Introduction

Camel husbandry is vital for numerous pastoralist groups in Africa and Asia.The camel's ability to survive and produce under harsh environmental conditions has made it possible to use marginal and desertified ecosystems; and over the centuries, the camel has been a symbol of stability for the pastoralists in the arid zones of the world (Abbas and Agab, 2002).

There is at present an increased awareness of the role of camels as the main source of milk and meat for pastoralists. The urban population of many countries (particularly in North Africa and the Middle-East) consumes camel milk and meat. Camel racing is popular in the Arabian Gulf countries and northern Africa. (Abbas and Agab, 2002).

Brucellosis is one of the world's major zoonotic problems ,though it has been eradicated in many developed countries in Europe, Australia, Canada, Israel, Japan and New Zealand (Gul and Khan, 2007).

Brucellosis remains an uncontrolled problem in regions of high endemicity such as Africa, Mediterranean, Middle East, parts of Asia and Latin America (Refai, 2002).

Almost all domestic species can be affected with brucellosis except cats which are resistant to *Brucella* infection. Considering the damage done by the infection in animals in terms of decreased milk production, abortions, weak offspring's, weight loss, infertility and lameness,brucellosis is one of the most serious diseases of livestock. It is also a major impediment for the trade. Death may occur as a result of acute metritis, followed by retained fetal membranes (Radostits *et al.*, 2000).

Brucellosis is caused by members of genus *Brucella*. These are small, non-motile, aerobic, facultative intracellular, Gram-negative coccobacilli.

The ability of *Brucella* to replicate and persist in host cells is directly associated with its capacity to cause persistent disease and to circumvent innate and adaptive immunity (Fichi, 2003).

The species of *Brucella* and their major hosts are *B. abortus* (cattle),*B. melitensis*(goats), *B. suis* (swine) and *B. ovis* (sheep). *B.abortus* alsocauses infection in horses and is commonly found in chronic bursal enlargements as a secondary invader rather than a primary pathogen (Radostits *et al.*, 2000).

The disease in dromedary camels can be caused by *B. abortus*, *B. melitensis* and *B.ovis* (Seifert, 1996). Different studies showed that *B. abortus* and *B. melitensis* are the most frequent isolates from camels brucellosis (Radwan *et al.*, 1992, Gameel *et al*., 1993, Agab *et al.*, 1994, Abou-Eisha, 2000 and Hamdy and Amin, 2002). *Brucella melitensis* and *B. abortus* are capable of infecting a wide range of hostsincluding man (Walker, 1999).

Brucellosis is transmitted from animals to humans by ingestion of raw milk, milk products, raw liver and contact with infected animals or handling of materials from such animals (El Tahir *et al.*, 2011).

From public health view point, brucellosis is considered to be an occupational disease that mainly affects slaughter-house workers, butchers, and veterinarians. Transmission typically occurs through contact with infected animals or materials with skin abrasions. Symptoms of human brucellosis can be highly variable, ranging from non–specific, flu-like symptoms (acute form) to undulant fever, arthritis, orchitis and epididymitis (Gul and Khan, 2007).

The economic and public health impact of brucellosis remain of particular concern in developing countries. The disease poses a barrier to trade of animals and animal products, represents a public health hazard, and is an impediment to free animal movement (Corbel, 2006).

The disease could seriously impair socio-economic development for

livestock owners, which represent a vulnerable sector in rural populations in general and pastoral communities in particular. It has a significant public health implication for a pastoral community in consequence of lifestyles, feeding habits, close contact with animals, low awareness, and poor hygienic conditions which favora infections (Schelling *et al.*, 2003).

Most surveys for prevalence of brucellosis are based on a number of standardized serological tests (Hesterberg et al., 2008). Rose Bengal PlateTest has been widely used (Cho *et al.*, 2010) and shown to be of significant sensitivity compared to other tests however, some surveys apply more confirmatory tests in addition to demonstration of *Brucella* in culture.

The objectives of the present study were to:

1\ Determine the seroprevalence status of the brucellosis in camels

2\Identify risk factors associated with the disease occurrence in Alzulfi Governorate.

Chapter One

Literature Review

1.1 Taxonomy and Distribution of Camels (*Camelusdromedarius*)

In zoological taxonomy, camelids are classified in the suborder *Tylopoda* (pad footed animals) that with the suborders *Suiformes* (pig-like) and *Ruminantia* (ruminants) and the order *Artiodactyla* (even-toed ungulates).Thus,camelids (family *Camelidae)* as ruminating animals are classified in proximity to ruminants but developed in parallel and are not part of the suborder *Ruminantia*.Some differences as foot anatomy, stomach system and theabsence of horns underline this fact (Schwartz and Dioli, 1992; Fowler, 1998). The family *Camelidae* is divided into three genera; the old world camels (genus *Camelus*) and the new world camels (genus *Lama* with the species *L. glama, L. guanicoe*, *L.pacos* and genus*Vicugna* with the species *V. vicugna*) (Wilsonand Reeder, 2005).

Two domesticated species of old world camels exist, the dromedary or one humped camel *(Camelus dromedarius)* that has its distribution in the hot deserts of Africa and Asia and the Bactrian or two-humped camel (*Camelusbactrianus)* that can be found in the cold deserts and dry steppes of Asia (Wilson,1984).

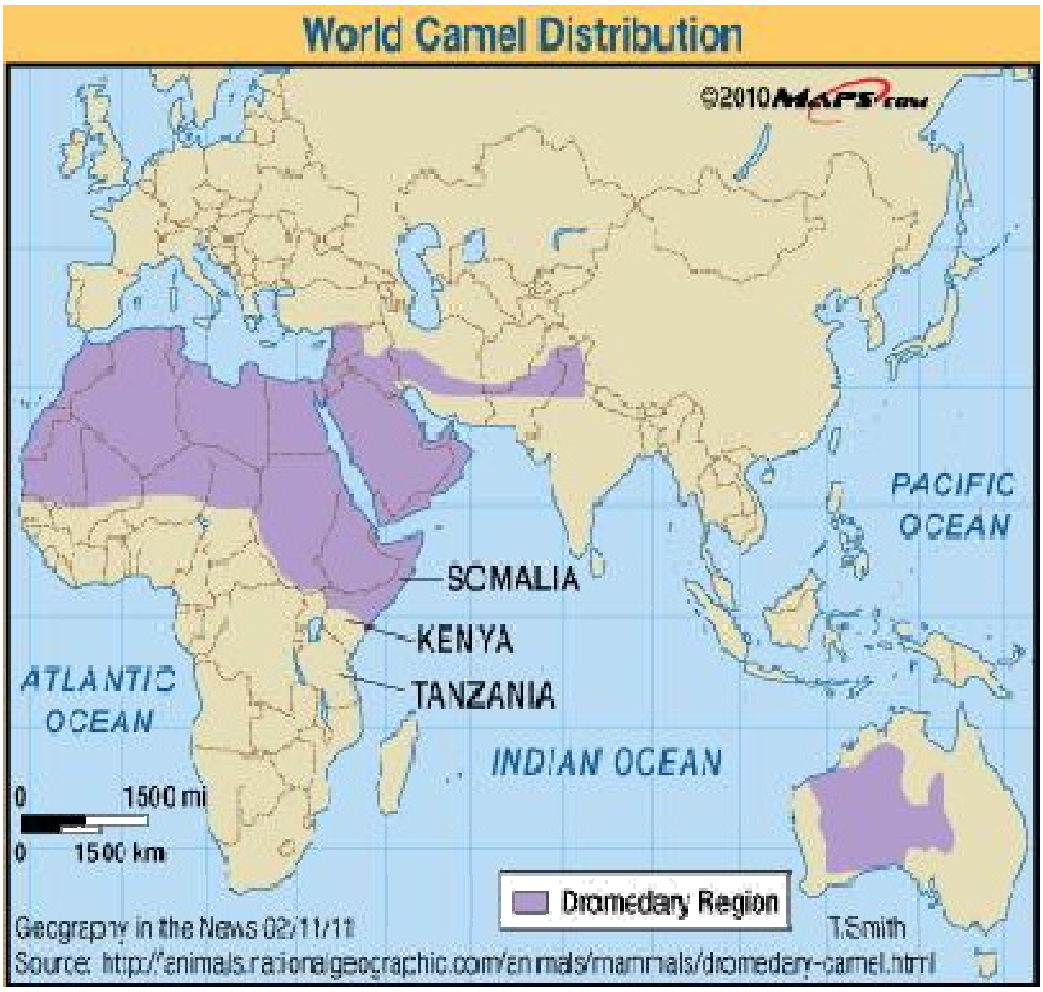

Figure 1. 1: World camel distribution

The world population of camels is about 20 million mainly in arid zones,of which, 15 million camels live in Africa and 5 million in Asia (Glipha, 2006). In 2001, the total camel population was 19 million,of which, 17 million were dromedaries and 2 million were Bactrian (Farah and Fischer, 2004).

In most countries, the camel population increased after a period of decreasing number due to the introduction of modern transport facilities (Table 1.1).

Table 1.1: Camel population is some selected countries

Country	Number (1000)	Density (No per km²)	Proportion to total National ruminants (%)
Djibouti	60	-	34
Egypt	170	0.16	5.8
Ethiopia	1030-1040	0.83	3.4
India	1100	0.33	0.4
Kenya	620-780	1.08	5.3
Niger	415	0.32	8.3
Saudi Arabia	165	0.00	14.9
Somalia	5800-6350	8.93	46.6
Sudan	2800-3100	0.99	11.1

Source: Adopted from Wislon et al., (1990); Schwartz and Dioli, (1992).

1.2 Potential Importance of Camels

As dromedaries are very drought tolerant, they thrive in arid zones of many countries in the world and provide food, hides and transport. Therefore, they had developed an increasing interest in arid countries, where other domesticated animals have difficulties to survive. Camels can graze on low productive pastures on which the production of milk is possible and economically profitable. For this reason, camels may reduce the dependence of pastoralists on other livestock that is usually much more vulnerable to drought than camels (Farah and Fischer, 2004).

Camel milk is one of the most valuable food resources for nomads in arid regions and can contribute to a better income for pastoralists, as in the last year's milk consumption among the urban population increased (Farah and Fischer, 2004).

Camel milk possesses superior keeping quality to cows' milk due to its high contents of proteins that have inhibitory properties against bacteria. This makes raw camel milk a marketable commodity, even under conditions of high temperatures.Therefore zoonoticrisks from camel milk must be considered in view of the traditional preference for raw milk consumption.

Besides milk, meat is one of the most important products of camels. It compares favorably with other livestock in yield and quality of the carcasses but camels are still not systemically bred for meat production in many regions as camels are considered too valuable for this production type. Usually males and infertilefemale camels are sold as slaughter animals by pastoralists. Nevertheless, selling these animals for meat production can be an important source of income. There has been an increasing demand of camel meat in people

and societies that do not breed camels, thus leading to a higher number of camel abattoirs and butcheries in several countries that mainly slaughter young animals (Farah and Fischer, 2004 and Finke, 2005).

Another important product is camel wool. It is one of the world's most expensive natural animal fibers. In some countries, camels are kept in the backyards of cities to gain wool, besides milk and meat. An adult camel usually produces 2 – 3 kg per shearing (Wernery, 2003). Camel hides are known for their strength and durability. They are used by camel breeders, but also as fashion accessories (Wernery, 2003). Other products used are dung as fertilizer and source of fuel for pastoralists and bones for production of jewellery or bone-meal for fertilizing purposes.

1.3 Brucellosis

1.3.1 Definition

1.3.2 Definition of the disease

Brucellosis is an infectious, contagious, and worldwide spread form of an important zoonotic disease caused by bacteria of the genus *Brucella*. In animals, the disease primarily affects cattle, sheep, goats, swine, and dogs, and is characterized by abortion or infertility and also affects people and other animal species (Ray and Steele, 1979). In human-beings, the disease is characterized by intermittent fever, chills, sweating, headache, myalgia, arthralgia, and a diversity of nonspecific symptoms (Young and Corbel, 1989).

1.3.2Synonyms

The historical synonyms of the disease in animals and human-beings is as follows:In domestic animals, brucellosis has been commonly known as enzootic abortion or bovine contagious infection, epizootic abortion, infectious abortion,

contagious abortion, slinking of claves, Bang's disease, and ram epididymitis.In the case of human brucellosis, it has been described by various names including undulant fever, Malta fever, Mediterranean fever, gastric fever, Mediterranean gastric fever, Gibraltar-Rock fever, Cyprus fever, Neapolitan fever, intermittent gastric fever or intermittent typhoid fever, pseudo typhus, febris typho-malariae, and fièvre sudorale (Ray and Steele, 1979).

1.3.3Historical Prospective

It is known from written resources that sheep and goats were the primary domestic animals in the Roman Empire. Small ruminants milk was used to make cheese, one of the primary ingredients in Roman cuisine.It was therefore hypothesized that milk and milk products were important sources of an infectious food-borne disease that was later know as the "Maltese fever" (i.e. brucellosis due to*Brucella melitensis* The Roman town of Herculaneum was destroyed by the tremendous volcanic eruption of Mount Vesuvius in August 79 A.D. Recently; L.Capasso found bone lesions typical of brucellosis in adult skeletal remains of people killed during the first volcanic surge of Mount Vesuvius (Capasso, 2002) He also demonstrated by scanning electron microscopy analysis of a buried carbonized cheese, the presence of cocco-like forms that were morphologically consistent with *Brucella* spp. (Capasso, 2002).

Sir David Bruce isolated in 1887 the organism (*Micrococcus melitensis*) responsible for Maltese fever from a British soldier who died from the disease in Malta (Bruce, 1887). This bacterium was renamed *Brucella melitensis* in his honor. In 1905, Zammit demonstrated, again in Malta, the zoonotic nature of *B. melitensis* by isolating it from goat's milk (Zammit, 1905).

1.3.4Zoonoses

Five out of the nine known *Brucella* species can infect humans and the most

pathogenic and invasive species for human is *B. melitensis*, followed in descending order by *B suis, B. abortus* and *B. canis* (Acha *et al*., 2003). The zoonotic nature of the marine brucellae (*B. ceti*) has been documented (Brew et al., 1999; McDonald *et al*., 2006; Sohn *et al*., 2003).

B. melitensis, B. suis and B. abortus are listed as potential bio-weapons by theCenters for Disease Control and Prevention in the USA. This is due to the highly infectious nature of all three species, as they can be readily aerosolized. Moreover, an outbreak of brucellosis would be difficult to detect because the initial symptoms are easily confused with those of influenza (Chain *et al*., 2005).

In places where brucellosis is endemic, humans can get infected via contact with infected animals or consumption of their products, mostly milk and milk products especially cheese made from unpasteurized milk of sheep and goats and rennet from infected lambs and kids. Some specific occupational groups including farm workers, veterinarians, ranchers, and meat-packing employees are considered at higher risk (Tabak *et al*., 2008). *B. abortus and B. suis* infections usually affect occupational groups, while *B. melitensis* infections occur more frequently than the other *Brucella* species in the general population (Acha *et al*., 2003; De Massis *et al*., 2005). Consumption of sheep or goat milk containing *B. melitensis* is an important source of human brucellosis worldwide and has caused several outbreaks. For example; in some countries including Italy, 99% of human brucellosis is caused by *B. melitensis* (De Massis *et al*., 2005; Wallach *et al*., 1997). The prevalence of human brucellosis acquired from dairy products in some countries is seasonal, reaching a peak usually after kidding and lambing (Dahouk *et al*., 2007).

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.

Although Brucella is considered highly infectious when encountered via the respiratory route (e.g. 10 bacteria required for infection in mice), inhalation of *Brucella* is not a common route of infection, but it can be a significant hazard for people in certain occupations, such as those people working in laboratories or abattoirs. In fact, *Brucella* spp. are considered as the most common laboratory acquired pathogens, and they are estimated to account for up to 2% of all laboratory- associated infections (Menseet *et al.,* 2001; Olle-Goig and Canela-Soler, 1987; Robichaud *et al*., 2004)

Table 1. 2:Zoonotic potential and host preference of brucella species

Species	Zoonotic Potential	Host Preference
Brucella .melitensis	High	Sheep, goat
Brucella. abortus	Moderate	Cattle
Brucella .suis	Moderate	Pig
Brucella .canis	Mild	Dog
Brucella .ovis	Absent	Sheep
Brucella neotomae	Absent	Desert wood rat
Brucella ceti	Mild	Cetaceans
Brucell a pinnipedialis	Mild	Seals
Brucella microti	Absent	Common voles

1.3.5 Economical Importance of Brucellosis

Brucellosis is characterized by abortion, non-viable offspring birth in female, and orchitis and epididymitis in male animals (Radostits *et al*., 1994; Seifert,

1996). Abortion is the major feature that is manifested in camels (Al-Khalaf and El-Khaladi, 1989). The disease is also associated with infertility and prolonged calving intervals, and has considerable impact on camel production. Chronic inflammation of epididymis, of the joints, tendon sheath and synovial bursae especially at the carpusmay also occur in camels (Abbas and Agab, 2002; Wernery and Kaaden, 2002). The disease can generally cause significant loss of productivity through late first calving age, long calving interval time, low herd fertility and comparatively low milk production, as in cattle may also happen in camels (Radostits *et al*., 1994). The disease can also have an impact on export and import of animals constraining livestock trade.

Afzal and Sakkir, (1994) have suggested that sub clinical brucellosis can pose problems in racing camels by reducing the performance and productivity of these animals in the Arabian Peninsula where camel racing is highly popular.

1.3.6 Public Health Importance of Brucellosis

Brucellosis in human represents a major public health hazard, which affects social and economic development in various countries. Groups at high risk for brucellosis are animal health workers, butchers, farmers, and those who habitually consume raw milk and come in contact with animals (Chukwu, 1987). In man, transmission occurs as a result of ingestion of milk, contact via skin abrasion, mucous membranes and inhalation (Radostits *et al*., 1994; Seifert, 1996). Masoumi *et al*., (1992) recorded higher prevalence rate among butchers and people who habitually consume raw milk.

Camel keepers consume camel milk as well as liver without heat treatment. This is even considered as delicacy (Gameel *et al.*, 1993). There is also a close contact between herdsmen and the animal during watering, grooming, riding, nursing sick ones and delivery assistance (Abbas *et al.,* 1987).

The isolation of the two major pathogenic *Brucella* species: *B. melitensis* and

B.abortus, from milk and other samples of camel origin (Gameel *et al.,* 1993 and Agab *et al*., 1994; Hamdy and Amin, 2002) clearly indicate the potential public health hazards of camel brucellosis (Straten *et al*., 1997). The disease in man may be misdiagnosed due to the prevailing malaria infections in dry areas (Abou-Eisha, 2000; El-Ansary *et al*., 2001).

1.4Epidemiology of Brucellosis

1.4.1 Aetiology

Brucellae are Gram-negative, facultative intracellular bacteria that can infect many species of animals and man. Six species are recognized within the genus *Brucella*:

Brucella abortus, *Brucella melitensis*, *Brucella suis*, *Brucella ovis*, *Brucella canis*,and *Brucella neotomae* (*Alton* et al.,1988; Corbel., *et al.,* 1984). This classification is mainly based on the difference in host preference and in pahogenicity. Distinction between species and biovars is currently performed by differential laboratory tests (Alton *et al*., 1988 and Corbel., *et al* 1984). Although it has been proposed that the *Brucella* species should be grouped as biovars of a single species based on DNAhybridization studies (Verger *et al*., 1985) and on the comparison of the genome of *B.melitensis* (Del Vecchio *et al*., 2002), and B. *suis* (Paulsen *et al.,* 2002), the currentclassification of brucellae in species according to differences in host preference and in pathogenicity should be preferred (Cloeckaert *et al*., 2001 and Moreno *et al*., 2002). Worldwide, the main pathogenic species for domestic animals are *B.abortus*, responsible for bovine brucellosis; *B. melitensis*, the main etiologic agent of small ruminant brucellosis; and *B. suis* responsible for swine brucellosis. These three *Brucella* species may cause abortion in their hosts. Because of the presence of brucellosis in a herd (or flock), a region or a country, international veterinary regulations impose restrictions on animal movements and trade, which result in huge

economic losses (Anonymous FAO/WHO, 1997; Anonymous OIE, 2003; Crawford *et al*., 1990). *B. ovis* and *B. canis* are responsible for ram epididymitis and canine brucellosisrespectively. For *B. neotomae* only strains isolated from desert rats have been reported. Albeit their respective host preferences, different *Brucella* strains have also been isolated from a great variety of wildlife species such as bison (*Bison bison*), elk*Cervus elaphus*), feral swine and wild boar (*Sus scrofa*), fox (*Vulpes vulpes*), hare(*Lepus capensis*), African buffalo (*Syncerus caffer*), reindeer (*Rangifer tarandus*), caribou (*Rangifer tarandus groenlandicus*), chamois (*Rupicapra rubicapra*) and ibex (*Capra ibex*) .Thus wildlife has to be considered as a reservoir forzoonotic brucellosis (Davis *et al.,* 1990; Godfroid,2002; Rhyan,2000). The broad spectrum of *Brucella* isolates has recently been enlarged to marine mammals. A number of recent reports describe the isolation and characterization of *Brucella* strains from a wide variety of marine mammals (Clavareau *et al*., 1998, Ewalt *et al*., 1994 and Foster *et al.,*2002). These strains have been identified as brucellae, however their overall characteristics are not as similarly to those of any of the six recognized *Brucella* species(Clavareau *et al.,* 1998; Cloeckaert *et al*., 2001; Jahanas *et al*., 1997).

Camels can be infected by *B. abortus and B. melitensis*. Different studies showed that *B. abortus* and *B. melitensis* are most frequently isolated from milk, aborted fetus and vaginal swabs of diseased camels (Radwan *et al.,* 1992; Gameel et al., 1993; Agab et al., 1994; Abou-Eisha, 2000; Hamdy and Amin, 2002).

1.4.2 Transmission

The spread of brucellosis depends on the *Brucella* species being prevalent in other animals sharing their habitat and on husbandry (Musa *et al.,* 2008).Both vertical and horizontal transmissions exist in animal brucellosis. Horizontal

transmission occurs through ingestion of contaminated feed, skin penetration, via conjunctiva, inhalation and udder contamination during milking. Congenital infection that happens during parturition is frequently cleared and only few animals remained infected as adult (Radostits *et al*., 1994). Spread of the disease is due to movement of infected animals to disease free herds. Proximity of infected herd to clean herds happens at water points where a number of camels come together. Epidemiologically important risk factors are large herd size, poor managements, and active abortions, milking more animals by single person and herding with other ruminants. Survival of the organisms in the environment may also play a role in the epidemiology of the disease (Abbas *et al.*, 1987; Radwan *et. al.*, 1992; Abuo-Eisha, 2000).In the environment the ability of brucella to persist out side mammalian host is relatively high compared with most non-sporing pathogenic bacteria under suitable condition. Dafni *et al.*, (1991) suggested that small ruminants act as extensive reservoir of *B. melitensis*, which constitutes a threat of infection to large ruminants including camels and man due to prolonged contact. The chance of transmission is higher during parturition and abortion when most of the *Brucella* contamination occurs (Abbas and Agab, 2002).

1.4.3 Host Factors

Infection may occur in animals of all age groups, but persists commonly in sexually mature animals (Radostits *et al*., 1994). Generally, infection is acquired after three years of age with increase in the subsequent age groups (Majid *et al.*, 1999; Abou-Eisha, 2000). Some studies revealed the equal distribution of *Brucella* antibodies among males and females (Abu Damir *et al*., 1984; Abbas et al., 1987; Radwan *et al.*, 1992). In other findings it appeared that females are more susceptible to the disease than males (Agab *et al*., 1997; Ajogi and Adamu,

1998). Higher susceptibility in female animals is attributed to physiological stresses (Walker, 1999). Female animals have essential epidemiological importance not only in susceptibility but also in disseminating the disease via uterine discharge and milk. The role of males in the spread of disease under natural condition is not important (Radostits *et al.,* 1994). The extent to which infection rate varies due to breed difference is not well known. (Wernery and Wernery, 1990) reported that breeding camels had lower brucellosis infection rate than racing animals. This was justified as due to racing camels (but not breeding animals) utilizing unpasteurized cow milk.

1.4.4 Environmental and Climatic Factors

Atmospheric conditions and seasons of the year may have influence on the management and contact of the infected and susceptible host. In dry areas, water resources are sparsely distributed (Helland, 1982). As a result, the congregation of a large number of mixed ruminants at water points facilitates disease spread. The coincidence of parturition in wet season (Schwartz and Dioli, 1992) enhances the viability of the organisms in the environment, thus increasing the chance of infecting susceptible animals (Corbel, 1990). (Baumann and Zessin, 1992) reported higher brucellosis reactors rate in too wet seasons than dry seasons. The incidence of brucellosis in camel population appears to be related to breeding and husbandry practices. Herd sizes, density of animal population, and poor management are directly related to prevalence (Wernery and Kaaden, 2002).

1.5Pathogenesis and Pathology

The pathogenic potential of *Brucella* spp. is highly dependent on its ability to enter and survive within host cells. *Brucella* does not have classic virulence factors such as exotoxins, capsule, or endotoxic lipo polysaccharide (LPS)

(Moreno., *et al* 2001).The major virulence mechanisms of *Brucella* already identified are those required for host cell invasion and intracellular survival or replication(Boschiroli., et al 2002;Edmonds., *et al* 2001; lapaque., et al 2005). Asuccessful entry of *Brucella* into the host is a crucial step in establishing infection. Considering that the digestive tract is the main entrance route of *Brucella,* some studies investigated possible virulence factors involved on successful infection through the digestive tract (Paixão., *et al* 2009; Bandara., et al 2007; Delpino., *et al* 2007). Following exposure, the organisms penetrate intact mucosal surface. In the alimentary tract the epithelium covering the ileal Peyer's patches are the preferred sites of entry. After penetration the organisms may be engulfed by phagocytic cells and localized to regional lymph nodes (Walker, 1999). Then they proliferate, disseminate haemogenously and localize in the reticuloendothelial and reproductive tract (Radostist *et al*., 1994). Various mechanisms are employed by *Brucella* organisms to survive inside the phagocytic cells: inhibiting phagolysosome fusion, blocking bactericidal action of phagocytes and suppressing the myeloperoxidase H2O2 halide system (Frenchick *et al*., 1985; Harmon *et al.*, 1988; Tizard, 1992; Walker, 1999). Little is known about the pathological changes in camels. Gross lesion may be found in the predilection sites uterus, udder, testicles, lymph nodes, joint bursa and placenta. Hydrobursitis was often observed in brucellosis positive dromedaries causing swelling of the bursa (Werney and Kaaden, 2002). The probable possibilities for the abortion in farm animals may be due to placentitis, direct effect of endotoxins or inflammatory response in fetal tissue (Walker, 1999).

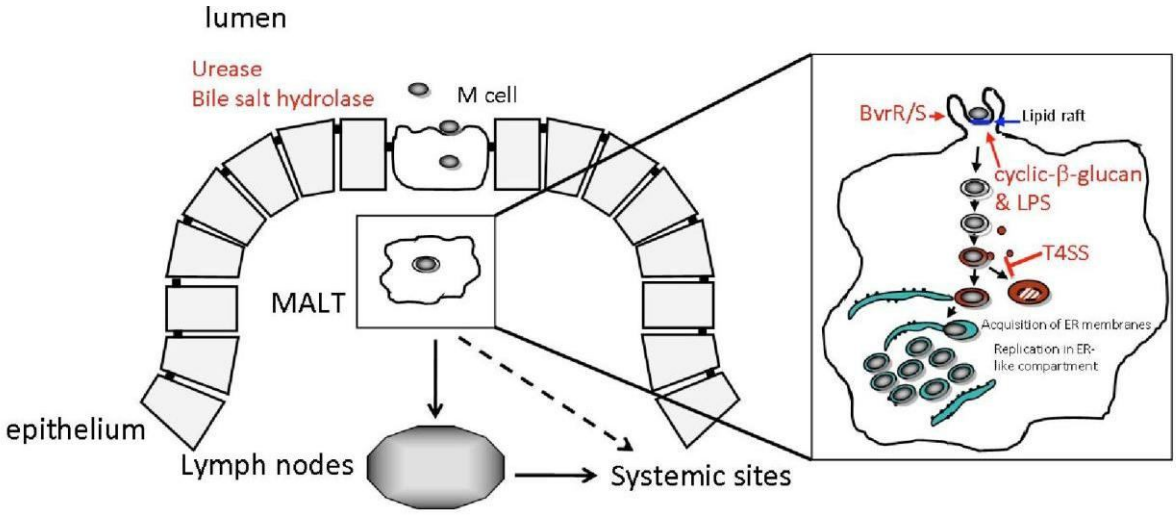

Figure 1. 2 Schematic representation of *Brucella* invasion through the digestive tract.

Entry is through M cells and subsequently the bacteria are taken up by macrophages of the mucosa associated lymphoid tissue (MALT). These macrophages transport the bacteria to the lymph node sand on to systemic sites. Blown up macrophage shows trafficking within the macrophage from entry via lipid rafts, through the endosomal pathway to the ER-like compartment in which *Brucella* replicates [10]. In red are *Brucella* virulence factors that are involved in establishing infection**.** (Xavier *et al*., 2010)

1.6 Immune Response

Brucella spp. are facultative intracellular pathogens which resist killing by neutrophilis,replicate inside macrophages and in “non-professional” phagocytes and maintain a long lasting interaction with the host cells(Dornand *et al*., 2002). As intracellular organisms, protection against *Brucella* infection requires cell-mediated immunity, which includes CD4+ and CD8+ T lymphocytes, Th1-typecytokines such as IFN- and TNF-, and activated macrophages and dendritic

cells (DC)(Golding *et al*., 2001). Therefore, host control of infection requires a set of cells and factors which together promote a complex response against *Brucella*. CD8+ T cells have the predominant role for optimal protection against *B. abortus* infection. This protection can be performed by a type 1 cytokine profile production, mainly IFN-, and lysis of *Brucella*-infected *macrophages* (Olivera *et* al.,1998; Splitter and Olivera, 1995).Lysis of this macrophages releases the bacteria to the extracellular milieu enabling uptake by other activated macrophages in a IFN-rich microenvironment. These cells presents augmented antibrucellae mechanisms and are able to destruct the pathogen, inhibiting *Brucella* spread (Jiang and Baldwin, 1993).Moreover, the type 1 cytokines produced by CD8+ T cells induce down-regulation of Th2 cytokines and IL-10. (Olivera *et al.*, 1995; Splitter and Olivera, 1998).

1.7 Diagnostic Methods

1..7.1 Bacteriological Methods

Great care should be employed during handling any material containing *Brucella* organisms. Generally, precautions to be taken include use of safety cabinet in laboratory; wearing gloves, protective cloth and facemask, autoclaving materials in contact with the organism and disinfecting contaminated surfaces (Alton et al., 1975). Commonly used basal media include: serum dextrose (Agab *et al.*, 1994), serum tryptose agar, glycerol dextrose agar, trypticase, and soya agar (Alton et al., 1975). Terzolo *et al*., (1991) suggested that Skirrow agar is a satisfactory medium for both *Brucella* species and *Campylobacterfetus*. Contamination is prevented by use of selective media containing actidione (30mg/l), bacitracin (25mg/l), polymixin B (5mg/l) and vancomycin (20mg/l) (Walker, 1999). Milk samples, vaginal swabs, semen and aborted fetus are useful for recovering the organisms at ante mortem. Samples collected at necropsy include multiple lymph nodes, spleen, udder, pieces of uterus and testicular tissue (Agab **et al**., 1994)

Tissue specimens are directly cultured on solid media whereas milk cultures are performed by centrifuging milk at 5900 to 7700 x g for 15 minutes (Walker, 1999). Cultures then, incubated at 37 with 5-10% CO2 enrichment for three days and above (Alton *et al*., 1975; Gameel *et al*., 1993; Agab et al.,1994). Characteristics colonies have small convex, smooth translucent appearance (Gameel *et al.*, 1993; Agab et al., 1994). Demonstration of the bacteria is by staining with Gram-negative stain or modified-Zeihl Neelsen staining. Animal inoculation (an old method) can also reveal characteristics lesion in liver, spleen and epididymis of a guinea pig (Walker, 1999). Further characterization is based on sereotyping, phage typing, dye sensitivity, and biochemical tests. Florescent antibody test and polymerase chain reaction methods have been described for *Brucella* species identification (Walker, 1999; Quinn *et al.*, 2002).

1.7.2 Serological Methods

Brucellosis was first diagnosed by a serological test by Wright and Smith, (1897) using a simple tube agglutination test. Subsequently, various modifications to the tube agglutination test and numerous other tests have been developed to increase test accuracy. The procedures are divided into two categories, the Conventional Tests and Primary Binding Assays. All conventional tests rely on the antibody performing a secondary function, for instance fixation of complement while in primary binding assays the only function of the antibody is attachment to its antigen. (Neilsen and Wu, 2010).

Conventional Tests *Agglutination tests

Slow tests requiring incubation from 8 to 24 hours:

a\ Standard tube (SAT).

b\ SAT with added reducing agents such as 2-mercaptoethanol or dithiothreitol SAT with addition of rivanol to precipitate glycoproteins.

c\ SAT with addition of ethylene diamine tetraacetic acid to reduce IgM binding (EDTA)d\ SAT with antiglobulin added to enhance agglutination

e\ Milk ring test

***Rapid agglutination tests performed in minutes:**

a\ Rose Bengal

b\ Modified Rose Bengal

c\ Buffered Antigen Plate Agglutination

d\ Card

e\ Antigen with rivanol added

f\ Heat treatment of serum

g\ Addition of 10% sodium chloride

***Precipitation tests**

a\ Agar Gel Immunodiffusion

b\ Radial Immunodiffusion

***Complement fixation tests:**

a\ Warm

b\ Cold

c\ Haemolysis

d\Indirect Haemolysis

***Primary Binding Assays**:

a\ Radioimmunoassay.

b\ Fluorescence Immunoassay

c\ Particle CountingFluorescence.

d\ Immunoassay.

e\ Indirect Enzyme Immunoassay.

f\ Competitive Enzyme Immunoassay .

g\ Fluorescence Polarization Assay

However, no test devised to date is 100% accurate so generally serological diagnosis consists of testing sera by several tests, usually a screening test of high sensitivity, followed by a confirmatory test of high specificity. .(Neilsen and Wu, 2010)

1.8Control and Prevention

The control and prevention of brucellosis in farm animals depend on animal species involved, *Brucella* species, management practices and availability and efficacy of vaccines. The options to control the disease include immunization, testing and removal, and improving management practices and movement control (Hunter, 1994; and Wernery and Kaaden, 2002).

Control of camel brucellosis should suite conditions in particular countries where camels are raised. In most of the developing countries where camels are raised by pastoralists, brucellosis prevalence is low. Thus control by herd immunization and vaccination of calves at 4 to 8 months of age is helpful. On the other hand, test and slaughter policy can be followed in

counties where intensification is practiced (Abbas and Agab, 2002).

1.8.1Immunization

The live attenuated *B. abortus* S19 and *B. melitensis* Rev-1 proved to be effective vaccine against the disease in camels and other ruminants. Both vaccines have disadvantages of causing abortion, being pathogenic to human beings and interference with serological tests . The non-smooth strains of *B. abortus* RB51 and *Melitensis vaccine*M111 have recently been introduced into some countries. These vaccines aresaid to be safe and do not interfere with serological tests (Wernery and Kaaden, 2002).

1.8.2Management practices and movement control

Improving management practices is one way of attempting to control brucellosis. This would aim to improve hygiene and reduce the chances of contact between infected and non-infected animals. Although it would not be easy under many circumstances, where resources are lacking and the movement of livestock is difficult to restrict, the following points can be attempted in reducing infection rates (Hunter, 1994; Radostits *et al.*, 1994): Public awareness is of vital importance in successful control and prevention of brucellosis; isolation of infected animals and female atparturition; proper disposal of aborted fetus, placental tissue and uterine discharge and disinfecting of contaminated areas.

1.9 Prevalence and Risk Factors of Camel Brucellosis in DifferentCountries

Brucellosis is considered to be one of the most important zoonotic camels diseases and other domestic animals in some countries of Asia and Africa.Several published literature regarding the prevalence of camel brucellosis from different countries were done.Musa and Shigidi, (2001)

investigated brucellosis in 3413 camels raised in areas of Sudan, where cattle, sheep and goats were intensively bred, bacteriological and serological examinations were performed. Among the camels, 3275 belonged to 110 herds, 35 were reared individually or with cattle, and 103 had been slaughtered at Nyala abattoir. The infection was found in 50 (45.5%) of 110 herds, with prevalence rates ranging from 1.4 to 89.5% in 72 (7.3%) out of 993 males and in 196 (8.1%) out of 2420 females. Of the positive camels 75% were adults over 4 years old and the remaining 25% were younger, from 6 months to 4 years old. Teshome *et al.*, (2003) examined 1442 camels from three different location in Ethiopia (Afar Somali and Borena) with RBPT, 82 (5.7%) of them were positive. The result of (CFT) on those positive with RPBT indicated (4.2%) prevalence of brucellosis in tested camels in Somali (2.8%) and Borena (1.2%) regions. Camels in Afar had a four timer higher risk of brucellosis with an odd ratic (OR) of 4.34 (confidence interval) 95%Cl = 1.76-10.72 , P< 0.001) compared to the risk in Borena .Likewise , afar had higher risk (OR = 1.76, 95%Cl = 1.13 – 2.74), P<0.05) than that in Somali . There was no significant difference in prevalence between the sexes (P>0.05). Although a higher prevalence (6.3%) was observed in camels over 3 years old in Afar, there was no significant over all age difference (P>0.05). Bati, (2004) conducted in his study on 3218 camels in 250 herds from Liben (2232) and Yabello (986 animals) districts. Of these 78.6% (2528 out of 3218) and 21.4% (690out of 3218)were female and male camels, respectively. All serum samples were initially screened by RBPT brucellosis and 72 of the sample were seropositive. All RBPT positive reactors were further tested by CFT for confirmation. CFT confirmed 58 seropositive cases out of 72 RBPT reactors. The study showed the

distribution of *Brucella* species antibodies in 1.8% (95% CI = 1.4 – 2.3) of the tested samples. Slightly higher seroprevalence was recorded in Yabello (2.0%, 95% CI = 1.2 – 3.1) than Liben district (1.7%, 95% CI = 1.2 – 2.3), though not statistically differing from each other ($p > 0.05$). Female camels had higher prevalence (2.06%, 95% CI = 1.5 – 2.7) than male animals (0.9%, 95% CI = 0.3 – 1.9). The effect of sex was observed to be significant for seroprevalence ($p < 0.05$) with the risk of infection 2.3 (95% CI = 1.1 – 5.3) times higher in females than male camels. Similarly, there was significant increase in seropositivity with respect to increasing herd size ($p < 0.05$) with chances of disease occurrence 1.4 times higher in herd of 11 – 20 camels and 2.4 times higher in herd above 20 animals compared to small sized herds (< 11 animals). Immature animals (2 – 4 years) had statistically lower reactors than adult camels ($p < 0.05$), the odds of infection being 2.2 (95% CI = 1.1 – 4.6) times lower in immature camels. Al – Majali et al., (2008) collected and screened for brucellosis 412 camel sera from 37 herds using RBPT, 47(11.4%) were positive and when these were confirmed by CFT, 39 (9.5%) were positive, there fore the seroprevalence of camel brucellosis as adjusted to RBPT and CFT sensitivities and specifities was 12.1%. The seroprevalence in southern part of Jordan was significantly higher ($P = 0.021$) than central or northern parts of Jordan. Chi square analysis on the individual camel data revealed 5 variables with $P<0.05$ (age, herd size, contact with small ruminants, addition of new camels and use of disinfectants), which were offered to the multivariable logistic regression model. Larger herd size and contact with small ruminants were identified as risk factors for camel brucellosis. Dawood, (2008) had carried a study of the prevalence of camel brucellosis in the south province of Jordan during the years 2006

and 2007. Six hundred forty camel sera from 44 herds were randomly collected and analyzed. Rose Bengal Plate Test was used to screen all serum samples. The positive samples were subjected to confirmation by Complement Fixation Test. The true prevalence of *Brucella* seropositive was 15.8%. Of the positive camels 64.8% were adult > than 4 years old and the remaining 35.2% were young ranging from 6 months to 4 years old. Ghanem *et al*., (2009) conducted a study to investigate the prevalence and risk factors of camel brucellosis in Northern Somalia (Somaliland) at three main districts of camel rearing regions (Awdal, Waqoyi Galbed and Togdheer) in the period from July to November, 2008. A total of 1246 camel blood sera were randomly collected from 42 sporadic small scale camel herds. Two serological tests were used to screen all serum samples, Rose Bengal Plate Test (RBPT) and indirect ELISA (I-ELISA). Multivariate logistic regression was constructed to study the risk factors associated with Brucella seropositive cases. The overall prevalence of camel brucellosis in districts under investigation was 3.9% by RBPT and 3.1% by (I-ELISA). Multivariate logistic regression on animal level showed that locality ($P<0.05$; OR: 6.254; CI, 1.186–32.976), herd size ($P<0.001$; OR: 5.493; CI, 2.956-10–207), rearing with other ruminants ($P<0.001$; OR: 12.433; CI, 3.957–39.060), and contact with other camels ($P<0.05$; OR: 5.311; CI, 1.093–25.800) were the potential risk factors. However, herd size ($P<0.05$; OR: 5.425; CI, 1.181– 24.932), and rearing with other ruminants ($P<0.05$; OR: 20.466; CI, 1.456–28.638) were recorded as risk factors on the herd level. Omer *et al*., (2010) studied brucellosis in 2225 camels in certain nomadic localities inSudan, using serum and milk samples. Serum samples were examined by Rose Bengal Plate Test (RBPT), modified RBPT (MRBPT), Serum Agglutination Test

(SAT) and Competitive Enzyme-linked Immunosorbent Assay (C-ELISA). Overall seroprevalence in camels (milk and serum samples) was 37.5%. The seroprevalence in males was 28.2% and in females 40.1%. Megersa *et al.*, (2011) sampled and tested 756 camels using RBPT and CFT, bucella anti bodies were prevalent in 2.2% (95% Cl = 1.4 – 3.7) of screened animals. Keeping ruminant species with camels at household level was found to be the riskfactor for camel brucellosis (OR= 5.3; 95%CI, 1.2–23.5). Mohammed *et al.*, (2011) screened in his study 573 camels from 88 herds. RBPT identified 11 sero positive reactors (1.9%). The positive reactors were further confirmed using CFT. Accordingly, nine (1.6%) seropositive camels were observed. Seroprevalence of brucelosis in female animals were 1.9% which is relatively higher than male camels 1.3% but there was no significant difference (P>0.05).The same scenario occurring in age and herd size. There was a significant association between seroprevalence and camels kept with small ruminants (P<0.05). Sadiq et al., (2011) collected sera samples from 254 adult camels in Brono State, Nigeria and tested them using RBPT and MTSA. Twenty four samples (9.4%) were positive by the two tests. There was no statically significant association between sex of camels, male with OR: 1.324 (95% Cl = 0.613 – 2.859), female OR: 0.970 (95% Cl = 0.0889-1.058) and serological reaction (P>0.05). Swai *et al.*, (2011) conducted a cross-sectional field survey to determine the seroprevalence and to identify risk factors for brucellosis seropositivity and udder health in camel from 8 geographical localities of northern Tanzania during the period of June to August 2010. The study populations comprised 193 camels of all age and sexes, selected from 14 traditional managed herds. Individual animal and herd-level data were collected using a structured

questionnaire. Mastitis was investigated based on microbiology and California Mastitis Test (CMT), while brucellosis was evaluated serologically for antibodies against *brucellosis* infection using Rose Bengal Plate Test (RBPT). The crude prevalence of antibodies to *Brucella* was 2.1% for individual camels and 21.4% for herds. Results of univariable logistic regression models identified body condition score and geographical location to be the major risk factors for individual herd seroprevalence. Poor condition score (16.6%; P<0.036) was associated with increased risk of seropositivity compared to animals with fair to good condition.

Table 1. 3 Prevalence of *brucellosis* in livestock in different countries

Country	**Species**	**Prevalence (%)**	***Brucella* species**	**Reference**
Algeria	Sheep	2.18	_	Refai (2000)
	Goat	12.00	_	
Egypt	Buffalo	10.00	*Br. abortus*	Refai (1989)
	Cattle	23.30	*Br. melitensis biovar 3*	and Montaser Hamoda (1998)
	Donkey	7.30		Montasser *et al.* (1999)
	Horse	5.88	_	
	Mule	71.42	_	
Eritria	Cattle	8.20	_	Omer *et al.* (2000)

	Sheep	1.40	_	
	Goat	3.80	_	
	Camel	3.10	_	
	Horse	0.00	_	
India	Equine	12.89	_	Sharma *et al*. (1979) Zowghi *et al*.
	Bovine	6.37	_	
	Sheep	3.42	_	
	Goat	5.53	_	
Iran	Cattle	0.85	_	(1990)
	Goat	10.18	*Br. Abortus*	
Camel		8.00	_	Zowghi and Ebadi (1988)
Iraq	Sheep	15.00	*Br. melitensis*	Al-Ani *et al.* (1998)
Cattle		3.00	*Br. Abortus*	
Camel		17.20	_	
Libya	Camel	4.10	*Br. melitensis biovar 1.*	Gameel *et al.* (1993)
Nigeria	Cattle	5.82	_	Cadmus *et al.* (2006)
	Goat	0.86	_	
Oman	Camel	8.00	*Br. abortus*	Anonymous (1998)
	Cattle	3.30	_	
	Sheep	1.60	_	
Goat		6.40	*Br. melitensis*	
Pakistan	Horse	5.78	_	Ahmed and Munir

				(1995a & b)
	Dog	9.33	_	
	Poultry	4.00	_	
	Buffalo	5.05	_	
	Cattle	5.46	_	
Camel		2.00	_	Ajmal *et al.* (1989)
Saudi			*Br. melitensis*	
Arabia	Camel	8.00	*biovar*	Memish (2001)
	Cattle	18.70	*2.*	
	Sheep	6.50	_	
Sri	Goat	9.70	_	Silva (2000) *Al*
Lanka	Cattle	4.7	_	*et al.* Yagoub *et* .
	Buffalo	4.2	*Br. abortus*	
Sudan	Camel	6.95	_	(1990)
				El-Ansary *et al.*
	Camel	0.00	*Br. abortus*	(2001)
	Cattle	5.00	_	
	Sheep	1.00	_	
	Goat	4.00	_	
United				an (199
Arab	Camel	2.00	_	Afzal d Sakkir 4)
Emirates	Cattle	1.30	*Br. abortus*	
	Sheep	2.00	_	
	Goat		_	

Chapter Two

Materials and Methods

2.1 Study Area

Alzulfi Governorate site strategically in the heart of Saudi Arabia (Region of Najd) between latitude '17 ° 26 north and longitude '48 ° 44 east and follow the administrative area of Riyadh, is away from the capital, Riyadh, almost 229 kilometers to the north-west and of maintaining an area of 5540 km2 and rising from the sea about 600 meters .The population is almost more than eighty thousands.

2.2 Study Population

The total animal population in the governorate is (1890400), 1500000 (79%) sheep, 300 thousand (15%) goat, 150000 thousands (8%) cow,4 hundred (0,02%) equine and 75 thousand (4%) camels.

The number of livestock in Alzulfi Governorate:

No	Species	Account
1	Sheep	1500000
2	Goat	300000
3	Camel	75000
4	Cattle	15000
5	Equine	400
6	Total	1890400

(Source of information Livestock division of the directorate of Agriculture Alzulfi). .

2.3 Study Design

Data was collected as part of astudy on the seroepidemiology of brucellosis infection in camels herding in Alulfi Governorate.Across-sectional study was carried out during December 2013 to estimate the seroprevalence of camel brucellosis and to investigate associated risk factors. Multistage random sampling was designed based on the governorate, locality, herd and animal, herds and individual animals based on the simple random sampling. Two localities were selected randomly during the study namelyMixedfarming and Desertgrazing.

2.4Sample Size

The sample size of the study animals was determined by using the formula given for simple random sampling method. The relevant formula for 95% confidence and 5% precision was:

$$n = \frac{(1.96)^2 \, Pexp(1-Pexp)}{d^2}$$

Where: n = required sample size

Pexp = expected prevalence

d= desired absolute precision

(Thrusfield 2005)

The expected prevalence in the present study was estimated as 50%. This was based on a previous study with prevalence rates ranging from 1.4 to

89.5% (Musa and Shigidi., 2001**)**.The average was 45% and inflated to 50% to widen the chance of observation and estimate the distribution of brucellosis Alzulfi governorate.

So the sample size was calculated as follows:

$$n = \frac{(1.96)^2 \times 0.5 \times 0.5}{(0.05)^2} = 700$$

The total calculated sample size was 700 however, 750 camels were screened from the study area.

2.5Sampling Technique

Blood samples of about 10 ml were aseptically collected using plain tubes from camels through jugular vein puncture. Serum was separated within12 hours of collection and transported to Algassim laboratory for Veterinary Diagnosis Ministry of Agriculture Saudi Arabia using an ice box where they were stored at -20c° until laboratory test was performed by RBPT, and C-ELISA.

2.6Questionnaire survey

Information of each camel sampled was obtained, this includes its location, age, gender, breed, body condition, whether reared individually or with other ruminant species, contact with other camel herds.

Selected camel owners were interviewed by using questionnaire. By doing so risk factors that had possible association with brucellosis among herds were investigated to support serological result. These includeherd size, management type, production type, source of drinking water , contact with other ruminant species,contact with other camels herds,health status (history of abortion,retained placenta,still birth and infertility)source of new

camel to the herd ,milking hygiene, herd man education, awareness of brucellosis, awareness of fetus and fetal membrane disposal and veterinarysupervision.

2.7Diagnostic Techniques

a\ RBPT: (Rose Bengal Plate Test)

All sera samples collected were initially screened by RBPT using RBPT antigen in Algassim laboratory for Veterinary Diagnosis,Ministry of Agriculture Saudi Arabia. Sera samples were kept in refrigerator at 4 °C before testing. Sera and antigen were left at room temperature for half an hour before the test to maintain to room temperature. The test procedure recommended by Alton *et al*. (1975) was followed: 30µl of RBPT antigen was added to each circle on the plate and 30µ l of test serum was placed alongside the antigen. The antigen and test serum were mixed thoroughly by wooden applicator. The plate was shacked for 4 minutes and the degree of agglutination reactions were read and recorded as + + ++ (coarse clumping and clearing),+ + + (clumping and some clearing), + + (visible fine agglutination), + (weak fine agglutinations using magnifying glass) in case of positive reactions, and 0 (no agglutinations) in negative reactions

.

b\ Competitive Elisa (C-ELISA)

The Competitive Enzyme- linked Immunosorbent Assay kit was obtained from Veterinary Laboratories Agency, Department of Environment, Food and Rural Affairs, Surrey, United Kingdom (Version 2.0 .June 2009). The test was conducted according to manufacturer's instruction. Initially the diluting buffer, washing solution, stopping solution, conjugate solution and controls were reconstituted as directed by the manufacturer. Test serum was added per each well of the micro titer plate which had sixty columns (wells). 100 μL of the prepared conjugate solution was then dispensed in all wells. The plate was then shaken for 2 minutes in order to mix the serum with the conjugate solution. The plate was then covered with the lid and incubated at room temperature for 3 minutes. The content of the plate was then discarded and rinsed 5 times with washing solutions and then dried. 100 μL of the substrate chromogen solution was added to all wells. The plate was kept at room temperature for 10 minutes. The reaction was slowed by adding 100 μL of the stopping solution to each well. Control Setup. 20mL of the negative controls was added to well A11, A12, B11, B12, C11, and C12, while another 20mL of the positive control was added to wells F11, F12, G11, G12, H11, and H12. D11, D12, E11, and E12 served as conjugated controls. Results:

The lack of color development indicated that the sample tested was positive. A positive / negative cut-off can be calculated as 60% of the mean of optical density (OD) of the 4 conjugate control wells. Any test sample giving an OD equal to or below this value should be regarded as being positive.

2.8Statistical Analysis

Data on tested serum and questionnaire were stored in Microsoft excel spread sheet (Microsoft corp. 1985- 2007) as data base. Statistical analysis wasperformed using Statistical Package for the Social Sciences(SPSS) , version 16.0 software for windows (SPSS Inc.,IL,USA).

The seroprevalence for animal level was calculated on the basis of RBPTpositivity, dividing the number of *Brucella* reactors by total number of tested animals. Similarly, herd level prevalence was calculated as the number of herds with at least one positive animal divided by the total number of herds tested. Data collected from the questionnaire survey was analyzed using descriptive statistic methods. Frequency distribution showed the frequency of occurrence of the observations in the present data set. Since the present data was categorical the frequency distribution of the variables comprised the frequency of occurrence of observations in every category. Crosstabulation was used in 2×2 tables and multi way tables to measure the degree of association between these tables and related statistics.Associations between the outcome variable (status of brucellosis) and its potential risk factors were first screened in a univariate analysis using the Chi-square. Potential risk factors with *P* value *<0* .20 were considered significant at this level. Significant risk factors in the univariate analysis were subjected to multivariate analysis using logistic regression.

Exp B was used to indicate the strength of association with risk factors involved in the occurrence of the disease.All risk factors with $p \leq 0.05$ were considered significant.

Chapter Three

Result

3.1 Frequency

3.1.1 Overall Serological Prevalence

In this study, 750camels were screened from 59 herds. RBPT (Rose Bengal Plate Test) identified 49 seropositive reactors out of 750 serum samples (6.5%) (Table 3.1). The seropositive camels obtained by RBPT (49) were subjected further more to C-ELISA (Competitive Enzyme Linked Immunosorbent Assay) Twenty four camels were positive (3.2%) (Table 3.2) .Out of 59examined herds 39 camel herds were positive for brucellosis (45.8%).

Table3.1 Frequency of *brucella* in 750 camels in Alzulfi Governorate examined by RBPT

RBPT Results		Frequency	Percent	Valid Percent	Cumulative Percent
	negative	701	93.5	93.5	93.5
	positive	49	6.5	6.5	100.0
	Total	750	100.0	100.0	

Table 3.2: :Frequency of *brucella* in 750 camels in Alzulfi Governorate examined by C-ELISA

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid				
negative	726	96,8	96,8	96,8
positive	24	3,2	3,2	100,0
Total	750	100,0	100,0	

3.1.2Serological Prevalence in Relationship to Risk Factors

3.1.2.1 General Risk Factors

a\ Localities: The study was conducted in 2 localities in the governorate namely(In farming and Around grazing),Camels were selected from Infarming 50 % (n=375) and Around grazing 50,5%(n=375) (Table 3,3). Seroprevalence of *brucellosis obtained* in Infarming was 9.9 % (n=37), higher than that in Around grazing 3.2 % (n=12) (Table 3,4). After that confrmed by C-ELISA, the results were as follows: In farming 50%(n=375) was 4,5% (no=17) , higer than that in Aroundgrazing 1,9 % (n=7) (Table 3,6). There was significant statistical difference between the prevalence in the two Localities, RBPT (P value=0.001) (Table 3.5) and C-ELISA (P value=0,038) (Table 3.7), in the univirate analysis.

3.1.2.2Individual Risk Factors

a\ Sex:All breeding male and female camels above 3 month of age were consideredin the analysis . from the total camelstested 71,9%(n=539) were females while 28,1 % (n=211) were male camels.(Table 3.3).Seroprevalence of Brucella in female animal using RBPT was 7,2% (n=39) rehigher than that of the male camels 4,2% (n=9)(Table 3.4).

Confirmation byC-ELISA showed that the results were as follows: In female animals were 3.6 (n=19) % relatively higher than that of the male camels 2,3% (n=5) (Table 3.6). There was signifigant difference observed in the univirate analysis ,RBPT(P value=0,145) (Table:3,5) and no significant C-ELISA (P=value 0,038) (Table3,7).

b\ Age: Age was one of the factors observed in the study .Categorization wasbased on the physiological maturity for breeding purpose where younggroup were considered below 5 years and adult group above 6-10 years old and last group over 10.Out of the total camels , sampled 27,3% (n=205) were young while 58,9%(n=442) were adult camels 6-10 and last group over 10 (n=103) 13,7%(Table3.3). In this observation seroprevalence of *Brucella* using RBPT was 3,9 % (n=8) in young and 7.7 % (n=34) in adult camels 6-10 and last group over 10 , 6.8% (n=7) (Table: 3.7). It was then confirmation tests the c-ELISA and the results were as follows :

In young 1, 0 % (n=2) in adult camels4,5% (n = 20) and last group 6-10 over10(n=2) 1,9% .(Table :3,6).

was statistical significance

There between 3 age groups, R B P T (P value = 0,191 (Table :3.5) and c-ELISA (P value = 0,043) (Table: 3.7),in the) univirate analysis.

c\ Breed: Individual camels selected in this study came from 2 breeds, Majaheemand Magatir . From the total camels screened 63,3 % (n=475) were Majaheem while 36,7 % (n=275) were Maqatir (Table :3,3). Of the Majaheem 7.8% (n=37) were found seropositive in the study using RBPT while of the Maqatir 4.4 % (n=12) (Table :3.4). Confirmation byC-ELISA and the results were as follow : Majaheem 3,8% (n=18) and Maqatir 2,2 % (n = 6) (Table :8). There was no statistical significance difference between the 2 breeds ,RBPT (P value=0,067) (Table5) and C-ELISA (P value=0,228) (Table :3,7),in the univirate analysis.

3.1.2.3 Managemental Risk Factors

a\ Herd size: Herd size was classified in to three categories (small < 10 ,medium 11 – 20 and large > 20 animals) as judged with respect to enclosure space and local herding condition context . Individual camels were 9,1 % (n=68) in small herds, 29.2 % (n=219) in medium herds and 61.7% (n=463) in large herds (Table3.3). Seroprevelance were 1.5 % (n=1) in small herds, 6.4 % (n=14) in medium herds and 7.3 % (n=34) in large herds (Table 3.6). After confirmation by c- ELISA results it was using RBPTin small herd 1,4% (n=1) 1,8 % (n=4) in medium herd ,4.3% in large herd (n =19) (Table :3.6) .Correspondingly there is an increase of sero positivity with increase in age ,but the difference is not signifant by RBPT (P:value=0,959) and C-ELISA(P: value 0.686)(Table 3.7) in the univirate analysis.

b\ Herd type: Observation during the survey revealed that there are three ways where male and female are kept alone ,female camels kept alone too or in mixed herds. Male camels herds 26.9% (n= 202) (Table 3.3).

The distribution of the disease based on RBPT was lowest in male herds 5,8%(n=8), lower in herds 6.6% (n=27) and in mixed camels herd 6,9%(n=14)(Table3.4).After confirmation by C-ELISA result it was also :All male

2,2% (n=3) lower in female herd 3,2%(n=13) and in mixed herd 4% (n=8) (Table 3,6). There was no stat significant difference between the 3 herds,RBPT (P value=0,916)(Table 3,5) and C- ELISA (P value= 0,655) in the unnivirate analysis (Table 3,7).

c\ Share Place: Other ruminants kept together with the camel herd were consideredas one of the putative factor for dissemination of infection. This was categorized based on the absence and presence of other ruminants together with the camel herds.Of the total camels sampled 69.7 % (n=523) kept without other ruminantswhereas 30.3 % (n=227) kept with other ruminants. (Table: 3,3). Using RBPT, camels reared with other ruminants showed seroprevalence 6.9 (n=36) % higher than that in camels kept alone 5.7 % (n=13) (Table: 3, 4). After conducting the c-ELISA test results were as follows:Camels with other ruminant 3,4 % (n=18) and the camels kebt alone 2,6%(n=6)(Table :3,6).There was no statistical significance difference between the 2 keeping practices, RBPT (P value=0.556) (Table,3,5) and C-ELISA (Pvalue= 0,568)in the univirate analysis (Table 3,7).

d\ Contact with other camels: Contact with other camel herds was considered ofputative risk factors. Individuals within herds in contact with other herd camels contributed 41.9 % (n=314) , while the other not in contact 58.1 % (n=436) (Table :3,3). The distribution of the disease in the first group (in contact) using RBPT was 8.0 % (n=25) and in the second group (not in contact) was 5.5 % (n=23) (Table: 3,4). After c-ELISA (in contact 2,9% n=9) and (no contact 3,4% n=19) . (Table :3,6) There was significant statistical difference between the two groups RBPT (Pvalue=0.179) (Table :3,5) and no significant C-ELISA (P=value0,659) in (Table;3,7) in the univirate analysis.

e\ Husbundary: was a potential risk factor for sero-prevalence of camelbrucellosis . Most of sero-positive was from the group of animals kept in the semi-intensive husbundary. In our analysis camels in semi- intensive husbundary contributed 74.0

% (n=555), on the other hand 26.0 % (n=195) in the intensive management system (Table :3,3).Camels raised in semi-intensive management system showed prevalence of 6,7% (n=37) and intensive 1,5% (n=3)(Table:3,4). After conducting the c-ELISA test result were as follows: Semi-intensive 3,8 % (n = 21) and intensive 1,5% (n=3) .(Tble : 3,6). There was no significant statistical difference between the result by obtained RBPT(P value=0,803)in the univirate analysis(Table 3,5) ,but significant difference was found among C-ELISA result (P value =0,125).

f\ Production type: During the survey camels were kept for milk, meat anddual. The production type was expected to be of the risk factors of the disease. Out of all camels examined 32 % (n=240) were milk camels, 44,9% (n=337) meat camels and 23,1% (n=195) dual camels (Table.3,3).

The occurrence of the disease was higher in milk 7,9. % (n =19) compared tomeat 7.1 % (n=24) and dual 3.5 % (n=6) (Table :3,4).After C-ELISA milk 4,2 % (n=10),meat 2,7% (n=9) and dual 2,9% (n=5) (Table 3,6).

There was statistical significant difference between production type RBPT (P value =0,165)(Table:3,5) and C-ELISA at the univirate level (P value = 0,582).

g\ Feeding : A ccording to feeding practice camels in the study were categorized to equipment feeding and ground feeding these were 68,1 % (n=511) and 31,9% (n=239) respectively (Table: 3,3). The seroprevelance was recorded 5,0% (n=12) in ground feeding lower than that equipment feeding 7,2%(n=37) using RBPT (Table3,4). After conformation of C-ELISA recorded 2,9% (n=7) in ground feeding lower than that in equipment feeding 3,3% (n=17) (Table 3,6). There was no statistical significance difference between the 2 categories RBPT (P value =0,252)(Table ;5) and C-ELISA(P value=0,773)(Table:3,7) at the univirate level.

h\ Watering: All camels screened drank from underground water, tap water with percentage 45.7% (n=343), 54.3 % (n=407) was respectively (table :2).

The brucellosis prevelance detected by RBPT lower in under ground water 4.4 % (n=15)compared to tap water 8,4% (n=34). (Table :3,4) . When conformation by the C-ELISAtest and the results were as follows : Under ground 2,6% (n=9) lower than atap water 3,7% (n=15) (Table :3,4). There was statistical significance difference between with RBPT (P value = 0.028) (Table: 3,5) and no significant difference byC-ELISA (P value = 0,411) (Table :3,5) at the univirate level.

k\ Source of new camels:Owners of Alzulfi governorate obtained their new camels by breeding from own herds or bought from the market .The camels tested had been obtiaoned from the market 25,9% (n=149) and from breeding herd were 74,1% (n=556)(Table:3,3) , the higher seroprevalence of brucellosis was seen in camels obtained from the using RBPT market herd 6,3%(n=35) and in herd 7,2%(n=14)(Table:4,4). Then it was conformed by C-ELISA test and the result were as follows : Obtained from the market 2,9% (n=16) and in herd 4,1% (n=8)(Table:3,6) . The association between the two groups was not significant RBPT (P value=0,655)(Table: 3,5)and C-ELISA (P value=0396) at the univirate level.

l\ Awareness of Brucellosis:During the study owners were interviewed on awareness of brucellosis. The ones found to be very knowledgeable on what brucellosis, its clinical signs, transmission**,** zoonotic impact and control measures were 44,1% (n=331) and the others with absence of knowledge were 55,9% (n=419) (Table:3,3). However, comparatively the first group had prevalence 5.0 % (n=21) lesser than that in thesecond group 8,5% (n = 28)among their came using RBPT (Table :3,4). After ELISA first group 1,9% (n=8) lesser than that in second group 4,8% (n=16) (Table 3,6).There was statistical significance difference between the 2 categories RBPT (P value=0,058)(Table 3,5) and C-ELISA (P value=0,024)(Table : 3,7)

3.2 Logistic Regression with RBPT

The Univariate Analysis by Chi-square on camel risk factors revealed 9 variables with P<0.2 (locality P=0.001, Awareness P=0.179, Breed P=0.076, Production P=0.028, Contact P=0.165, Watering P=0.028, sex P= 0,145 and Age P=0.191 are found significant at univirate level(Tabels: 3,5 and 3,6).

Sex and locality were only identify as signifant (P=0,38 and P=0,001) respectively at the multivirate level.

OR=1.39; 95% CI: 0.756, 2.886) (Table :3,7).

Locality(P>0.05;OR=3.13; 95% CI: 1.600, 6,130), Awareness(P>0.05;OR=;0,153 95% CI:0,783 , 2,999), Contact (P>0.05;OR=0.144; 95% CI: 0.746, 2.791),Production (p=0,05;0R=2,52 95% cl: 0,815 7,817) ,Breed (P>0.05;OR= 1,06 95 % cl: 0,810 3,140) .

Sex (P>0.05;OR=2.38; 95% CI: 1.049, 5.386) (Table 3,6), feeding p> P>0.05;OR=1.48 ; 95% CI: 0.756, 2.886watering(no significant)

3.3Logistic Regression with C-ELISA:

The univirate analysis by Chi –square on camels risk factor reveald 4 significant variable with p<0,2, locality P= 0,038 ,age P = 0,43, husbandary P= 0,125, awareness of brucellosis p = 0,024, when subjected to multivirate analysis.In the multivirate analysis location (P=0,001) and sex (P=0,038) were found as significant risk factor for camels brucellosis (Table:3,8).

Table 3.3: Frequencies and distributions of tested serum samples by individual and management risk factors in Alzulfi Governorate

Risk Factor	**No. of tested samples**	**% of tested samples**
Location		
In	375	50.0
Around	375	50.5
Breed		
Majaheem	475	63.3
Maqatir	275	36.7
Sex		
Female	539	71.9
Male	211	28.1
Age		
≤ 5	205	27.3
6 - 10	442	58.9
>10	103	13.7
Herd size		

≤ 10	68	9.1
11 - 20	219	29.2
>20	463	61.7
Herd type		
All males	138	18.4
All females	410	54.7
Mixed	202	26.9
Production		
Milk	240	30.0
Meat	337	44.9
Dual	173	23.1
Hasbundary		
Intensive	195	26.0
Semi-intensive	555	74.0
Total	750	100

Table 3.3: Frequencies and distributions of tested serum samples by management risk factors in Alzulfi Governorate

Risk Factors and its levels	Number of tested samples	% of tested samples
Feeding		
Equipement	511	68.1
Ground	239	31.9
Watering		
Tap	407	54.3
Underground	343	45.7
New camels		
By breeding	194	25.9
Purchase	556	74.1
Contact		
No	436	58.1
Yes	314	41.9
Shared place		
No	227	30.3
Yes	523	69.7
Awareness		
Good	331	44.1
Not good	419	55.9
Total	750	100

Table 3.4: Estimated Seroprevalences of brucellosis in camels by individual and management risk factors in Alzulfi Governorate using RBPT.

Risk Factor	No. of tested samples	No. of positive samples	Sero-prevalence	95% CI Lower - Upper
Location				
In	375	37	9.9[a]	7.25 - 13.3
Around	375	12	3.2[b]	1.84 - 5.51
Breed				
Majaheem	475	37	7.8	5.70 - 10.6
Maqatir	275	12	4.4	2.51 - 7.47
Sex				
Female	528	39	7.2[a]	2.51 - 7.47
Male	222	9	4.2[a]	2.46 - 8.09
Age				
≤ 5	205	8	3.9[a]	1.99 - 7.51
6 - 10	442	34	7.7[a]	5.55 - 10.6
>10	103	7	6.8[a]	3.33 - 13.4
Herd size				
≤ 10	68	1	1.5[a]	3.18 - 16.1
11 - 20	219	14	6.4[a]	3.84 - 10.4
>20	463	34	7.3[a]	4.58 - 9.10
Herd type				
All males	138	8	5.8[a]	2.97 - 11.0
All females	410	27	6.6[a]	4.57 - 9.42
Mixed	202	14	6.9[a]	4.17 - 11.3
Production				
Milk	240	19	7.9[a]	5.13 - 12.0
Meat	337	24	7.1[a]	4.83 - 10.4
Dual	173	6	3.5[a]	1.60 - 7.36
Hasbundary				
Intensive	195	12	6.2[a]	3.55 - 10.4
Semi-intensive	555	37	6.7[a]	4.88 - 9.06
Total/Overall	750	49	6.5	4.97 - 8.53

Table 3.4: Estimated seroprevalences of brucellosis in camels by management risk factors in Alzulfi Governorate using RBPT.

Risk Factors	Number of tested sample	Number of positive samples	Sero-Prevalence (%)	95% CI Lower - Upper
Feeding				
Equipement	511	37	7.2[a]	5.30 - 9.82
Ground	239	12	5.0[a]	2.89 - 8.57
Watering				
Tap	407	34	8.4[a]	6.04 - 11.5
Underground	343	15	4.4[a]	2.67 - 7.09
New camels				
By breeding	194	14	7.2[a]	4.35 - 11.8
Purchase	556	35	6.3[a]	4.56 - 08.6
Contact				
No	436	24	5.5[a]	3.72 - 8.05
Yes	314	25	8.0[a]	5.45 - 11.5
Shared place				
No	227	13	5.7[a]	3.38 - 9.55
Yes	523	36	6.9[a]	5.01 - 9.38
Awareness				
Not Good	331	28	8.5[a]	5.92 - 12.0
good	419	21	5.0[a]	3.30 - 7.54
Total/Overall	750	49	6.5	4.97 - 8.53

different superscripts indicate significant difference at $p \leq 0.05$

Table 3.5: Univeriate Analysis for the Association between brucellosis in camels by individual and management risk factors using the Chi square test in Alzulfi Governoratewith RBPT-positive status.

Risk Factors with Levels	Number of tested	Number of positive	% positive	Chi square	df	p-value
Location				13.65	1	0.001
In	375	37	9.9			
Around	375	12	3.2			
Breed				3.347	1	0.067
Majaheem	475	37	7.8			
Maqatir	275	12	4.4			
Sex				2.126	1	0.145
Female	528	39	7.4			
Male	222	10	4.5			
Age				3.308	1	0.191
≤ 5	205	8	3.9			
6 - 10	442	34	7.7			
>10	103	7	6.8			
Herd size				0.084	2	0.959
≤ 10	68	1	1.4			
11 - 20	219	14	6.4			
>20	463	34	7.3			
Herd type				0.177	2	0.916
All males	138	8	5.8			
All females	410	27	6.6			
Mixed	202	14	6.9			
Production				3.605	2	0.165
Milk	240	19	7.9			
Meat	337	24	7.1			
Dual	173	6	3.5			

Hasbundary				0.062	1	0.803
Intensive	195	12	6.2			
Semi-intensive	555	37	6.7			

Table 3.5: Univeriate Analysis for the Association between brucellosis in camels by management risk factors using the Chi square test in Alzulfi Governorate with RBPT-positive status.

Risk Factors with Levels	Number samples	Number of positives	% positives	Chi square	df	p-value
Feeding				1.314	1	0.252
Equipement	511	37	7.2			
Ground	239	12	5.0			
Watering				4.830	1	0.028
Tap	407	34	8.4			
Underground	343	15	4.4			
New camels				0.200	1	0.655
By breeding	194	14	7.2			
Purchase	556	35	6.3			
Contact				1.805	1	0.179
No	436	24	5.5			

Yes	314	25	8.0			
Shared place				0.347	1	0.556
No	227	13	5.7			
Yes	523	36	6.9			
Awareness				3.599	1	0.058
Good	331	28	8.5			
Not good	419	21	5.0			

Table 3.6: Multiveriate Analysis for the Association between brucellosis in camels by individual and management risk factors using the Chi square test in Alzulfi Governorate with RBPT-positive status.

Risk Factors	Number tested	Number of positive (%)	Exp(B)	*p-value*	95% CI for Exp(B) Lower - Upper
Location					
Around	375	12	Ref		
In	375	37	3.13	0.001	1.600 - 6.130
Breed					
Maqatir					
Majaheem	275	12	Ref		
	475	37	1.60	0.177	0.810 - 3.140
Sex					
Male	222	10	Ref		
Female	528	39	2.38	0.038	1.049 - 5.386
Age					

≤ 5	205	8	Ref		
6 - 10	442	34	2.29	0.066	0.947 - 5.515
>10	103	7	1.39	0.574	0.441 - 4.385
Production					
Dual	173	6	Ref		
Milk	240	19	2.51	0.108	0.816 - 7.744
Meat	337	24	2.52	0.108	0.815 - 7.817
Feeding					
Ground	239	12	Ref		
Equipement	511	37	1.48	0.254	0.756 - 2.886
Watering					
Underground	343	15	Ref		
Tap	407	34			
Contact					
No	436	24	Ref		
Yes	314	25	1.44	0.275	0.746 - 2.791
Awareness					
Not good	419	21	Ref		
Good	331	28	1.53	0.213	0.783 - 2.999

* indicates significant risk factors

Table 3.6: Estimated Seroprevalences of brucellosis in camels by individual and management risk factors in Alzulfi Governorate using C-ELISA.

Risk Factors	No. of tested samples	No. of positive samples	Sero-prevalence	95% CI Lower - Upper
Location				
In	375	17	4.5[a]	2.85 - 7.14
Around	375	7	1.9[a]	0.91 - 3.81
Breed				
Majaheem	475	18	3.8[a]	2.41 - 5.91
Maqatir	275	6	2.2[a]	1.00 - 4.68
Sex				
Female	528	19	3.6[a]	2.32 - 5.55
Male	222	5	2.3[a]	0.96 - 5.16
Age				
≤ 5	205	2	1.0[a]	0.27 - 3.49

6 - 10	442	20	4.5[a]	2.94 - 6.88
>10	103	2	1.9[a]	0.53 - 6.80
Herd size				
≤ 10	68	1	1.4[a]	0.26 - 7.87
11 - 20	219	4	1.8[a]	1.56 - 6.45
>20	463	19	4,1	2.14 - 5.54
Herd type				
All males	138	3	2.2[a]	0.74 - 6.19
All females	410	13	3.2[a]	1.86 - 5.35
Mixed	202	8	4.0[a]	2.02 - 7.62
Production				
Milk	240	10	4.2[a]	2.28 - 7.50
Meat	337	9	2.7[a]	1.41 - 5.00
Dual	173	5	2.9[a]	1.24 - 6.59
Hasbundary				
Intensive	195	3	1.5[a]	0.53 - 4.43
Semi-intensive	555	21	3.8[a]	2.48 - 5.71
Total/Overall	750	24	3.2	2.16 - 4.72

Table 3.6: Estimated Seroprevalences of brucellosis in camels by management risk factors in Alzulfi Governorate using C-ELISA.

Risk Factors	**Number of tested sample**	**Number of positive samples**	**Sero-Prevalence (%)**	**95% CI Lower - Upper**
Feeding				
Equipement	511	17	3.3[a]	2.09 - 5.27
Ground	239	7	2.9[a]	1.43 - 5.92
Watering				
Tap	407	15	3.7[a]	2.25 - 6.00
Underground	343	9	2.6[a]	1.38 - 4.91
New camels				
By breeding	194	8	4.1[a]	2.10 - 7.92

Purchase	556	16	2.9[a]	1.78 - 4.63
Contact				
No	436	19	3.4[a]	2.81 - 6.71
Yes	314	9	2.9[a]	1.52 - 5.36
Shared place				
No	227	6	2.6[a]	1.21 - 5.64
Yes	523	18	3.4[a]	2.19 - 5.37
Awareness				
NotGood	331	16	4.8[a]	2.99 - 7.70
good	419	8	1.9[a]	0.97 - 3.72
Total/Overall	750	24	3.2	2.16 - 4.72

different superscripts indicate significant difference at $p \leq 0.05$

CI by Epi tool

Table 3.7: Univeriate Analysis for the Association between brucellosis in camels by individual and management risk factors using the Chi square test in Alzulfi Governorate with C-ELISA-positive status.

Risk Factors with Levels	Number of tested	Number of positive	% positive	Chi square	df	p-value
Location			4.304		1	0.038
In	375	17	4.5			
Around	375	7	1.9			
Breed			1.453		1	0.228
Majaheem	475	18	3.8			
Maqatir	275	6	2.2			
Sex			0.914		1	0.339
Female	528	19	3.6			
Male	222	5	2.3			
Age			6.306		2	0.043

≤ 5	205	2	1.0		
6 - 10	442	20	4.5		
>10	103	2	1.9		
Herd size			0.754	2	0.686
≤ 10	68	1	1.5		
11 - 20	219	7	3.2		
>20	463	16	3.5		
Herd type			0.847	2	0.655
All males	138	3	2.2		
All females	410	13	3.2		
Mixed	202	8	4.0		
Production			1.082	2	0.582
Milk	240	10	4.2		
Meat	337	9	2.7		
Dual	173	5	2.9		
Hasbundary			2.349	1	0.125
Intensive	195	3	1.5		
Semi-intensive	555	21	3.8		

Table 3.7: Univeriate Analysis for the Association between brucellosis in camels by management risk factors using the Chi square test in Alzulfi Governorate with C-ELISA-positive status.

Risk Factors with Levels	**Number samples**	**Number of positives**	**% positives**	**Chi square**	**df**	**p-value**
Feeding				0.083	1	0.773
Equipement	511	17	3.3			
Ground	239	7	2.9			
Watering				0.677	1	0.411
Tap	407	15	3.7			

Underground	343	9	2.6			
New camels				0.721	1	0.396
By breeding	194	8	4.1			
Purchase	556	16	2.9			
Contact				0.194	1	0.659
No	436	19	3.4			
Yes	314	9	2.9			
Shared place				0.326	1	0.568
No	227	6	2.6			
Yes	523	18	3.4			
Awareness				5.106	1	0.024
Good	331	16	4.8			
Not good	419	8	1.9			

Table 3.8: Multiveriate Analysis for the Association between brucellosis test in Alzulfi Governorate with C-ELISA-positive status.

Risk Factors with Levels	Number tested	Number of positive (%)	Exp(B)	*p-value*	95% CI for Exp(B) Lower - Upper
Location					
Around	375	7	Ref		
In	375	17	5.76	0.001	2.162 - 15.333
Breed					
Maqatir	275	6	Ref		
Majaheem	475	18	1.84	0.232	0.677 - 4.997
Age					
≤ 5	205	2	Ref		
6 - 10	442	20	6.55	0.016	1.425 - 30.07
>10	103	2	2.27	0.429	0.298 - 17.27
Hasbundary					
Intensive	195	3	Ref		
Semi-intensive	555	21	5.80	0.009	1.539 - 21.86
Awareness					
Not good	419	8	Ref		
Good	331	16	1.68	0.271	0.666 - 4.259

* indicates significant risk factors

Table 3.8: Agreement between the RBPT and C-ELISA

	cELISA-Negative	**cELISA-Positive**	**Total**
RBPT-Negative	701	0	**701**
RBPT-Positive	25	24	**49**
Total	**726**	**24**	**300**

Chapter Four

Discussion

Brucellosis is a widespread disease in camels. The infection rate is higher in intensive camel production system where large numbers of animals are kept in a farm. In countries with extensive form of husbandry the rate is low (Abbas and Agab, 2002). The disease is known to cause abortion and birth of non-viable offspring in female, and orchitis and epididymitis in male animals and infertility in both cases (Radostits *et al.,* 1994; Agab, 1997; Straten *et al.,* 1997). In production system where livestock diversification is practiced, the disease circulates in sheep, goats and cattle, and further spreads to dromedaries (Andreani et al., 1982; Radwan *et al.,* 1992). Five out of the nine known *Brucella* species can infect humans and the most pathogenic and invasive species for human is *B. melitensis*, followed in descending order by *B. suis*, *B. abortus and B. canis* (Acha *et al.,* 2003).The zoonotic nature of the marine brucellae *(B. ceti)* has beendocumented (McDonald *et al.,* 2006). In places where brucellosis is endemic, humans can get infected via contact with infected animals or consumption of their products, mostly milk and milk products. Some specific occupational groups including farm workers, veterinarians, ranchers, and meat-packing employees are considered at higher risk (Tabak *et al*., 2008).*B. abortus and B. suis* infections usually affect occupational groups, while*B. melitensis* infections occur more frequently than the other.*Brucella* species in the general population (Acha *et al*., 2003).

Most common symptoms of brucellosis in Man include undulant fever in which the temperature can vary from 37°C in the morning to 40°C in the afternoon; night sweats with peculiar odor, chills and weakness. common symptoms also include malaise, insomnia, anorexia, headache, arthralgia, constipation, sexual impotence, nervousness and depression (Acha *et al.,* 2003).Despite the advances made in surveillance and control, the prevalenceof brucellosis is increasing in many developing countries due to varioussanitary, socioeconomic, and political factors (Pappas et al., 2006). In this study and based on the results of RBPT, the prevalence of *Brucellosis* of examined camels was (6.5 %). This result was in accordancewith that recorded in Ethiopia (Teshome *et al*., 2003). However, higher prevalence was recorded in Sudan (Musa and Shigidi 2001and Omer et al., 2010), Saudi Arabia (Abbas and Agab 2002), Jordan (Al- Majali et al., 2008 and Dawood 2008), and Nigeria (Sadiq *et al.*, 2011). The complexity of disease epidemiology and the lack of exact camel population concerning detailed demographic data, besides lack of cattle, sheep and goats *brucellosis* control program including vaccination may contribute to this prevalence of camel brucellosis. The differences in the prevalence of camel brucellosis from different countries may be attributed to varying husbandry and management practices, the number of susceptible camels, the virulence of the organisms, presence of reactor animals in the region, absence of veterinary service, lack of awareness about the disease in camels and continuous entry of infected camels into a susceptible camel herd (Radostits *et al.*, 2007).

In this study by the univariate analysis, the presence of seropositive camels was significantly associated ($P<0.20$) with the variables:

location, age, breed, , water sources (watering) ,awareness,contact with the other ruminant ,sex,and type of production. The occurrence of the disease was higher in Infarming 9,9% (n = 37), aroundgrazing3,2 %(n=12),.This result is in agreement with that recorded by Teshome et al., (2003),Al- Majali et al., (2008) and Swai *et al.*, (2011).Teshome *et al.*, (2003) attributedthe effect of locality on *Brucella* infection to husbandry, management practice, absence of veterinary service, lack of awareness, and uncontrolled movement of camels from place to another. This finding is also supported by Radostits *et al.*, (2007) who stated that the movement may worsen the epizootic situation of brucellosis in any location .Husbundary camels raised in semi-intensive management system showed prevalence of 6,7% (n=37) and in intensive management system 6,2% (n=12).After conducting the C-ELISA test result were as follows: Semi-intensive 3,8%(n=21) and intensive 1,5% (n=3),similar findings were recorded previously by Teshome et .,(2003),Al-Majali et al., (2008) and Mohammed et al ., (2011). The present result is supported by Abbas *et al* ., (2000) and Al-Majali *et al* .,*(*2008). Herd size was not found to affect significantly the seropositivity of *Brucella* on animal level (P=0.959). Herds with more than 20 camels weremore frequently affected. Seroprevelance was 7.3 % (n=34) in large herds, 6.4 % (n=11) in medium sized herds and 1.5 % (n=1) in small herds. This result was in agreement with that previously reported by Abbas and Agab (2002), Bati (2004), Al-Majali *et al.*, (2008) and Mohammed et al., (2011). It was suggested that more contact between camels may occur in large herds than smaller ones.

The prevalence was lower among the young animals screened in this study compared to the older ones . In this observation seroprevalence

of*Brucella* was 3,9 % (n=8) in young and 7,7% (n=34) in adultcamels,over age camels 6,8%(n=7). The same results was recorded by Musa and Shigidi (2001), Bati (2004), Al- Majali *et al.*, (2008), Dawood (2008), Omer *et al*., (2010) and Swai *et al*., (2011). Usually young animals are protected by maternal immunity until when the immunity disappears, thus susceptibility seems to be low among them. Also, older camels are more exposed. The presence of growth factors such as erythritol and hormones favor infection in mature animals. The high prevalence seen in the older animals was demonstrating the chronic nature of brucellosis. Multivariate analysis showed that herd size comprising more than 20 camels was significantly associated with seroprevalence of camel brucellosis in logistic regression (OR=9.324; 95% CI: 1.1 – 74.2, P<0.05). The same scenario recorded by Bati (2004) (OR=1.5; 95% Cl: 1.0 –2.2, P<0.05), Al- Majali *et al.*, (2008) (OR=1.5; 95% Cl: 1.1 – 3.7, P<0.05) and Ghanem *et al*., (2009) (OR=5.425; 95% Cl: 2.9 – 10.2 , P<0.001). The increase in herd size increases the chance of contact between animals leading to more chances of infection. In large herd size the presence of mature animals is of great importance particularly during the breeding season. Calving or aborted female camels contaminate the environment and increase the infection rate of brucellosis. Also, older camels are more exposed to infection. The herd size and density of animal population together with poor husbandry, management practices, the number of susceptible camels, the virulence of the organisms, presence of reactor animals in the region, absence of veterinary service and lack of awareness about the disease in different locations directly increasing the infection rate of brucellosis. Mixed herding and frequent contact

with small ruminants and cattle are contributing factors to infection rate. In large size camel herds there is high chance of brucellosis transmission from these ruminants to dromedaries asthey live free in pasture and at water points. Specially, contact between dromedaries and small ruminants that incriminated for the transmission of brucellosis to camels.

Conclusion

The current study has shown the overall prevalance of *Brucella* antibodies as 6.5% of the tested dromedaries in Alzulfi governorate. Despite the fact that the overall seroprevalence of brucellosis in this study was low, animals and owner family members of those infected herds are all at risk. In univariate analysis location, contact, age groups ,breed, water sources (watering), sex,type of production and awareness categories have shown significant association with seroprevalence of camel brucellosis. The multivariate analysis of presumed risk factors indicated awareness as a major risk factor associated with camel brucellosis. Results of the present study clarified the status of camel brucellosis in Alzulfi governorate and the risk factors that contribute to the occurrence of the disease in dromedaries as well as the possible zoonotic implications in human beings.

Recommendations

Based on the results of this study the followings are recommended:

1/ Although the prevalence of brucellosis in camel population is probably related to husbandry- practice, there is lack of information regarding the pathogenesis and epidemiology of brucellosis in camels.

2/ However education of herdsmen about animal diseases modern managementpractices and sanitary measures could play a major role in lowering the prevalence of the disease.Isolation and identification of species and biotypes of *brucellosis* involved in camel brucellosis are needed.

3/ A routine vaccination for cattle, sheep and goats should be considered in areas where camels are kept together with these animals.In the future, study is necessary to investigate the risk factors and the public health issues related to camel brucellosis .

4/ The need for governmental and non-government organization to enhance their

capabilities in camel research ,veterinary services and to establish adequate veterinary infrastructures concerning camel dairy production.

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