19.69%) and 78 (21.7%). Among slaughterhouse workers in India and Pakistan, respectively. This study agreed with our study.

This might be correlated to low prevalence of small ruminant brucellosis reported in the abattoirs. Since seropositively of brucellosis in human is largely affected by the presence of the disease among domestic animals (MK, O, SkjerveE,2002*).*Additionally, the brucellosis sero-prevalence was reported to be 10.3% (using SAT) in Uganda and 7.9% (using ELISA) in Iran. While the sero-prevalence of brucellosis can be influenced by several factors such as the status of infection in slaughtered animals and laboratory methods employed to analyze the samples (Licensee, MDPI, 2018).

We found that, among butchers activities like accidental skin injuries during animal slaughtering were associated with higher rates of *Brucella* infection. We found in our study that, out of 200 participants there were 63(31.5%) consumed of raw milk but there is no significant with brucellosis.

In this study the prevalence of brucellosis increased with age reach the peak at the age group 45 and more which correlates with a study done by Saleh(2000) in Yemen he founded high prevalence in > 40 years, also in a study in Iran which reported the highest seroprevalence of brucellosis among slaughterhouse workers was in 41-50 years (Mohammad *et al* 2012).

In this study we found 4% of participants were had done *Brucella* test. We found in previous studies among slaughterhouse workers that the use of personal protective equipment was associated with a lower *Brucella* sero-prevalence rate. However, these studies did not investigate the role of wearing protective glasses in brucellosis prevention. Contact with the conjunctive of the eyewear ought to prevent this disease's transmission. *(Swai et al* 2009).Difference in serologic evaluation criteria and/ or in working environments may influence these results.

We conducted and demonstrated the evidence of human brucellosis in our study from butchers and associated risk factors.

Chapter Five

Conclusion and Recommendations

5 Conclusion and Recommendations

5.1 Conclusion

In our study the results of seroprevalence of *Brucella* infection among butchers and non-butchers in Sana'a city, Yemen was high26%among all of the participants by using the SAT (standard agglutination test). The most affected people were butchers.

The study reported the evidence of human brucellosis in Sana'a city, Yemen and revealed that the *Brucella.abortus* was more common in butchers where is *B. abortus* and *B. meletinesis* were the most in non butchers.

Those people with ≥ 45 old years were at high risk for brucellosis

The majority of participants were had not any knowledge or practice about brucellosis.

5.2 Recommendations

1- Butchers should apply personal protective equipment and increase the awareness of *brucella*.

2- We recommend that public health should do an educational program and leaflet should be established to aware the people by the risk of *Brucella* infection.

3- Routinely brucellosis test for patients especially butchers who suffering of fever (headaches, fatigue, malaise, chills) should be wide used for the benefits of early diagnosis and early treatment of brucellosis cases.

4- Should do tests for *Brucella* for animals in ports of Yemen.

5- We recommend doing studies in the future by using ELISA technique in more population including female.

6- Presently there is clear need for doctors in Yemen to be made aware of the frequency of this infection, the means available for clinical and laboratory diagnosis and effective treatment.

7- The health authorities should recognize these efforts by researchers and should be prepared to help them for more studies in infectious diseases and particularly on brucellosis in the future.

8- Emphasis for inter the Serological diagnosis of Brucellosis in human , especially Rose Bengal Test within the routine work of the general and private laboratory.

9- Need for cooperation between the relevant authorities in Human and Veterinary medicine to assess the disease and give it more attention because of the increase in cases of Brucellosis , which necessitates the need to eliminate the disease in animals because it is the main source of transmission of the disease to human.

Chapter Six

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6 References

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Appendix

Questionnaire

Seroprevalance of broucellosis among butchers and non-butchers in Sana'a city in Yemen.

Name (optional)..... Age ( )
Sex ( ) Date 2021 / /
Telephone No ( ) Serial No ( )

Duration of working at the abattoir(Years)			
Have you exposed cut your skin during the working?	Yes	NO	
	How many		
		Yes	No
Have you ever heard of broucellosis?			
Have you ever tested for human broucellosis?			
Have you heard of a vaccination against animals broucellosis?			
Do you use of protective equipment during working?			
Have cattle at home?			
Have you exposed for animals outside abattoir and home?			
When the last time exposed to fever(, headaches, fatigue, malaise, chills)?			
Have you suffering from fever this week?			
Have you suffering from fever this month?			
Have you use antibiotics?			
Types of antibiotics :			

Arabic summary

الملخص العربي

الخلفية: داء البروسيلات يعتبر أحد الامراض الحيوانية الرئيسة التي تنتقل الى الانسان المنتشرة عالميا خاصة في الدول النامية حيث يتعامل الناس مع الحيوانات.

الأهداف:

هدفت هذه الدراسة الى

١- تقيم الانتشار المصلي لداء البروسيلات بين الجزارين وغير الجزارين في مدينة صنعاء، اليمن.

٢- مقارنة الانتشار المصلي لداء البروسيلات بين الجزارين وغير الجزارين.

٣- معرفة عوامل الخطورة المرتبطة بداء البروسيلات.

٤- تقييم معرفة وممارسة الناس عن داء البروسيلات.

الطرق: كشفت هذه الدراسة المقطعية عن الانتشار المصلي لداء البروسيلات بين الجزارين وغير الجزارين في ١٠ مديريات مدينة صنعاء، عينات الدم التي تم جمعها من المشاركين تم فصلها بجهاز الطرد المركزي وتم فحص الامصال باستخدام شريحة اختبار التخثر. وتم جمع البيانات من المشاركين في استبيان معد مسبقا والذي تضمن العمر وأسئلة عن المعرفة بداء البروسيلات والفحص للبروسيلات وعوامل الخطورة مثل جرح اليد اثناء ذباحة الحيوانات وأيضا استهلاك الحليب الغير مبستر.

النتائج: بلغ الانتشار المصلي لداء البروسيلات ٥٢(٢٦%) عينة إيجابية من بين ٢٠٠ عينة.

من بين ١٠٠ عينة للجزارين وجدت٣٣ عينة إيجابية، ومن بين ١٠٠ عينة غير جزارين وجدت ١٩ عينة إيجابية، حيث كانت هذه النتائج ذات دلالة إحصائية (p. value= 0.024).

الخلاصة: يمكننا الاستنتاج من هذه الدراسة ان بروسيلات الانسان في مدينة صنعاء كانت منتشرة بين السكان، وكان الجزارين هم اكثر إصابة بالبروسيلات.

الكلمات المفتاحية: البروسيلات، داء البروسيلات، مصل، صنعاء، اليمن.

الملخص العربي

الجمهورية اليمنية
جامعة ٢١ سبتمبر للعلوم الطبية والتطبيقية
كلية الطب ألمخبري

قسم الأحياء الدقيقة الطبية

تقييم داء البروسيلات بين الجزارين وغير الجزارين في مدينة صنعاء، اليمن

بحث تخرج مقدم إلى كلية الطب ألمخبري كاستيفاء لشروط الحصول على درجة البكالوريوس

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