

**ADDIS ABABA UNIVERSITY**

**COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE**

**SERO-PREVALENCE STUDY OF BRUCELLOSIS IN SMALL RUMINANTS AND  
CAMELS AS WELL AS RISKY PRACTICE OF PASTORALISTS FOR ZOO NOTIC  
BRUCELLOSIS IN SELECTED DISTRICTS OF AFAR REGION**

**MSc THESIS**

**BY**

**WESINEW ADUGNA**

**JUNE, 2012**

**DEBRE-ZEIT, ETHIOPIA**

**SERO-PREVALENCE STUDY OF BRUCELLOSIS IN SMALL RUMINANTS AND  
CAMELS AS WELL AS RISKY PRACTICE OF PASTORALISTS FOR ZOONOTIC  
BRUCELLOSIS IN SELECTED DISTRICTS OF AFAR REGION**

A thesis submitted to school of Graduate Studies of Addis Ababa University in partial fulfillment  
of the requirements for the Degree of Masters of Veterinary Science in Tropical Veterinary  
Microbiology

**BY**

**WESINEW ADUGNA**

**JUNE, 2012**

**DEBRE-ZEIT, ETHIOPIA**

**SERO-PREVALENCE STUDY OF BRUCELLOSIS IN SMALL RUMINANTS AND  
CAMELS AS WELL AS RISKY PRACTICE OF PASTORALISTS FOR ZOONOTIC  
BRUCELLOSIS IN SELECTED DISTRICTS OF AFAR REGION**

**BY**

**WESINEW ADUGNA**

**Board of Examiners**

**Signature**

1. Professor Tesfu Kassa    Aklilu Lemma Institute of Pathobiology, AAU \_\_\_\_\_
2. Dr. Kelay Belihu        FAO- Awash Field Office \_\_\_\_\_
3. Dr. Alemu Yami        Ethiopian Sheep and Goat Production Improvement Project \_\_\_\_\_

**Academic Advisor**

**Signature**

Dr. Tesfaye Sisay (DVM, MSc, PhD, Associate Prof.) \_\_\_\_\_

## ACKNOWLEDGEMENT

I deem it as my utmost pleasure to avail this opportunity to express the heartiest gratitude and deep sense to my dedicated advisor Dr. Tesfaye Sisay, for his valuable suggestions, keen interest, dexterous guidance, enlightened views, constructive criticism, unfailing patience and inspiring attitude during my studies, research project, and writing of this manuscript.

A big share of thanks goes to Dr. Shiferaw Jembrae, and his lab fellow W/ro Wubet G/medhin, from NVI, Serology section for their technical guidance in laboratory work and for giving me a chance to work with them.

I gratefully acknowledge invaluable help render by Dr. Ahimed Issa through providing diagnostic reagents; and all Semera Veterinary laboratory staffs particularly Dr. Anteneh Hailu, Mr. Mekonin Ali, Mr. Hussen Mohamed, and Mr. Hassen Ali for helping me during sample collection and laboratory work.

I would like to express my heartiest honor to my parents whose affections and encouragement enabled me to finish this research work. I am also thankful to my family members, especially my wife W/ro Habil Aklilu for her patience and understanding during the period of my study.



# TABLE OF CONTENTS

|                                                     | PAGE |
|-----------------------------------------------------|------|
| ACKNOWLEDGEMENT .....                               | I    |
| TABLE OF CONTENTS .....                             | II   |
| LIST OF TABLES .....                                | IV   |
| LIST OF FIGURES .....                               | V    |
| LIST OF APPENDICES .....                            | VI   |
| LIST OF ABBREVIATIONS .....                         | VII  |
| ABSTRACT .....                                      | IX   |
| 1. INTRODUCTION .....                               | 1    |
| 2. LITERATURE REVIEW .....                          | 4    |
| 2.1. Brucellosis .....                              | 4    |
| 2.1.1. Etiology .....                               | 4    |
| 2.1.2. Epidemiology .....                           | 8    |
| 2.1.3. Pathogenesis .....                           | 12   |
| 2.1.4. Virulence factors .....                      | 13   |
| 2.1.5. Immune responses .....                       | 17   |
| 2.1.6. Diagnosis .....                              | 19   |
| 2.2. Brucellosis in sheep, goats and camels .....   | 25   |
| 2.3. Human brucellosis .....                        | 26   |
| 2.4. Disease control and prevention .....           | 29   |
| 3. MATERIALS AND METHODS .....                      | 33   |
| 3.1. Description of study area .....                | 33   |
| 3.2. Study population .....                         | 36   |
| 3.3. Study design .....                             | 36   |
| 3.4. Sampling method .....                          | 37   |
| 3.5. Study methodology .....                        | 38   |
| 3.5.1. Questionnaire survey .....                   | 38   |
| 3.5.2. Serological survey .....                     | 39   |
| 3.6. Data Processing and Statistical Analysis ..... | 40   |
| 4. RESULTS .....                                    | 42   |

|                                                                                                        |     |
|--------------------------------------------------------------------------------------------------------|-----|
| 4.1. Seroprevalence of small ruminant and camel brucellosis .....                                      | 42  |
| 4.2. Effect of risk factors on seroprevalence of small ruminant and camel brucellosis .....            | 43  |
| 4.3. Univariate logistic regression analysis of risk factors .....                                     | 46  |
| 4.3.1. Effect of risk factors on small ruminants seroprevalence .....                                  | 46  |
| 4.3.2. Effect of risk factors on camels seroprevalence .....                                           | 47  |
| 4.4. Multivariable stepwise logistic regression analysis of risk factors for brucella reactivity ..... | 48  |
| 4.5. Results of questionnaire survey .....                                                             | 49  |
| 5. DISCUSSION .....                                                                                    | 52  |
| 6. CONCLUSION AND RECOMMENDATIONS .....                                                                | 57  |
| 7. REFERENCES .....                                                                                    | 59  |
| 8. ANNEXES .....                                                                                       | 83  |
| 9. CURRICULUM VITAE .....                                                                              | 101 |
| 10. SIGNED STATEMENT OF DECLARATION .....                                                              | 103 |

|                                                                                                               |    |
|---------------------------------------------------------------------------------------------------------------|----|
| Table 1: Seroprevalence of brucellosis in sheep, goat and camel in some countries .....                       | 9  |
| Table 2: Seroprevalence of small ruminant and camel brucellosis in Ethiopia.....                              | 10 |
| Table 3: Study population and sample size per district .....                                                  | 37 |
| Table 4: Herd level seroprevalence of small ruminants and camel brucellosis in the study area .               | 42 |
| Table 5: Chi-square test showing the cumulative effect of the three species with brucellosis reactivity ..... | 43 |
| Table 6: Association of risk factors with brucellosis reactivity in small ruminants.....                      | 44 |
| Table 7: Association of risk factors with brucellosis reactivity in goats in the study areas .....            | 44 |
| Table 8: Association of risk factors with brucellosis reactivity in sheep in the study areas .....            | 45 |
| Table 9: Association of risk factors with brucellosis reactivity in camel of the study areas.....             | 45 |
| Table 10: Effects of risk factors on the individual level sero-prevalence of sheep and goats brucellosis..... | 46 |
| Table 11: Effects of risk factors on the overall sero-prevalence of small ruminants' brucellosis.             | 47 |
| Table 12: Effects of risk factors on the overall individual level sero-prevalence of camel brucellosis.....   | 48 |
| Table 13: Multivariable stepwise logistic regression analysis of risk factors .....                           | 49 |
| Table 14: Awareness about zoonotic transmission of livestock diseases .....                                   | 50 |
| Table 15: Animal products handling and consumption habits .....                                               | 51 |



|                                                                                 |    |
|---------------------------------------------------------------------------------|----|
| Figure 1: The Map of Afar region showing the locations of the study areas ..... | 35 |
| Figure 2: The Principle of Complement Fixation Test.....                        | 90 |



|                                                                                                                                                      |     |
|------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Annex 1: Rose Bengal Plate Test (RBPT) procedure.....                                                                                                | 83  |
| Annex 2: Complement Fixation Test (CFT) procedure.....                                                                                               | 85  |
| Annex 3: Questionnaire for the assessments of brucellosis in animals and zoonotic risk to human<br>.....                                             | 91  |
| Annex 4: Figure showing sample collection.....                                                                                                       | 94  |
| Annex 5: Picture showing laboratory activities.....                                                                                                  | 95  |
| Annex 6: Picture showing RBPT and CFT Results.....                                                                                                   | 96  |
| Annex 7: Picture showing the raw milk consumption habit of the pastoral communities .....                                                            | 97  |
| Annex 8: Picture showing the existing livestock managemnt system which allows mixing of<br>small ruminants and camel at different circumstances..... | 98  |
| Annex 9: Photos taken during primary data collection using questionnaire .....                                                                       | 100 |

## LIST OF ABBREVIATIONS

|         |                                                |
|---------|------------------------------------------------|
| APC     | Antigen Presenting Cells                       |
| ARFEB   | Afar Region Finance and Economic Bureau        |
| BPAT    | Buffered Plate Antigen Test                    |
| cELISA  | Competitive Enzyme Linked Immuno Sorbent Assay |
| CFU     | Colony Forming Unit                            |
| CI      | Confidence Interval                            |
| CSA     | Central Statistics Authority                   |
| DNA     | Deoxyribo Nucleic Acid                         |
| FAO     | Food and Agriculture Organization              |
| FPA     | Fluorescence Polarization Assay                |
| FPSR    | False Positive Serological Reactions           |
| I-ELISA | Indirect Enzyme Linked Immunosorbent Assay     |
| IFN-g   | Interferon-gamma                               |
| Ig      | Immunoglobulin                                 |
| IL      | Interleukin                                    |
| kDa     | Kilodalton                                     |
| KDO     | 2-keto-3-deoxyoctulosonic acid                 |
| LPS     | Lipopolysaccharide                             |
| MoARD   | Ministry of Agriculture and Rural Development  |
| mRBPT   | modified Rose Bengal Plate Test                |
| NGOs    | Non Governmental Organizations                 |
| OIE     | Office International des Epizooties            |
| OMP     | Outer Membrane Protein                         |

|               |                                  |
|---------------|----------------------------------|
| OR            | Odds Ratio                       |
| PA            | Pastoralist Association          |
| RBPT          | Rose Bengal Plate Test           |
| RFM           | Retained Fetal Membrane          |
| S-LPS         | Smooth-Lipopolysaccharide        |
| SSA           | Sub-Saharan Africa               |
| STAT          | Standard Tube Agglutination Test |
| Spp           | Species                          |
| TLR2          | Toll-like receptor 2             |
| TNF- $\alpha$ | Tumor Necrosis Factor- alpha     |
| TNF- $\beta$  | Tumor Necrosis Factor- beta      |
| WHO           | World Health Organization        |



## ABSTRACT

A cross-sectional study was carried out in four districts of Afar region from November 2011 to April 2012 to determine the prevalence of brucellosis in small ruminants and camels and to identify risky practice that would facilitate the transmission of zoonoses to humans. The study involved testing 324 sheep, 726 goats and 461 camels and interviewing 120 livestock owners. Modified Rose Bengal Plate Test (mRBPT) and Complement Fixation Test (CFT) were used as screening and confirmatory tests, respectively. The SPSS 16.0 software was used to establish the overall prevalence for each species, while potential risk factors for seropositivity were analyzed using a multivariable logistic regression analysis. The results showed that 7.1% of the sheep, 5.4% of the camels and 13.6% of the goats ( $X^2 = 24.91$ ,  $P < 0.05$ ) and 5.11% and 24% of the small ruminant and camel herds tested had antibodies against *Brucella* species. District level seroprevalence ranged from 3.6% to 13.6%, 3.3% to 6.7% and 11.7% to 15.5% in sheep, goats and camels, respectively. The final logistic regression model for small ruminants identified goats (OR=2.36; 95% CI: 1.46-3.82) showing higher risk of brucella infection when compared to sheep. In addition small ruminants greater than two years (OR= 3.132; 95%CI: 1.6-6.15), females (OR=1.79; 95%CI: 1.02-3.13) and larger than 30 herd size (OR=2.04; 95%CI: 1.35-3.1) are at higher risk of brucella infection than their counter categories. Moreover in camels, herd size > 20 animals (OR = 2.8; 95% CI: 1.16-6.62) and greater than four years (OR = 4.9; 95% CI: 1.45-16.82) show higher risk of brucella infection when compared to small herds and those  $\leq 4$  years old. The questionnaire survey showed that most respondents had no accurate knowledge about the transmission of zoonoses. It was also found that some of their practice would potentially facilitate the transmission of zoonotic pathogens to human, such as handling of aborted materials and raw animal product consumption. The results of this study demonstrated that livestock brucellosis is widely prevalent in the study areas. Hence, the study suggests the need for implementing control measures and raising public awareness on prevention methods of brucellosis.

*Key words:* Afar region, Brucellosis, CFT, mRBPT, Sheep, Goat and Camel

# 1. INTRODUCTION

Brucellosis is considered by the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the Office International des Epizooties (OIE) as one of the most widespread zoonoses in the world (Schelling *et al.*, 2003). According to OIE, it is the second most important zoonotic disease in the world accounting for the annual occurrence of more than 500,000 cases (Pappas *et al.*, 2006). The disease can affect almost all domestic species and cross transmission can occur between cattle, sheep, goats, camels and other species (Ghanem *et al.*, 2009). It is an important zoonotic disease and causes significant reproductive losses in sexually mature animals (Radostits *et al.*, 1994). The disease is manifested by late term abortions, weak calves, still births, infertility and characterized mainly by placentitis, epididymitis and orchitis, with excretion of the organisms in uterine discharges and milk. It also causes morbidity and considerable loss of productivity (Pappas, 2006). *Brucella melitensis*, *B. abortus*, and *B. suis* are zoonotic pathogens which can infect humans. *B. canis* may cause infections in immunosuppressed individuals (Young, 2000).

The clinical manifestations of brucellosis are different in humans, resulting in intermittent fevers, chills, sweats, weakness, myalgia, osteoarthricular complications, endocarditis, depression and anorexia. The severity of the symptoms and signs vary depending on the species of *Brucella*: *B. melitensis* causes the most severe and acute symptoms, followed by *B. suis*, while *B. abortus* and *B. canis* tend to produce milder disease and subclinical infections in humans (Young, 1995).

Human brucellosis was once thought to be predominantly transmitted through animal contact. However, it is now being realized increasingly that animal products such as milk and meat products also play an important role in the disease transmission. Dairy products prepared from unpasteurized milk such as soft cheeses, yoghurts, and ice creams may contain high concentration of the bacteria and consumption of these is an important cause of brucellosis. Other routes of infection include skin abrasions or inhalation of airborne animal manure particles. Contamination of skin wounds may be a problem for persons working in slaughterhouses or meat packing plants or for veterinarians (Mantur and Amarnath, 2008).



Brucellosis is a zoonosis that exists worldwide and is more or less endemic within most African countries (Mangen *et al.*, 2002). Globally this disease is under-reported because of its vague clinical symptoms, difficult laboratory diagnosis and lack of familiarity by the medical professionals (Corbel, 2006). Within Sub-Saharan Africa (SSA), many of the known infectious diseases occur commonly and are poorly controlled, both in livestock and in human populations (Mangen *et al.*, 2002; McDermott and Arimi, 2002). It has been stated that in SSA, the epidemiology of brucellosis in humans and livestock are not well understood and available data are limited (McDermott *et al.*, 1999; Schelling *et al.*, 2003).

In Ethiopia pastoralism and agropastoralism are an important means of livelihood for more than four million people, with most pastoralists living in the Somali, Afar, Oromiya and Southern Nations regions. Ethiopia's arid or semi-arid pastoral lands comprise approximately 63% of the total land area. The Ministry of Agriculture and Rural Development (MoARD) estimates that nationally, pastoralists own 73% of the goats (equivalent to 7.05 million head), 25% of the sheep (equivalent to 4.25 million head), 20% of the cattle (equivalent to 7.70 million head) and a substantial proportion of the camels (approximately 1 million head)(MoARD, 2008).

Under Ethiopian context, livestock of different species usually share pastures and dwellings. This may play a role in maintenance and transmission of endemic diseases such as tuberculosis and brucellosis (Amenu *et al.*, 2010). More over brucellosis is common in rural areas because farmers live in close contact with their animals and often consume fresh unpasteurized dairy products. However, the vending of dairy products may also bring the disease to urban areas (Mantur and Amarnath, 2008).

Likewise Afar region is known for its large number of small ruminants and camels that are raised under extensive production system. *Brucellosis* is widely distributed and is still somewhat problematic in the region, with small ruminants remaining the most prevalent hosts. Previous works in different districts of the region revealed a prevalence ranging from 0-11.7% (Ashenafi *et al.*, 2007). In camels previous serological surveys showed overall prevalence rates of 4.4% (Domenech, 1977), 5.5% (Richard, 1980) and 4.2% (Teshome *et al.*, 2003) in different camel



rearing areas of Ethiopia. Specifically in Amibara district of Afar region Woldegebriel (2011) reported a prevalence rate of 7.6% in camels.

In the region activities such as; the habit of consuming raw milk and its products, rearing of diversified animal species together, herding of large number of animals collectively is widely practiced. Moreover because of repeatedly occurring natural phenomena such as concurrent drought, earthquake and flooding in the region the introduction of new animals from outside the districts as a replacement stock by different NGOs and food security programs for vulnerable communities and to hazard victims is a common practice. Therefore, because of the aforementioned observable fact the region is at high risk of disease transmission. As the disease has veterinary, public health and economic importance, it is necessary to assess the current status of brucellosis in small ruminants and camels in selected districts of Afar region.

Therefore the objectives of this study were:-

- ❖ To determine the sero-prevalence of brucellosis in camel, goats and sheep in four districts of Afar Region.
- ❖ To determine the effects of different risk factors on the prevalence of brucellosis in animals.
- ❖ To assess the awareness level of the pastoral community on zoonotic brucellosis.

## 2. LITERATURE REVIEW

### 2.1. Brucellosis

#### 2.1.1. Etiology

Over 100 years after Lieutenant Colonel David Bruce, a physician in the Royal Army, discovered “*Micrococcus melitensis*” in the spleens of British soldiers residing on the island of Malta in the Mediterranean Sea (Moreno and Moriyon, 2002), Del Vecchio *et al.* (2002a) presented the genomic sequence of *Brucella melitensis*, which was renamed after Dr. Bruce (Spink 1956). This revelation brought new insight into the biology and pathology of the organism. The genus *Brucella* belongs phylogenetically to the proteobacteria, a group that contains bacterial species with a wide variety of lifestyles, including symbionts of animals and plants (*Wolbachia*, *Sinorhizobium*), as well as obligate or facultative intracellular and extracellular pathogens, such as *Rickettsia*, *Brucella* and *Agrobacterium* (Tsolis, 2002).

Six species are currently recognized within the genus *Brucella*: *B. melitensis*, *B. abortus*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomae*. This classification is mainly based on differences in biochemical characteristics, pathogenicity and host preferences. Each of these species of *Brucella* is adapted to a specific host, but not exclusively (Alton *et al.*, 1975; Alton, 1990).

In 2000, a new species of the genus, with different features when compared to other known species, was isolated from the rodent *Microtus arvalis*, and named of *B. microti* (Scholz *et al.*, 2008a). Recently, a novel *Brucella* species has been isolated from post partum uterus of pregnant baboons with history of stillbirth (Schlabritz-Loutsevitch *et al.*, 2009).

In recent years, an important controversy has developed concerning the taxonomy of the genus *Brucella*. Although *Brucella* species can be differentiated by conventional phenotypic tests, these species display a high degree of DNA homology in DNA-DNA hybridization assays (>90% identity), including the recently recognized marine mammal strains (Verger *et al.*, 1998).



Therefore, it has been proposed that the genus *Brucella* should be a monospecific genus, with *B. melitensis* as the sole species and the other species should be considered as biovars (Verger *et al.*, 1985). Conversely, several molecular genotyping methods have been developed and applied to characterize *Brucella* species, indicating that significant DNA polymorphisms occur between species, which favor the current multi-species classification of *Brucella* (Halling *et al.*, 2005).

Importantly, comparison of genome sequences of *B. suis* and *B. melitensis* demonstrated the existence clusters of genes that are unique in both species (designated genetic islands). It is reasonable to hypothesize that these unique genes may contribute to the differences in host specificity between *Brucella* species (Tsolis, 2002). Furthermore, recent studies based on comparative whole genome analysis of several *Brucella* species indicate that there is limited divergence with a large number of pseudogenes. Interestingly, these genomic analyses do not clearly explain the host preferences of *Brucella* spp. (Foster *et al.*, 2009; Wattam, 2009). One of these studies indicates that *B. ovis* is the basal lineage to the rest of the *Brucella* spp., and that apparently most *Brucella* species diverged from their common *B. ovis* ancestor in the past 86,000 to 296,000 years (Foster *et al.*, 2009).

It is noteworthy that the International Committee on Systematics of Prokaryotes, Subcommittee on the Taxonomy of *Brucella* has taken a clear position recommending a taxonomic classification that includes different species within the genus, either classical or new, which are still considered as individual species. Therefore, the genus is currently grouped into nine species, namely *B. melitensis*, *B. abortus*, *B. suis*, *B. ovis*, *B. canis*, *B. neotomae*, *B. ceti*, *B. pinnipedialis*, and *B. microti* (Mariana *et al.*, 2009). The newly isolated *Brucella* species from baboons (Schlabritz-Loutsevitch *et al.*, 2009) has not yet been classified nor included in the above mentioned list.

*Brucella* species are short, non-motile, non-sporulating, non encapsulated, gram-negative aerobic rods. They are facultative intracellular pathogens of animals and humans (Corbel, 1997). The genus *Brucella* consists of six species, each with a preference for a primary host and varying degrees of pathogenicity. *B. melitensis* primarily infects goats and is the most pathogenic for humans. *B. abortus* infects cattle; *B. canis* causes infection in dogs; *B. ovis* is pathogenic for sheep; *B. suis* infects pigs; and *B. neotomae* causes infection in the desert wood rat. In recent



years, there have also been *Brucella* species isolated from marine mammals, mostly seals and cetaceans (Bricker *et al.*, 2000). The *Brucella* genus is highly homogeneous with all members showing greater than 90% homology in DNA-DNA pairing studies (Verger *et al.*, 1985; Vizcaino *et al.*, 2000).

### *Brucella melitensis*

*Brucella melitensis* is a world-wide disease which remains most problematic in developing countries. Although considered to be eradicated in the United States, *B. melitensis* is a continuing cause for concern because of its status as a potential agent of biological warfare (Moreno, 2002).

The primary host of *B. melitensis* is the caprine host as well as the ovine host, although the pathogen can be problematic in cattle, camels, and humans as secondary hosts (Nielson and Dunkan, 1990). In female goats and sheep the primary symptom is abortion, but larger doses of the pathogen cause mastitis and joint problems. In male goats, orchitis is uncommon, and if observed it is generally unilateral. In male sheep, however, the disease is often asymptomatic, although orchitis and hygromas are a rare observation (Alton, 1990).

The only approved vaccine against *B. melitensis* is the Rev.1 vaccine, developed from a laboratory strain of *B. melitensis* 16M. This vaccine, although effective, is potentially abortigenic and produces anti-LPS antibodies that can interfere with diagnostic tests (Blasco, 2006; Gonzalez *et al.*, 2008). Vaccines developed from strains of *B. abortus* are not effective in providing protection against infection with *B. melitensis*.

*B. melitensis* is the most virulent strain in human infection, with only 1 to 10 organisms needed for infection and with undulant fever as the primary symptom (Mantur *et al.*, 2007). There is no human vaccine for brucellosis and the *B. melitensis* Rev.1 vaccine for animals is infectious to humans (Seleem *et al.*, 2009). *Brucella melitensis* is highly contagious and can be easily disseminated with devastating effects to public and agricultural health. *B. melitensis*, as well as *B. suis* and *B. abortus*, have been deemed potential bioterrorism agents by the United States Centers for Disease Control and Prevention.

*Brucella abortus* is the agent responsible for the majority of bovine brucellosis cases. It was discovered in 1897 by Bernhard Bang and the disease was originally called “Bang’s disease” (Mochman *et al.*, 1988). In 1918 Alice Evans linked *B. abortus* with the previously discovered *B. melitensis* and showed that both were agents of brucellosis in humans and animals (Parascandola, 1998).

Primary hosts include cattle, bison and elk, but goats, sheep, pigs, and humans are also susceptible as secondary hosts. Affected cattle typically experience a permanent infection, shedding the bacterium for the duration of their lives (Nielson *et al.*, 1990). The disease is spread easily through direct contact and an entire herd can quickly become compromised with the inclusion of a single infected animal. Bovine brucellosis is a disease that primarily targets the reproductive system of cattle (Enright, 1990). Infected cows typically experience joint ailments, a decrease in milk production, sterility, and abortion of the first pregnancy that occurs after the onset of infection (Nielson and Duncan, 1990).

Vaccination against *B. abortus* has proven an important tool in eradication of the disease. The first brucellosis vaccine approved for commercial use was labeled Strain 19 (S19) and was derived from a field strain of *B. abortus* (Sanmartino, 2005). However, S19 is a smooth organism and induces the production of antibodies that interfere with diagnostic tests. For this reason, a rough *B. abortus* vaccine, termed RB51, was developed from a laboratory strain of *B. abortus* 2308 and was shown to be equally efficacious as S19 and did not hinder serological testing (Schurig, 1991; 2002).

*Brucella abortus* is moderately infectious to humans, with an estimated  $1 \times 10^5$  organisms needed for infection and the primary symptom is undulant fever. While *B. melitensis* is a more frequent and wide-spread zoonosis, *B. abortus* infects mainly occupational groups working closely with the organism or infected animals. *Brucella abortus* vaccines approved for use in animals, such as S19 and RB51, are infectious to humans and there remains no vaccine available for human use against *B. abortus* (Seleem *et al.*, 2009).



## 2.1.2. Epidemiology

### Occurrence

Brucellosis occurs worldwide except in those countries where bovine brucellosis has been eradicated. This is usually defined as the absence of any reported cases for at least five years. These countries include Australia, Canada, Cyprus, Denmark, Finland, the Netherlands, New Zealand, Norway, Sweden and the United Kingdom (OIE, 2004). The Mediterranean countries of Europe, northern and eastern Africa, near east countries, India, central Asia, Mexico and Central and South America are specially affected (WHO, 2006).

The occurrence of brucellosis is increasing in developing countries in an aggravating epizootiological situation. This depends on the policy of many countries in importing exotic breeds without having the required veterinary infrastructure and the appropriate level of development of the socio-economic situation of the animal holder. Furthermore, the increasing international animal trade, with increasing movement of animals and the trends towards intensification of animal production has facilitated the spread of brucellosis (Seifert, 1996). The reason for high prevalence is probably due to the fact that many countries have not yet started control or eradication schemes (Chukwu, 1987). The prevalence of the disease in many countries ranges from 1% to 24% in sheep, 3.4% to 31% in goats and 2%-37.2% in camels (Table 1).

Differences in sensitivity and specificity of different serological tests used are important factors that contribute to the variation. Other factors associated with the high and variable prevalence in these extensive livestock production system are large herd sizes, extensive movements of animals, and common grazing and watering points (Kadohira *et al.*, 1997). Different studies showed that brucellosis is prevalent in various regions of Ethiopia (Table 2).



**Table 1:** Seroprevalence of brucellosis in sheep, goat and camel in some countries

| Country      | Species | Prevalence (%) | <i>Brucella</i> species        | Reference                      |
|--------------|---------|----------------|--------------------------------|--------------------------------|
| Algeria      | Sheep   | 2.18           |                                | Refai, 2000                    |
|              | Goat    | 12             |                                |                                |
| Egypt        | Camels  | 9.3            |                                | Abdel Monghney, 2004           |
|              | Camels  | 7.4            |                                | El-Boshy <i>et al.</i> , 2009  |
| Eritrea      | Sheep   | 1.4            |                                |                                |
|              | Goat    | 3.8            |                                | Omer <i>et al.</i> , 2000      |
|              | Camel   | 3.1            |                                |                                |
|              | Camel   | 3.5            |                                | Azwai <i>et al.</i> , 2001     |
| India        | Sheep   | 3.42           |                                |                                |
|              | Goat    | 5.53           |                                | Sharma <i>et al.</i> , 1979    |
| Iran         | Goat    | 10.18          |                                | Zowghi <i>et al.</i> , 1990    |
|              | Camel   | 8              |                                | Zowghi and Ebadi, 1988         |
|              | Camel   | 10.8           |                                | Ahmad and Nemat, 2007          |
| Iraq         | Sheep   | 15             | <i>B. melitensis</i>           | Al-Ani <i>et al.</i> , 1998    |
|              | Camel   | 17.2           |                                |                                |
| Libya        | Camel   | 4.1            | <i>B. melitensis</i> biovar 1. | Gameel <i>et al.</i> , 1993    |
|              | Goat    | 31             |                                |                                |
|              | Sheep   | 24             |                                | Ahmed <i>et al.</i> , 2010     |
|              | Camel   | 14             |                                |                                |
| Nigeria      | Goat    | 5.82           |                                | Cadmus <i>et al.</i> , 2006    |
|              | Camel   | 9.4            |                                | Sadiq <i>et al.</i> , 2011     |
|              | Sheep   | 14.5           |                                |                                |
|              | Goats   | 16.1           |                                | Bertu <i>et al.</i> , 2010     |
| Pakistan     | Camel   | 2              |                                | Ajmal <i>et al.</i> , 1989     |
| Saudi Arabia | Sheep   | 6.5            |                                |                                |
|              | Goat    | 9.7            |                                | Memish, 2001                   |
|              | Camel   | 8              | <i>B. melitensis</i> biovar 2. |                                |
| Sudan        | Sheep   | 1              |                                |                                |
|              | Goat    | 4              |                                | El-Ansary <i>et al.</i> , 2001 |
|              | Camel   | 37.2           |                                | Omer <i>et al.</i> , 2010      |
|              | Camel   | 6.95           | <i>B. abortus</i>              | Yagoub <i>et al.</i> , 1990    |

**Table 2:** Seroprevalence of small ruminant and camel brucellosis in Ethiopia

| Study area                    | Species | Prevalence (%) | Reference                     |
|-------------------------------|---------|----------------|-------------------------------|
| Afar and Borena               | Camel   | 5.7            | Teshome <i>et al.</i> , 2003  |
| Afar and Somali Region        | Goat    | 13.2           | Teshale <i>et al.</i> , 2006  |
| Afar and Somali Region        | Sheep   | 5.6            |                               |
| Afar Region                   | Goat    | 5.8            | Ashenafi <i>et al.</i> , 2007 |
| Afar Region                   | Sheep   | 3.2            |                               |
| Amibara District(Afar Region) | Camel   | 7.6            | Woldegebrel, 2011             |
| Around Dire Dawa city         | Camel   | 1.6            | Mohammed <i>et al.</i> , 2011 |
| South Omo Zone                | Goat    | 4.2            | Ashagrie <i>et al.</i> , 2011 |
| South Wollo Zone              | Sheep   | 1.5            | Yesuf <i>et al.</i> , 2010    |
| Telalak District              | Goat    | 10.4           | Muleta, 2011                  |
|                               | Sheep   | 10.5           |                               |

#### Source of infection and mode of transmission

The concentration of *Brucella* organism is highest in pregnant uterus. In addition, the foetus and foetal membranes are considered as major source of infection. Aborted foetuses, placental membranes or fluids, and other vaginal discharges are all highly contaminated with infectious *Brucella* organisms. Infected animals also shed organisms in the milk; therefore raw milk or raw milk products of bovine origin are sources for infection in humans. Although exposure through skin, the conjunctival and genital mucosa routes can occur, ingestion is the most common route of entry (Radostits *et al.*, 1994; Walker, 1999). Contaminated feed can spread the infection from infected pastures over long distances during purchasing and selling activities. The use of infected bulls for artificial insemination poses an important risk and spreads the infection to many herds (Seifert, 1996; Acha and Szyfers, 2001).

## Risk factors

### *Management*

Increase in herd size and animal density are directly related to the high prevalence of the disease and difficulty in controlling infection in a population (Walker, 1999). Calving practices also play major roles in the spread of brucellosis. Separate calving pens allow for minimizing exposure of uninfected animals. Replacement animals from other sources increase the potential for the introduction of the disease into herds (Nicoletti, 1980; Walker, 1999). Poor hygienic measures and susceptible animals favour the rapid spread of the disease and subsequently resulting in high prevalence of the disease in that particular herd (Nicoletti, 1980; Radostits *et al.*, 1994).

### *Host*

Susceptibility to infection depends on age, sex, breed, and pregnancy status of the animal. Male and female calves up to six months of age are not very susceptible and generally experience only transient infection. Sexually mature animals are much more susceptible to infection, regardless of gender. After sexual maturity, the status of pregnancy has a greater influence on the degree of susceptibility. Bulls are more resistant than sexually mature heifers, cows and less resistant than sexually immature heifers. Some animals, even the most susceptible groups, never become infected, or if they do, their infection is transient, and this accounts for individual difference in disease resistance (Walker, 1999; Acha and Szyfers, 2001).

### *Environment*

The resistance of the pathogen to the environment depends on whether they are protective against sunlight and high temperature. Survival is prolonged by low temperatures and the organism remains viable for many years in frozen tissues. Neutral soil PH and a moist protected environment, which are rich in organic materials, are favourable elements for survival of *Brucella* (Seifert, 1996). It is generally accepted that *Brucella* organisms do not multiply in the



environment, instead they merely persist and their viability is influenced by the existing environmental conditions (Radostitis *et al.*, 1994).

### 2.1.3. Pathogenesis

Once an animal or individual becomes infected with the *Brucella* pathogen, the bacterium travel in the lymph to the nearest draining lymph node and then into the blood stream of the host animal. *Brucella* spp. avoids destruction by the host's innate immune system with an ability to avoid lysosomal degradation after being engulfed by host macrophages (Celli, 2006). Furthermore, *Brucella* spp. thrives and proliferates inside of the host macrophage, and ultimately causes the death of that macrophage, releasing the multiplied bacteria back into host tissues. Once the host becomes bacteremic, the pathogen migrates throughout the body to different organs and tissues (Cheers, 1984).

The most common pathology of brucellosis in animals is the abortion of the first pregnancy that occurs after the onset of the disease. Most *Brucella* spp. proliferate efficiently in the cells of the body with high erythritol levels, such as the cells found in the urinary and reproductive tracts (Enright, 1990). Once in the uterus, the bacteria can penetrate the epithelial cells of the embryonic membrane and cause placentitis, which can lead to a decrease in the blood supply to the fetus. Fetuses recovered from brucella infected mothers have also been shown to develop edema and congestion in their lungs and hemorrhages in their epicardium. Whether the cause is a decrease in oxygenated blood due to an inflamed placenta or the detrimental lesions formed by the infection of the fetus, the final outcome of pregnancy is either an abortion or a weak fetus that dies shortly after birth (Enright, 1990).

Other effects on the reproductive system of animals with brucellosis include the swelling of the testes and epididymus of male animals. As in the uterus, *Brucella* in the male urogenital tract can also cause inflammation and swelling of the male reproductive organs. Orchitis and epididymitis are common symptoms of brucellosis in animals, causing sterility in infected males. Most, if not all, *Brucella* species can be isolated from semen, and males can shed the bacterium for prolonged periods. Brucellosis is also responsible for arthritis of the joints and spondylitis in many infected

animals as well as hygroma formation on the knees. Brucellosis can also be responsible for mastitis in females, neurologic swelling and splenic abscesses with edema, congestion, and hemorrhage in the lung and heart (Radostitis *et al.*, 1994).

Granulomatous inflammation is also observed during persistent *Brucella* infections. The exudate of granulomatous inflammation is generally composed of mature macrophages, which gravitate to the area of the body affected by the causative agent (Tizard, 2004). Here, macrophages have the potential to form into multinucleate giant cells. Multinucleate giant cells are a hallmark of infections involving pathogens that thrive within phagocytes, as infected macrophages are identified and engulfed by other macrophages in a process that results in the formation of a giant cell composed of several responding phagocytes (Abbas, 2005). Fibrosis and necrosis are often associated with granulomatous inflammation, especially in the case of granuloma formation. Granuloma formation is primarily a product of chronic delayed-type hypersensitivity and occurs in cases of chronic brucellosis. In a persistent infection and subsequent immune response, the lytic and anti-pathogenic actions of non-specific phagocytes begin to damage host tissue. In a chronic infection, this is sometimes manifested as the formation of fibrosis and scar tissue surrounding the site of inflammation, which has the potential to cause tissue damage that may disrupt normal tissue function (Merck, 2007). Necrosis formation in the center of granulomas may occur due to anoxic conditions and may function to eliminate infected macrophages, destroying the pathogen within (Abbas, 2005).

#### 2.1.4. Virulence factors

As is the case for other bacterial pathogens, several cellular components contribute to the survival and virulence of the *Brucella*. However, the identity of most of these components has not been elucidated. Research concerning the virulence factors expressed by the *Brucellae* has focused primarily on the structural components of the outer membrane. Components are included as virulence factors on the basis of the following classification (Lory and Tai, 1984): (1) components that when inactivated by mutation markedly decreases virulence, (2) components that mimic pathological effects when administered in purified form and (3) components that elicit a partially protective immune response. In contrast to other gram-negative pathogens, the outer



surface of the *Brucellae* does not have complex structures, such as pili or fimbriae, nor does it have capsular material. The outer membrane contains only two components that have been identified as virulence factors: the lipopolysaccharide (LPS) and the outer membrane proteins (OMPs) (Lory and Tai, 1984).

### Lipopolysaccharide (LPS)

The LPS has been classified as a virulence factor according to each of the categories described above. Of the potential virulence factors expressed by the *Brucellae*, the LPS is the best defined (Moreno *et al.*, 1979). The availability of natural isolates lacking LPS and exhibiting reduced virulence, the toxic effect of purified LPS, and the protective immunity stimulated by this fraction suggest that LPS is a primary virulence determinant of the *Brucellae*. Virulence is associated with the smooth colony morphotype, designated S, which we now know contains the full complement of *Brucella* LPS. The rough colony morphotype, designated R, arises by dissociation from the smooth form and its accumulation is typically observed *in vitro*. Dissociation is stimulated by changes in environmental conditions, including different nitrogen sources or exposure to D-alanine (Braun, 1947; Goodlow *et al.*, 1952). The R morphotypes are characterized by their opaque appearance and lusterless colony morphology (Henry, 1933).

In general, for most *Brucella* species, smooth strains are virulent and rough and mucoid strains are not. The LPS of smooth strains consists of lipid A, a core region containing flucose, mannose, quinovosamine and 2-keto-3-deoxyoctulosonic acid (KDO) and an O chain composed of a homopolymer of about 100 residues of N-formylated perosamine (Caroff *et al.*, 1984). Two distinct epitopes occur on the O chain depending upon the species/biovar of the strain. In those typified by *B. abortus* biovar 1, the A epitope is present and probably represents terminal N-formylated perosamine residues which are linked by the 1 and 2 carbon atoms throughout the chain. In strains typified by *B. melitensis* biovar 1, the M epitope is present. In this case, the O chain consists of repeating units of four 1, 2-linked N-formylated perosamine residues and one 1, 3-linked residue (Perry *et al.*, 1986). The A, and M epitopes can be detected by cross-absorbed polyclonal antisera. Other antigens present on or near the surface include the outer membrane proteins. These, like the smooth LPS complex, are involved in the protective immune response to



*Brucella*. Intracellular antigens include a variety of proteins, glycoproteins and polysaccharides. The latter may include the polysaccharide B and native hapten present in some strains, which have been employed in the radial diffusion test. The lower molecular weight polypeptide fraction of the intracellular antigens plays an important role in eliciting delayed hypersensitivity reactions in the intradermal test (Bundle *et al.*, 1987).

The toxic effects of the *Brucella* LPS are attributable to the lipid A portion (Galanos *et al.*, 1972). Increased survival of smooth organisms compared with rough avirulent organisms in macrophages suggests that the LPS play a role in uptake and survival (Braun *et al.*, 1958; Kreutzer and Robertson, 1979; Riley and Robertson, 1984). Differences in uptake of smooth and rough organisms have been demonstrated in experiments using guinea pig and bovine monocytes. LPS plays a role in enhancing intracellular survival. Experiments performed by Frost *et al.* (1972) indicate that *in vivo* grown, smooth organisms may have an additional membrane component that increases survival and virulence. The mouse model system has been investigated extensively with respect to the protective immunity induced by antibodies directed against LPS. The protective immunity elicited by LPS in the cow has not been clearly established. Winter *et al.* (1988) have demonstrated effective protection in the mouse model using a combination of O-polysaccharide covalently linked to *Brucella* porin. Under identical conditions, porin linked to R-LPS did not stimulate a significant level of protection. Montaraz *et al.* (1986) were able to demonstrate effective immunization by passive transfer of monoclonal antibodies directed against the O-antigen, while monoclonal antibodies directed against a number of outer membrane proteins induced no significant protection.

#### Outer Membrane Proteins (OMPs)

The OMPs have been classified as virulence factors according to category 3 of Lory and Tai (1984). Characterization of the OMPs as virulence factors has been hampered by the inability to identify variants lacking expression or exhibiting altered profiles on SDS-PAGE gels. Furthermore, careful examination of these profiles is hampered by the presence of the LPS, which interferes with the migration of the OMPs. Thus, the role of these proteins in the survival and virulence can only be hypothesized. Protective immunity stimulated by OMPs has been

demonstrated following vaccination with rough or mucoid organisms or cell envelope fractions that lack LPS. Verstrete *et al.* (1982), Santos *et al.* (1984), and Douglas *et al.* (1984) have identified three major groups of proteins present in the outer membrane of the *Brucellae*. These were designated as group 1 (88 to 94 kDa), group 2 (35 to 40 kDa), and group 3 (25 to 30 kDa). Little variation among species has been observed in the OMP profiles (Santos *et al.*, 1984). All three groups of proteins appear to be recognized by the bovine immune system during the course of infection. Protective immunity stimulated by OMPs has been documented for several Gram-negative organisms (Wang and Frash, 1984; Isibasi *et al.*, 1988).

Protection stimulated by *Brucella* OMPs is still a matter of debate, and despite considerable experimental effort, is far from resolved. The doses employed have either been insufficient to produce an immune response or presentation of the antigen in the killed vaccines does not mimic the situation obtained with a living vaccine strain. Also, the reliability of the mouse as a model for bovine brucellosis, despite their experimental practicality, is questionable. Vaccines tested with good success in the mouse have been subsequently tested without success in cattle. The development of an improved laboratory animal model for bovine brucellosis would be a major step in the resolution of this problem. Recent vaccine trials performed in cattle suggest that under the appropriate conditions, protective immunity can be stimulated by rough cell envelopes, implying that an immune response directed against the OMPs is protective (Adams *et al.*, 1987).

### Toxins and Other Factors

To date, no concrete evidence exists that toxins similar to those produced by other organisms (i.e., *Vibrio cholera*, *Corynebacterium diphtheriae*, *E. coli*, *Shigella dysenteriae*, *Clostridium botulinum*, *C. tetani*) are produced by the *Brucellae*. A toxin has been postulated to exist due to the severe effects of the disease on the central and autonomic nervous systems. Endotoxin, consisting of LPS, has been suggested as the causative factor.

The *Brucellae* also produce catalase. Huddleson and Stahl (1943) determined that a catalase activity decreases with changes from smooth to rough morphology. Although the biochemical advantage of expressing catalase in *Brucella* has not been identified definitively, it may not only



protect the organisms from peroxide, but could also be important in maintaining oxygen tension required for survival of the organism (McCullough, 1970).

#### 2.1.5. Immune responses

The host immune response to an infectious agent is divided into innate and adaptive. The first one consists on preexisting or rapidly responding chemical and cellular defense mechanisms and is non-specific, while the second one is specific against the infected agent but takes more time to become effective (Tizard, 2004). Immunity against brucellae involves cellular and humoral response (Huang *et al.*, 2003; Giambartolomei *et al.*, 2004).

##### Cellular immunity

The immediate innate immune response to *Brucella* spp. in naïve animals begins when *Brucella* lipoproteins (LP) contacts nonactivated antigen-presenting cells (APC), mainly dendritic cells and local macrophages, through CD14 and Toll-like receptor 2 (TLR2) dependent mechanisms (Huang *et al.*, 2003; Giambartolomei *et al.*, 2004). This initial contact generates a receptor induced signaling that stimulates infected cells to secrete cytokines such as TNF $\alpha$ , IL1 $\alpha$ , IL6 and IL12 that modulate the development of specific Type I cellular immune response to promote a clearance of the bacteria (Zaitseva *et al.*, 1995; Dornand *et al.*, 2002).

The set of cytokines secreted by *Brucella*-infected APC stimulate a T helper 0 cells (Th0), a subpopulation of CD4<sup>+</sup>  $\alpha/\beta$  T lymphocytes, to differentiate them in Th1 cells, which result in IL2, IL18, TNF $\beta$  and IFN $\gamma$  secretion. All together, these cytokines stimulate activation of macrophages, promote B-cell switching of immunoglobulin and recruit more immune cells to the site of infection (Jiang and Baldwin, 1993). Natural killer (NK) cells, neutrophils, CD8<sup>+</sup> and  $\gamma\delta$ T lymphocytes are part of the secondary innate immune response and migrate immediately to the infection site to contribute to resolve the infection (Dornand *et al.*, 2002).

Characteristic chronic granulomatous lesions develop in infected tissue where macrophage, neutrophils and lymphocytes respond to *Brucella* antigens. As the organisms are facultative



intracellular organisms, phagocytes play a key role in initiating T-cells by processing and presenting antigens. Sensitized T-cells release cytokines that activate macrophages which in turn combat *Brucella* by reactive oxygen intermediate. Both CD4 and CD8 subsets are involved in cell-mediated protection. Cytokines also play a role in controlling *Brucella* infections (WHO, 1997). Neutrophils effectively utilize the myeloperoxidase-hydrogen peroxide halide system in killing *Brucella*. However, the organisms inhibit degranulation and the respiratory oxidative burst, and are able to survive in the cells (Riley and Robertson, 1984). Macrophages readily ingest *Brucella* when opsonized with either complement or specific antibodies. The survival of the organisms in macrophages may result from a failure of phagosome-lysosome fusion and resistance to oxidative killing by producing superoxide dismutase and catalase (Frenchick *et al.*, 1985; Harmon *et al.*, 1988; Quinn *et al.*, 2002). Tatum *et al.* (1992) suggested that anti-oxidant Cu-Zn superoxide dismutase plays a role in the survival of *Brucella* species in phagocytic cells.

### Humoral immunity

Secondary infection boosts a *Brucella*-specific immune response. At that point humoral response plays a major role, being *Brucella*-specific antibodies available to bind and opsonize the agent, facilitating uptake by phagocytic cells (Eze *et al.*, 2000). On the other hand, specific CD4<sup>+</sup> T cells (Th1) secrete INF $\gamma$  that activates macrophages and enhances their killing activity (Jones and Winter, 1992; Jiang and Baldwin, 1993), and specific CD8<sup>+</sup> T lymphocytes (cytotoxic T cells) that recognize and kill *Brucella*-infected cells by binding them through the microbial antigen displayed on the surface (Oliveira and Splitter, 1995). These cytotoxic T cells not only secrete proteolytic enzymes that trigger apoptosis in the infected cells, releasing the intracellular microbes that can then be phagocytosed by activated macrophages, but they also produce substantial amounts of INF $\gamma$  involved in macrophage activation (Zhan *et al.*, 1993).

Naturally infected and vaccinated animals can be serological reactors. After infection, the level of immunoglobulin isotypes: IgM, IgG and IgA will significantly increase in serum (Radostits *et al.*, 1994). IgM antibodies, which appear initially after infection and low levels of IgG, will cause complement-mediated lysis of *Brucella*. Secretory IgA tends to be abundant in milk whereas IgG is high in serum (Walker, 1999). The O-chain of smooth lipopolysaccharide complex of the

cell envelope together with the outer protein epitopes have contributory role as protective immunogens. On the other hand, the immunogenicity of the non-smooth variant is relatively low (Corbel, 1990; WHO, 1997). The O-chain specific antibodies play a major role in protective immunity, but don't eliminate the organisms as they are protected being intracellular (WHO, 1997). This indicates lack of correlation between protection and high antibody level (Walker, 1999).

Natural resistance to brucellosis in domestic animals has been observed since 1940 (Cameron *et al.*, 1942; Templeton *et al.*, 1990). Bovine *Slc11a1* (former *NRAMP1*) gene has been pointed out as a candidate element for controlling intracellular replication of *B. abortus* in macrophages (Barthel *et al.*, 2001); however, its biochemical function and how it contributes to resistance to the infection remain unclear (Wyllie *et al.*, 2002). Inheritance of *Brucella* infection resistance involves more than one gene (Adams and Templeton, 1998), but no other genetic element responsible for control of infection has been identified.

#### 2.1.6. Diagnosis

##### Bacteriological diagnosis

A technique, involving a biphasic (solid and liquid) medium in the same bottle, is recommended for the culture of blood and other body fluids. For the solid phase, trypticase soy agar, tryptose agar, or *Brucellae* agar may be used on the other hand the liquid phase consists of the same basal medium without agar, and also Serum Dextrose Agar can also be used to culture *Brucellae* (Nielson and Duncan, 1990).

Incubation should be in an atmosphere of 5% to 10% CO<sub>2</sub> at 35 °C to 37 °C. *Brucella* colonies usually appear after 4 to 5 days, but cultures should be kept for 30 days before they are discarded as negative. In vitro growth of *Brucella* from patient specimens requires careful and informed laboratory processing of materials. For primary culture, direct inoculation of materials on solid media is recommended to facilitate recognition and isolation of the developing colonies and to limit the establishment of non-smooth mutants. Delicate translucent colonies of 2-3 mm in



diameter grow on blood or glucose agar. *B. ovis* grows always in the M (mucoid) form, *B. abortus* and *B. melitensis* grow at the beginning in the S (smooth) form and later dissociate into the R (rough) and the M (mucoid) form commercial blood culture bottles may also be used for culturing *Brucella*; however, subcultures must be made every 4 to 5 days (Cherwonogradsky *et al.*, 1987). Laboratory processing of materials, such as animal tissue or milk that may be heavily contaminated with other microorganisms, requires either animal inoculation or the use of selective media containing antibiotics (Schwabe, 1984).

### Serological diagnosis

Testing modalities for brucellosis include antigen and antibody detection as well as agent isolation through culture. These methods have different advantages and disadvantages in the laboratory and in their use in disease control and eradication programs. The serological tests depend on a reaction between *Brucella* antigen and antibodies produced in response to the infection. A number of classes and subclasses of antibody (isotypes) may occur in positive sera. The various serological tests vary in their ability to detect different isotypes.

#### *Standard Tube Agglutination Test (STAT)*

Historically, the Standard Tube Agglutination Test (STAT) has been recognized as the principal serological test used for the diagnosis of brucellosis. IgM isotypes of antibody is the most active agglutinin at neutral pH (Rice and Boyes, 1971; Corbel, 1972; Nielsen *et al.*, 1984). Therefore the STAT is susceptible to false positive reaction by cross-reacting antibodies (Corbel, 1988; Nielsen, 2002). Due to low specificity by original tube agglutination test, a large number of modifications have been made to destroy or inactivate IgM agglutinins. Of these modifications, the acidified antigen, rivanol precipitation and 2-mercaptoethanol are in common use in various laboratories for inactivating IgM (Nielsen, 2002).

In Rose Bengal Plate Test (RBPT) antigen is used at a pH of 3.65. The low pH prevents some agglutination by IgM and encourages agglutination by IgG1 thereby reducing non-specific

interactions (Corbel, 1972). The RBPT is considered to be suitable for screening of individual animals, however, some cross-reacting antibodies have been detected by this test and false negative reaction may occur mostly due to prozoning (OIE, 2004).

Numerous variations of the Indirect Enzyme Linked Immunosorbent Assay (I-ELISA) have been described employing different antigen preparations, antiglobulin-enzyme conjugates and substrates/chromogens. Several commercial I-ELISAs are available that have been validated in extensive field trials and are in wide use. The I-ELISA is a highly sensitive test but sometimes not capable of differentiating between antibody resulting from S 19 vaccination or other false positive serological reactions (FPSR) and that induced by pathogenic *Brucella* strains. The I-ELISA should, therefore, be considered more as a screening test rather than a confirmatory test for testing of vaccinated cattle or herds affected by FPSR problems (OIE, 2004).

Thus no single test appears to be free from demerits. This has prompted many workers to carry out comparative studies and to determine the efficacy of different tests. Nielsen (2002) and Gall and Nielsen (2004) after reviewing various serological tests concluded that no individual test found perfect, however, error could be minimized using the most reliable test.

Serologic testing is critical to brucellosis disease control and eradication programs. Culture requires a bio-safety level three laboratory (OIE, 2008a), which makes the ability to culture samples prohibitive. Instead, serologic screening and confirmatory tests are used to identify infected animals. The World Organization for Animal Health (OIE) prescribes the use of a buffered *Brucella* antigen test called the buffered plate antigen test and the Rose Bengal Test (RBT) as approved screening tests and the complement fixation test as the confirmatory test. For bovine brucellosis the prescribed test by the OIE are buffered brucellosis antigen tests, the complement fixation test, the indirect and competitive enzyme-linked immunosorbent assay (ELISA) and the fluorescence polarization assay. In sheep and goats the prescribed tests are the buffered brucellosis antigen test and the complement fixation tests. The brucellin skin test and the fluorescence polarization assay are considered alternative tests (OIE, 2008c).



## *Rose Bengal Test (RBT) and the Buffered Plate Antigen Test (BPAT)*

The two most commonly used acidified antigen agglutination tests are the Rose Bengal Test (RBT) and the Buffered Plate Antigen Test (BPAT). The RBT and BPAT use acidified antigens to reduce the binding of IgM antibodies and to encourage the IgG1 binding. Cross-reactions of the IgM antibodies can occur with *Yersinia enterocolitica* O:9 (OIE, 2008a, b). The standard antigen used to test for *B. abortus*, *B. melitensis* and *B. suis* is from a laboratory *B. abortus* strain. This can be done because the O-polysaccharide on the smooth-lipopolysaccharide (S-LPS) surface of *B. abortus*, *B. melitensis* and *B. suis* are antigenetically similar because their epitopes are similar. *B. ovis* and *B. canis* tests utilize antigens from each specific species (Nielson, 2002). More specifically, serologic tests use antigens from the laboratory *B. abortus* strains 1119-3 and 99 stained with a dye to make it visible. When the prepared antigen and serum with antibodies are mixed, an antigen-antibody complex is formed and the agglutination is visible due to the dyed antigen. Thus agglutination indicates that the tested animal is positive for brucellosis (OIE, 2008a). The buffered plate antigen test is more sensitive than the RBT, but requires more precise equipment, making it more difficult to do in the field (Nielson, 2002; Gall and Nielson, 2004; OIE, 2008a, b).

There is a difference in the use of the RBT with sheep and goats as compared to cattle. With sheep and goats 75µl of serum and 25µl of reagent are used as compared to the 30µl of serum and reagent used in the bovine RBT. The adjustment is done in sheep and goats to increase the sensitivity, especially when used without a confirmatory test (OIE, 2008a, b). Overall the buffered *Brucella* antigen tests have a higher sensitivity but also have a less reliable specificity; this results in a reduced number of false negatives and a significant number of false positive. Therefore a confirmatory test with higher specificity is needed (Nielson, 2002; OIE, 2008b).

## *Indirect enzyme linked immunosorbent assay*

Indirect enzyme linked immunosorbent assays (I-ELISA) tests are also approved for use by the OIE as a brucellosis screening test because of its high sensitivity. Currently, it is a prescribed test in bovine brucellosis diagnosis and an alternative test for swine brucellosis. The reason that it is

not approved for international trade in small ruminant testing is due to a lack of *B. melitensis* reagent standardization and its inability to differentiate between antibodies produced in response to vaccination versus natural infection (OIE, 2008b).

The I-ELISA used in bovine brucellosis also suffers from cross-reactions and the inability to distinguish between vaccine antibody and natural infection antibody. Yet, it does have standardized antigens. The I-ELISA uses a standard *B. abortus* antigen (S-LPS), a serum sample and an anti-bovine IgG1 antibody that has a horseradish peroxidase attached to it. If the anti-S-LPS antibody is in the serum sample, then the anti-bovine IgG1 with an attached peroxidase causes a color change in the assay, which indicates a positive test (Nielson, 2002; Corbel, 2006; OIE, 2008a). Finally, since swine brucellosis does not have an effective vaccine, the I-ELISA does not have to distinguish between vaccine-associated and natural infection antibodies. Therefore, the I-ELISA is a useful diagnostic test in swine. An alternative test for confirmation of *B. melitensis* in small ruminants is the brucellin skin test. This test evaluates the cell-mediated immunity of the tested animal against brucellosis. It has a high sensitivity and a higher specificity than agglutination tests since it does not cross-react with other bacteria (OIE, 2008b). It is effective for use in non-vaccinated animals. A rough strain of *B. melitensis* (B115) is used to avoid the presence of an S-LPS antigen that would react with antibodies and create a local area of inflammation (OIE, 2008a). The brucellin derived from the lysed B115 cells are injected intradermally into the lower eyelid and read 48 hours later. A positive test is indicated by a thickening of greater than 2 mm (OIE, 2008b).

### *Complement Fixation Test (CFT)*

A commonly used confirmatory test is the complement fixation test (CFT). This is the approved confirmatory test for international trade as prescribed by the OIE. The CFT uses the complement activation system's ability to lyse cells, and in this case erythrocytes. If the serum sample contains antibody against S-LPS it will bind the reagent S-LPS antigen on the *B. abortus* cells. This antibody-antigen complex then activates the available complement in the test reagent. So, when sheep red blood cells sensitized with rabbit anti-sheep antibodies are added to the mixture, the complement in the reagent has already been activated and the blood cells do not lyse. If the



test serum did not have antibodies, the S-LPS antigen would remain unbound and the sensitized red blood cells would have activated the complement causing their lysis. So, a test result showing no red blood cell lysis is a positive test (OIE, 2008a, b). The high sensitivity and specificity (Nielson *et al.*, 2008) of this test makes it a very good confirmatory test, but it is intensive to run, requiring many reagents. None of the serologic tests are capable of distinguishing S19 and Rev 1 vaccination related antibodies present in cattle and small ruminants, respectively from antibodies due to natural infection (Gall and Nielson, 2004). This is a considerable hindrance in control and eradication programs when the livestock's disease status is not known prior to vaccination and when they are not permanently marked to indicate them as a vaccinated animal.

#### *Fluorescence Polarization Assay (FPA)*

The fluorescence polarization assay (FPA) helps address the issue of seroconversion in vaccinated animals. The concept behind this test utilizes the physics principle that the rate of rotation of an object is inversely proportional to the size of the object. The rate of rotation differs for antigen alone and for antigen bound to an antibody and this can differentiate between animals that have sero-converted and those that have not. A standardized antigen that is 22 kilo-Daltons in size and a component of the S-LPS, the *O*-polysaccharide is used. The FPA is highly sensitive and specific, is quick to run and can be done in the field (Nielson, 2002; Nielson *et al.*, 2008). It is also capable of determining a field strain and vaccine strain positive (Gall and Nielson, 2004).

#### *Competitive Enzyme Linked Immuno Sorbent Assay (cELISA)*

Another test that is able to distinguish between sero-conversion due to natural infection and vaccination by Rev 1 or S19 for small ruminants and cattle respectively is the competitive ELISA (cELISA). The cELISA uses the concept that vaccine-induced antibodies have a lesser affinity for the antigen than antibodies that arise from natural infection. Theoretically, this lesser affinity develops due to the shorter exposure time that the humoral immune system has to develop antibodies in a vaccination exposure as compared to a natural infection. The increased amount of time of pathogen exposure to the humoral immune system in a natural infection creates an antigen-antibody complex with a greater affinity (Nielson, 2002; Gall and Nielson, 2004). The

cELISA is a prescribed test for cattle trade and an alternative test for swine trade (Nielson, 2002). It is a highly sensitive and specific test as well (Nielson *et al.*, 2008).

## 2.2. Brucellosis in sheep, goats and camels

There is a great deal of variability of disease seen among species. In livestock species (cattle, sheep, goats, swine, and camel), the most frequent clinical sign following infection with *Brucella* is abortion (Acha and Szyfers, 2003).

Reproductive issues are seen in all infections, but the severity and the sex-association of the most severe reproductive issues vary based on the *Brucella* spp. causing the infection. Most clinical disease manifests as fertility-related issues in cattle and small ruminants. Yet, in small ruminants, the less virulent *B. ovis* presents differently than *B. melitensis*. Small ruminants are the reservoir for both *B. ovis* and *B. melitensis*. In *B. ovis* infections, which are primarily infections of sheep, the rams are more affected than the ewes. Rams develop orchitis and epididymitis due to infection and associated inflammation of the epididymes and testes. Semen quality and the viable spermatozoa in a sample of ejaculate decrease significantly. In ewes, a placentitis can occur that results in the birth of weak lambs, stillbirths or abortions (Blasco, 1990; Radostits *et al.*, 2007).

As for infection with *B. melitensis*, the impact of disease is greater amongst the ewes than the rams. The most common sign of disease is late term abortion in naïvely infected pregnant animals. In endemic flocks, abortions are less common and most infected ewes give birth to weak offspring as sheep rarely abort twice (Olsen and Stoffregen, 2005). Retained placentas are commonly found among ewes in endemic flocks as well. Rams experience orchitis and epididymitis. In addition, animals with polyarthritis can also be seen in endemic flocks (Alton, 1990; Garin-Bastuji *et al.*, 1998; Corbel, 2006; Radostits *et al.*, 2007). Ovine brucellosis can be divided into classical brucellosis and ram epididymitis. Ram epididymitis is caused by non-zoonotic agent *B. ovis*, while classical brucellosis is caused by *B. melitensis* and constitutes a major public health threat equal to goat brucellosis (Acha and Szyfers, 2003).



Camels are also prone to infection from *B. abortus* and *B. melitensis* (Crawford *et al.*, 1990; McDermott and Arimi, 2002; Refai, 2002; Corbel, 2006). Infections in camels can result in abortion in females and epididymitis and orchitis in males (Teshome *et al.*, 2003). They can be infected by *B. abortus* and *B. melitensis* when they are pastured together with infected sheep, goats and cattle. Milk from infected camels represents a major source of infection that is underestimated in the Middle East (Musa *et al.*, 2008).

### 2.3. Human brucellosis

Human brucellosis is a neglected disease of poverty often found in highly agrarian, livestock dependent societies (WHO, 2006). It is a purely zoonotic disease in that animals infect humans but there is not human-to-human transmission (Corbel, 2006). Humans are a dead-end host and very rarely infect another human. Brucellosis can be transmitted to humans through contact with *Brucella* infected animals or their products. The incidence of human brucellosis results from four primary routes: (1) ingestion of unpasteurized and contaminated milk or cheese; (2) inhalation; (3) penetration of ocular or oral mucosa; and (4) direct inoculation into the bloodstream through abrasions in the skin or vaccination (Walker, 1999).

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 and Szyfers, 2003). The zoonotic nature of the marine brucellae (*B. ceti*) has been documented (Brew *et al.*, 1999; Sohn *et al.*, 2003; McDonald *et al.*, 2006). *B. melitensis*, *B. suis* and *B. abortus* are listed as potential bio-weapons by the Centers 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 and Szyfers, 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).

Because person-to-person transmission rarely occurs, infected persons do not pose a threat to their surroundings; consequently, eradication of the disease from the natural animal reservoirs leads to a dramatic decrease in the incidence of human infection. For example, in the United States the national bovine brucellosis eradication program has resulted in the number of reported human cases dropping from 6321 in 1947 to about 100 per year by 1998; most of the cases were acquired overseas or due to consumption of infected milk products from Mexico (Cook *et al.*, 2002). In Denmark, where some 500 cases per year reported between 1931 and 1939, human brucellosis had disappeared by 1962 as a result of eradication of brucellosis in cattle (Acha and Szyfers, 2003). In France with the introduction of brucellosis eradication program more than 800 cases of human brucellosis were reported in 1976, declining to 405 cases in 1983, 125 cases in 1990, and 44 cases in 2000 (Pappas *et al.*, 2006b).

The incubation period of brucellosis normally is 1–3 weeks, but it can be several months before showing signs of infection. *B. melitensis* is associated with acute infection whereas the infections with other species are usually subacute and prolonged (Mantur *et al.*, 2007). Most common symptoms of brucellosis 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, depression (Acha and Szyfers, 2003) and formation of granulomas that most often result in osteo-articular signs and endocarditis (Franco *et al.*, 2007).



Human brucellosis is also known for complications and involvement of internal organs and its symptoms can be very diverse depending on the site of infection and include encephalitis, meningitis, spondylitis, arthritis, endocarditis, orchitis, and prostatitis (Acha and Szyfers, 2003). Spontaneous abortions, mostly in the first and second trimesters of pregnancy, are seen in pregnant women infected with *Brucella* (Khan *et al.*, 2001). Although rare complication, *Brucella* endocarditis (<2% of cases) is most commonly associated with *B. melitensis* infection and is the most severe complication. It accounts for at least 80% of deaths due to brucellosis (Peery and Belter, 1960; Reguera *et al.*, 2003). Lack of appropriate therapy during the acute phase may result in localization of brucellae in various tissues and organs and lead to subacute or chronic disease that is very hard to treat (Young, 1995). Symptoms and signs of brucellosis usually referred as fever of unknown origin can be confused with other diseases including enteric fever, malaria, rheumatic fever, tuberculosis, cholecystitis, thrombophlebitis, fungal infection, autoimmune disease and tumors (Mantur *et al.*, 2007).

Live animal vaccines *B. melitensis* Rev. 1 and *B. abortus* strain 19 are known to cause disease in humans. The course of the disease with vaccine strains is usually shorter and more benign (Acha and Szyfers, 2003). Direct person-to-person spread of brucellosis is extremely rare. Mothers who are breast-feeding may transmit the infection to their infants and sexual transmission has also been reported (Arroyo *et al.*, 2006; Kato *et al.*, 2007).

Although brucellosis is not typically considered a fatal disease, human cases left untreated can result in mortality (Franco *et al.*, 2007; Park *et al.*, 2007). It is believed that many cases of human brucellosis remain undiagnosed and thus unreported. This may be due to the similarity of initial symptoms to those of influenza (Chain *et al.*, 2005). Human brucellosis is diagnosed using serological testing and by culturing bacteria from blood, lymph, or cerebrospinal fluid (Seleem *et al.*, 2009). Disadvantages of this method include the slow growth of *Brucella* in culture and a potentially low number of colony forming units (CFU) present in clinical samples due to the stage of infection or to the use of antibiotics prior to sample collection (Franco *et al.*, 2007; Seleem *et al.*, 2009). The Rose Bengal test is also useful for human diagnosis, as well as specially designed ELISAs (Orduna *et al.*, 2000; Acha and Szyfers, 2003). The tube agglutination test was the first test used for diagnosis of brucellosis in humans and was later adapted for use in animals.

In that test, serum is diluted and added to a tube containing a standard quantity of killed *Brucella* cells. The occurrence of clearing and agglutination following incubation is considered a positive result (Beran, 1994). Wright's serum agglutination test, which measures the titer of anti-brucella antibodies (Mert *et al.*, 2003), and Huddleson's slide agglutination test, in which serum agglutination can be rapidly detected (Spink, 1956), are successful diagnostic tools for brucellosis. Studies surrounding the use of molecular-based diagnosis using the Polymerase Chain Reaction (PCR) have also been explored with varied results (Kattar *et al.*, 2007).

Treatment for human brucellosis is most commonly through combined antibiotic regimens, as treatments using single antibiotics have shown higher relapse rates (Solera *et al.*, 1997; Pappas *et al.*, 2005; 2006a). The recommended combination is doxycycline coupled with rifampin, but doxycycline with streptomycin or gentamicin have also been effective treatments (Solera *et al.*, 1997; Seleem *et al.*, 2009). Prior to 1986, the World Health Organization reported that a doxycycline-streptomycin regimen was the preferred method of treatment for brucellosis in human adults. Currently, however, the WHO Committee on Brucellosis has updated their recommendation to a combined oral treatment of 600-900 mg of rifampicin daily coupled with 200mg/day of doxycycline for a period of 6 weeks for the treatment of acute brucellosis in human adults (WHO, 2006). Regardless of therapeutic regimen used, relapse rates of approximately 5-10% have been observed in both adults and children treated for brucellosis (Hall, 1990).

## **2.4. Disease control and prevention**

Reducing brucellosis zoonosis requires reducing the exposure of humans to the disease agent. There are two primary exposure patterns of importance that can be targeted for intervention. The first is occupational-associated exposure. These individuals contract disease through direct contact with infected and shedding animals, the contaminated environment or through inhalation associated with aerosolization of the bacteria in dense animal holding areas. The second route of exposure is through contaminated food such as fresh and soft cheese made from raw milk, raw milk and other dairy products made from raw milk with a high water content, low salinity, and low curing time in high salinity and a pH > 5 (Corbel, 2006).



Occupational disease is controlled through the animal population. There is no human vaccine available to directly protect these workers (Shurig *et al.*, 2002), but there are effective *B. melitensis* vaccines for small ruminants and *B. abortus* vaccines for cattle. Unfortunately, there is no a *B. suis* vaccine commonly used (Radostits *et al.*, 2007). The small ruminant *B. melitensis* vaccine is the Rev 1 modified live vaccine and the *B. abortus* cattle vaccines are the S19 and RB51 modified live vaccines. Self-inoculation can cause disease in humans and vaccination of pregnant animals can result in abortion. The Rev 1 and S19 vaccines interfere with serologic tests, complicating disease control and eradication programs (Shurig *et al.*, 2002).

There are a few prerequisite programs that need to be in place prior to any intervention, disease control program or eradication program. For success in eradication, local stakeholders need to be engaged and in support of the effort and the collaboration between the farmers, their customers and government entities at local, state and national levels (Blasco and Molina-Flores, 2011). Such programs take a large investment of time, resources and money. Further, region-specific seroprevalence needs to be determined and interventions need to be tailored to the epidemiologic unit of interest or region. It is a mistake to assume uniformity of disease prevalence or epidemiology within a country (Blasco and Molina-Flores, 2011).

The Rev 1 vaccine is a modified live *B. melitensis* vaccine that confers protection to 65% of the animals vaccinated and the vaccine immunity can last from three to five years (Olsen and Stoffregen, 2005; Zinnstag *et al.*, 2005). The use of reduced dose vaccination ( $1 \times 10^4$  to  $10^6$  CFUs) rather than full dose ( $1 \times 10^9$  CFUs) can be protective against abortion, but it does not result in adequate immunity (Blasco, 1997; Olsen and Stoffregen, 2005). Despite the potential abortions associated with vaccination, mass vaccination is the most effective method for control of the disease (Garin-Bastuji *et al.*, 1998; Olsen and Stoffregen, 2005; Blasco and Molina-Flores, 2011). Ideally vaccination with Rev 1 in small ruminants occurs in animals aged three to four months, in adults prior to breeding season or while lactating, but in females not in mid-gestation (Blasco and Molina-Flores, 2011). The use of conjunctival inoculation will also reduce the interference with serology (Olsen and Stoffregen, 2005) and can protect against but not prevent abortion (Blasco, 1997; Garin-Bastuji *et al.*, 1998; Blasco and Molina-Flores, 2011). Rather than

a systemic reaction that occurs with subcutaneous injections, the conjunctival injection results in the antibody response being located in the cranial lymph nodes (Olsen and Stoffregen, 2005). Thus, the conjunctival route of administration is the recommended method (Blasco, 1997; Blasco and Molina- Flores, 2011).

*Brucella abortus* can also be controlled in cattle populations using vaccination. There are two vaccines in use today; the S19 and RB51. The S19 vaccine was developed in the 1930s and 1940s in the United States. The attenuation of a virulent S19 strain of *B. abortus* occurred when it was accidentally left at room temperature for one year. Like the Rev 1 vaccine for *B. melitensis*, the S19 vaccine uses the S-LPS antigens to initiate an immune response. This is the same antigen used in serologic tests (Shurig *et al.*, 2002).

In fact it is not biochemically different from *B. abortus* biovar 1 (Blasco and Molina- Flores, 2011). Because of its close relationship to pathogenic strains of *B. abortus* the S19 vaccine induces a serological response that interferes with testing. Initially the immune response to vaccination is the production of IgM antibodies, but is followed shortly by IgG1 and IgG2 antibody production. Sero-conversion back to negative status can occur. This sero-conversion back to sero-negative post-vaccination is slow in older, pregnant cattle that were vaccinated with larger doses of vaccine, but calves vaccinated between three and eight months of age will be sero-negative within nine months of the inoculation (Nicoletti, 1990).

Another method to reduce the serological response and testing interference of the S19 vaccination in calves is to use the reduced dose vaccination ( $3$  to  $10 \times 10^9$  CFUs) rather than full dose ( $2.5$  to  $12 \times 10^{10}$  CFUs). This vaccination still protects 65% to 75% of animals for their lifetime. In pregnant adults, S19 can induce abortions, but only does so in 1% to 2.5% of pregnant animals (Shurig *et al.*, 2002). Reduced dose vaccination of  $0.3$  to  $3 \times 10^9$  CFUs can be used to infer immunity and protect against abortion (Olsen and Stoffregen, 2005).

The RB51 vaccine was developed after the S19. The *Brucella abortus* strain 45/20 vaccine showed that a rough strain-based vaccine could confer protection against the zoonotic smooth-strain *Brucella* spp. infection. This led to the finding of the *B. abortus* strain 2388 with rifampicin



resistance. The 2388 strain produces very little O-polysaccharide, the antigen against which host antibodies are made when an individual is infected with zoonotic S-LPS species of *B. abortus*, *B. melitensis* and *B. suis*. Therefore, vaccinated animals do not produce O-chain antibodies and thus the vaccination does not result in interference with serological testing (Shurig *et al.*, 2002; Vemulapalli *et al.*, 2002). The benefits of the vaccination is that it does not have abortifacient effects; it induces similar immunity to S19 and it does not interfere with serology when a  $1$  to  $3.4 \times 10^{10}$  CFU dose is given subcutaneously in calves and  $10^9$  dose in pregnant cows (Shurig *et al.*, 2002). RB51 confers immunity through induction of the host's cell-mediated immunity using Th1 cells rather than a humoral immunity (Vemulapalli *et al.*, 2002).

There is some evidence that when using S-LPS based ELISA diagnostic tests in RB51 vaccinated animals a false positive can result. This false positive is due to exposure of core epitopes of the agent for which the RB51 vaccinated animal has antibodies. Agglutination-based serological tests use whole cell antigen and ELISA does not; it uses epitopes, thus accounting for the potential false positive results (Blasco and Molina-Flores, 2011). Nonetheless, RB51 has become the only vaccine used in the United States for eradication of *B. abortus* (Shurig *et al.*, 2002; Vemulapalli *et al.*, 2002).

### 3. MATERIALS AND METHODS

#### 3.1. Description of study area

The Afar National Regional State (ARS) is located in the northeast part of the country. Administratively, the region is divided into five zones, which are further subdivided into 32 woredas and 358 kebeles. Pastoralism and agro-pastoralism are the two major livelihood ways practiced in the region and according to the official population statistics, the region's population is estimated to be 1.2 million; of which 90% are pastoralists and 10% agro-pastoralists (a mixture of livestock rearing with rain fed and irrigated crop production). The total surface area of the region is estimated to be 97,970 Km<sup>2</sup> (ARFEB, 2007). The study was conducted in four districts namely, Afambo, Assaiyita, Teru and Awura, which are located in zones one and four (Figure 1).

##### Assayita District

Assayita is part of the administrative Zone 1. It is located at about 70 kms east of Semera town. It is bordered on the east by the Shinnele zone, on the west by the Dubiti, on the north by Elidar, and on the south by Afambo Districts. The district has 11 rural peasant associations (PAs) and 2 urban PAs and it is a lowland area with an altitude of about 350 meters above sea level. It has a mean minimum and maximum temperature of 28°C and 45°C, respectively. The district is a destination for pastoralists from zone four and neighboring districts of zone one in search of pasture for their livestock because the district is endowed with several large scale irrigation farms using Awash River which attracts the pastoralists to feed agricultural leftovers.

##### Afambo District

Afambo is one of the 8 districts in zone one. It is bordered on the North East by Djibouti, Southwest by Dubiti the East by Shinnele zone, on the West and Northwest by Assayita district, respectively. The area is characterized by high humidity and temperature which range between 27°C to 45°C. The district has one urban and eight rural PAs of which three (Alasa Bolo,



Humodoyeta and Mego) are totally agro-pastoralists PAs. The elevation of the district is between 280-850 meter asl with average annual temperature of 120mm. The land coverage of the district is about 1926km<sup>2</sup> and the district is known by Lake Abbi which is the final destination for Awash River (ARFEB, 2007).

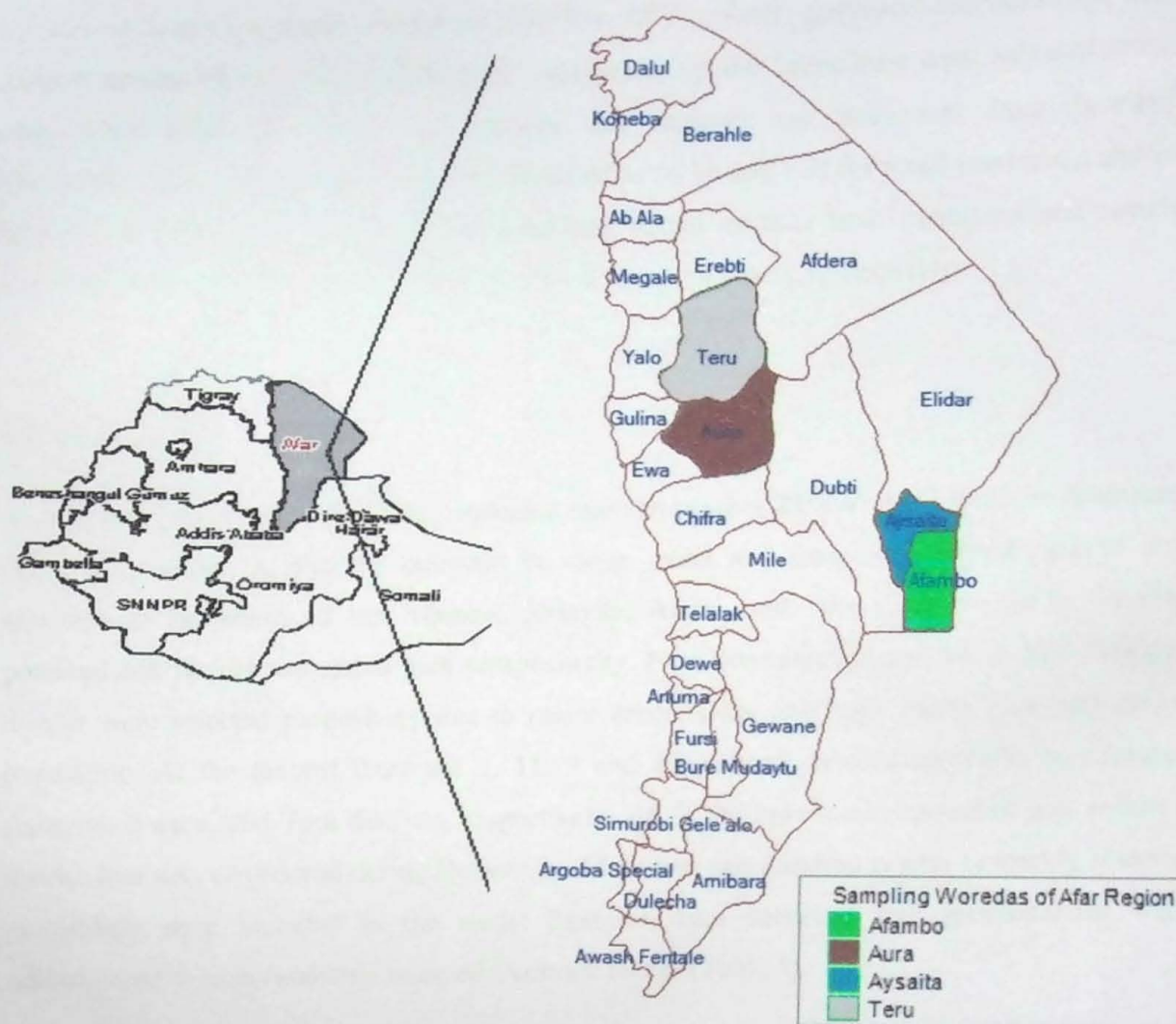
#### Teru District

Teru district has only one livelihood zone in Teru Pastoral livelihood zone. Teru district is one of the 32 districts with in afar-National regional state in zone 4. The district has border with Afdera in the North, with Awura in south, Dubti in the eastern and with Golina, Yalo, and Megalle at west, With Erebti at North East. Teru is located about 350 Km from Semera. Teru Woreda is established with 12 Kebeles. The principal livelihood of the 12 Kebeles' people is pastoralism whose economic set up is based on extensive production of livestock. The topography is flat and mountainous landscape. It is found in the low land of Afar. The climate is arid wit minimum and maximum temperatures of 28°C and 50°C, respectively. The woreda has two main rainy seasons, karma (July-Sept) and sugum (March-April) and also short rainy season called Dadaa with in January. There are dry seasons called Gillal and Hagay. Gillal season is relatively colder than the others season. The annual rainfall is from 200mm-800mm. There are no perennial rivers. The main water sources are seasonal rivers, Ela, and pond. The woreda is covered by sparse Accacia species and extensive grazing land (ARFEB, 2007).

#### Awura District

Awura district has only one livelihood zone called Teru pastoral livelihood zone. Awura district is one of the 32 districts with in Afar National regional state found in Northern Afar, bordering Golina and Teru Woreda in the North, Golina and Kobo (Guraworke) in the West, Ewa in the south and Dubti and Teru Woreda in the East. It is 250km from Semera. Awura district has 3096km<sup>2</sup> land coverage and it is established with eleven kebele centers. The total population of the woreda is about 28,704 from this 53% male and 47% female. The principle livelihood of the people is pastoralism whose economic set up is based on extensive production of livestock. Some kebeles practice agricultural activity and they are on the transit to agro-pastoral. The climate is

generally arid to semiarid with high temperature. The woreda have two main rainy season Karma (July-september) and Sugum (March-April) and also a short rainy season called dadaa between January and February. In addition, there are two dry seasons Hagay (April-July) and Gillal (Sept-Feb). Gilla is relatively a colder season and Hagay the hottest season (ARFEB, 2007).



**Figure 1:** The Map of Afar region showing the locations of the study areas



### 3.2. Study population

The approximate number of sheep, goats and camels in Awesi-Resu (zone one) were 687,551, 1,083,567 and 165,776, respectively (CSA 2010/11); while in Fanteyna Resu (zone four) approximately 418,206 sheep, 398,127 goats and 136,720 camel populations exist in the area and this was considered as study population (ARFEB, 2007). Sheep, goats and camels which were above 6 months of age, with no history of vaccination against brucellosis were included in the study. Then individual animal age, species, sex category and flock size were recorded. Afterwards, herd size per household was classified as ( $\leq 30$  and  $>30$  for small ruminants) and ( $\leq 20$  and  $>20$  for camels). Moreover, based on their sexual maturity small ruminants and camels were classified into ( $\leq 2$  years;  $>2$  years) and ( $\leq 4$  years;  $>4$  years), respectively.

### 3.3. Study design

A cross-sectional study design was conducted from November 2011 to April 2012, to determine the seroprevalence of brucella infection in sheep, goats and camel in selected pastoral and agropastoral residences of the Afambo, Assayita, Awura and Teru districts and to identify potential risk factors associated with seropositivity. First, four districts and about 30% PAs per district were selected purposively due to easier accessibility and huge sheep, goat and camel population. At the present there are 7, 11, 9 and 12 pastoral associations (PA) in Afambo, Assayita, Awura, and Teru districts, respectively. PA is the lowest administrative unit within a district that was considered during the survey. Moreover one hundred twenty randomly selected pastoralists were included in the study. Samples were collected and questionnaires were administered to each randomly selected livestock owner (Table 3).

**Table 3:** Study population and sample size per district

| Zone  | District | No of PAs | No of PAs selected | PAs selected | Livestock population in the districts* |        |       | Animals sampled per district |      |       | Number of respondents |
|-------|----------|-----------|--------------------|--------------|----------------------------------------|--------|-------|------------------------------|------|-------|-----------------------|
|       |          |           |                    |              | Sheep                                  | Goat   | Camel | Sheep                        | Goat | Camel |                       |
| 1     | Afambo   | 7         | 4                  | Alasabolo    | 12152                                  | 15592  | 1137  | 100                          | 237  | 60    | 30                    |
|       |          |           |                    | Mego         |                                        |        |       |                              |      |       |                       |
|       |          |           |                    | Humodoyeta   |                                        |        |       |                              |      |       |                       |
|       |          |           |                    | Deka         |                                        |        |       |                              |      |       |                       |
| 4     | Assayita | 11        | 3                  | Galifage     | 16943                                  | 23086  | 13277 | 69                           | 193  | 151   | 30                    |
|       |          |           |                    | Henele       |                                        |        |       |                              |      |       |                       |
|       |          |           |                    | Urmiytu      |                                        |        |       |                              |      |       |                       |
|       |          |           |                    | Deritu       |                                        |        |       |                              |      |       |                       |
| 4     | Awura    | 9         | 3                  | Lekoma       | 114952                                 | 131040 | 29668 | 110                          | 128  | 100   | 30                    |
|       |          |           |                    | Mesgid       |                                        |        |       |                              |      |       |                       |
|       |          |           |                    | Uidolul      |                                        |        |       |                              |      |       |                       |
|       |          |           |                    | Asabera      |                                        |        |       |                              |      |       |                       |
|       | Teru     | 12        | 3                  | Alelo        | 209552                                 | 233093 | 29668 | 45                           | 168  | 150   | 30                    |
|       |          |           |                    |              |                                        |        |       |                              |      |       |                       |
|       |          |           |                    |              |                                        |        |       |                              |      |       |                       |
|       |          |           |                    |              |                                        |        |       |                              |      |       |                       |
| Total |          | 39        | 13                 |              |                                        |        |       | 324                          | 726  | 461   | 120                   |

\* ARFEB, 2007

### 3.4. Sampling method

Multistage sampling technique was used in the survey of sheep, goat and camel brucellosis. The PA was regarded as primary unit, the herds as secondary units and individual animals as tertiary units. An average of 30% of small ruminants and 40% of camels aged 6 month and above were picked randomly from each selected herd till the calculated sample size has achieved.

Sheep, goats and camel herds in 13 PAs from four districts were sampled during the study based on the livestock population of each district. In order to determine the desired sample size of sheep, goat and camels, there was no past knowledge about the previous year's prevalence of brucellosis in the districts except the 0% (n=32) report by Ashenafi *et al.* (2007) in small ruminants of Assayita. Hence, the average expected prevalence rate was assumed to be 50% for



the area within 95% Confidence Intervals (CI) at 5% desired accuracy. Subsequently, the formula used to calculate sample size (n) was as follows:

$$n = \frac{1.96^2 \times P_{ex} \times (1-P_{ex})}{d^2} \quad (\text{Trusfield, 2005})$$

Where n = sample size, d = desired absolute precision (0.05),  $P_{ex}$  = expected prevalence (50%), thus the desired sample size for  $P_{ex} = 0.5$  is  $n = 384$ . But, it was safe to inflate the sample size by two to three folds as far as practically possible. Hence,  $n = 1150$  small ruminants and  $n = 461$  camels were sampled. Sampling was proportionally distributed based on the total small ruminant and camel population in the study districts and PAs. (Table 3).

### 3.5. Study methodology

#### 3.5.1. Questionnaire survey

Verbal consent was obtained from the respondents and the objective of the survey explained to them before start of the interview. The interviews were conducted in local language (Afar). One hundred twenty randomly selected livestock owners from the four districts were interviewed by using structured questions. Two questionnaire formats; one for serum sampled individual animal history and the other structured questionnaire format for herders were developed and used in this study. The questionnaire focused on animal feeding and housing practices, knowledge about zoonotic diseases, habit of animal product consumption and handling, and dead-animal/ aborted foeti disposal practices. In total, 120 pastoralists from whom their animals tested for brucellosis were interviewed. By doing so, risk factors that have possible association with the brucellosis occurrence were investigated and used to support serological results (Annex 3 and 9).

### 3.5.2. Serological survey

Blood samples were collected from jugular vein of each animal of selected herds using plain vacutainer tubes. The blood samples were allowed to clot at room temperature. Then, serum was separated from clotted blood by decanting to other tubes. Separated sera were stored at  $-20^{\circ}\text{C}$  until laboratory test was performed by both RBPT and CFT (Annex 4).

#### Rose Bengal Plate Test (RBPT)

The modified Rose Bengal Plate Test (mRBPT) was done in Semera Regional Veterinary Laboratory in order to screen positive samples by RBPT using RBPT antigen (Institut Pourquier 325, rue de la galère 34097 Montpellier cedex 5, France) Positive sera were then retested using Complement Fixation Test (CFT) at the National Veterinary Institute (NVI), Debre Zeit. Samples were considered positive for brucellosis if they were positive for RBPT and CFT (Annex 1 & 5).

For the mRBPT, the procedure described by Alton *et al.* (1975) was followed. The sera and antigen were taken out from the refrigerator and left at room temperature for at least 30 minutes before the test was done. Then, 75  $\mu\text{l}$  of test sera were dispensed on each of the 12 circles of the plate. The antigen bottle was gently shaken and a drop of RBPT antigen (25  $\mu\text{l}$ ) was placed alongside the serum. The antigen and serum were mixed thoroughly using applicator stick and the plate was rocked manually for about 4 minutes. Finally, results were read in a good light source or using magnifying glass when micro-agglutinations were suspected.

Reactions were categorized as 0, +, ++, +++, according to Nielsen and Dunkan (1990), where: 0 = means no agglutination, + = barely perceptible agglutination (using magnifying glass), ++ = fine agglutination, some clearing, and +++ = clumping, definite clearing. Those samples identified with no agglutination (0) were regarded as negative, while those with +, ++ and +++ were regarded as positive (Annex 6).



## Complement Fixation Test (CFT)

Positive sera with RBPT were further tested with CFT for confirmation using Standard *Brucella abortus* antigen (New Haw, Addlestone, Surrey KT15 3NB, UK). The CFT test proper and reagent preparation procedures were following the procedures outlined by Alton *et al.* (1975) and OIE (2000). The 1% sheep red blood cells suspension was prepared before the beginning of the test. Test sera with  $\geq 50\%$  sedimentation at a dilution of 1:5 and above are considered to be positive. The reading was as complete fixation (no hemolysis) with water clear supernatant was recorded as + + + +, nearly complete fixation (75% clearing) as + + +, partial hemolysis (50%) + + and some fixation (25% clearing) as +. Complete lack of fixation (complete hemolysis) was recorded as 0. For positive reactions final titration was recorded.

Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above were classified as positive (OIE, 2004). The test was examined by the eye; sedimentation of the sensitized RBCs was considered as positive and the complete lysis of the sensitized RBCs was taken as negative (Annex 2 & 6).

### 3.6. Data Processing and Statistical Analysis

The data were summarized and individual animal level seroprevalence was calculated on the bases of RBPT and CFT positive results divided by total number of animals tested. Coded data were stored in Microsoft Excel 2007 spread sheet and transferred to SPSS® Version 16 for statistical analysis. Descriptive and analytic statistics were computed using software SPSS® V16. Logistic regression and Chi-square test ( $\chi^2$ ) were employed to see the association of risk factors with that of seropositivity to brucella antibody; the degree of association was computed using Odds ratio (OR) and 95% confidence interval (CI). A test value was considered as statistically significant when  $P < 0.05$ . Odd ratio (OR) was used to indicate the degree of risk factor association with the disease occurrence signified by 95% confidence intervals. Odds ratio is the ratio of the odds of disease occurring among individuals exposed to a variable and the odds of the

disease occurring among individuals not so exposed (Thrusfield, 2005). Variable reduction was performed by fitting univariate logistic regression for each covariate and variables with p-value > 0.25 were dropped.



## 4. RESULTS

### 4.1. Seroprevalence of small ruminant and camel brucellosis

A total of 1050 and 461 sera samples were collected from 132 and 100 herds of small ruminants and camels, respectively with no history of previous vaccination against brucellosis. Out of 1511 tested animals; 164 (10.9%) were found positive with mRBPT. Out of these, 147 were positive with CFT. Thus, the subsequent data analyses were based on the one hundred forty seven sera that were positive to both the RBPT and CFT test results. An overall individual animal level seroprevalence of 9.73% (147/1511) was recorded from sheep, goat and camels of the study area. Thus, the number of seropositive sheep, goats and camels in study districts was 7.1% (23/324), 13.6% (99/726) and 5.4% (25/461), respectively. This difference in seroprevalence between species is statistically significant ( $X^2=24.91$ ,  $P<0.05$ ). In addition the herd level seroprevalence of 51.1% and 24% were found in small ruminants and camels, respectively. There was no statistically significant difference in seroprevalence between the administrative locations ( $P>0.05$ ) (Table 4-5).

**Table 4:** Herd level seroprevalence of small ruminants and camel brucellosis in the study area

| Zone | District | Species | Number tested | RBPT positive (%) | CFT positive (%) | Herd Level    |              |
|------|----------|---------|---------------|-------------------|------------------|---------------|--------------|
|      |          |         |               |                   |                  | Number Tested | Positive (%) |
| One  | Afambo   | Ovine   | 100           | 12(12)            | 9(9)             |               |              |
|      |          | Caprine | 237           | 34(14.35)         | 30(12.66)        | 43            | 22(51.16)    |
|      |          | Camel   | 60            | 5(8.33)           | 4(6.67)          | 16            | 4(25.00)     |
|      | Assayita | Ovine   | 69            | 5(7.24)           | 4(5.8)           |               |              |
|      |          | Caprine | 193           | 32(16.58)         | 30(15.54)        | 33            | 17(51.5)     |
|      |          | Camel   | 151           | 6(3.97)           | 5(3.31)          | 34            | 5(14.71)     |
| Four | Awura    | Ovine   | 110           | 4(3.64)           | 4(3.64)          |               |              |
|      |          | Caprine | 128           | 18(14.06)         | 15(11.71)        | 29            | 12(41.38)    |
|      |          | Camel   | 100           | 6(6)              | 6(6)             | 19            | 6(31.58)     |
|      | Teru     | Ovine   | 45            | 6(13.33)          | 6(13.33)         |               |              |
|      |          | Caprine | 168           | 25(14.88)         | 24(14.28)        | 27            | 17(62.96)    |
|      |          | Camel   | 150           | 11(7.33)          | 10(6.67)         | 31            | 9(29.03)     |
|      | Total    | Ovine   | 324           | 27(8.33)          | 23(7.1)          |               |              |
|      |          | Caprine | 726           | 109(15.01)        | 99(13.64)        | 132           | 68(51.51)    |
|      |          | Camel   | 461           | 28(6.1)           | 25(5.42)         | 100           | 24(24.0)     |

#### 4.2. Effect of risk factors on seroprevalence of small ruminant and camel brucellosis

This study showed a higher seroprevalence of brucellosis in goats than either in sheep or camel. The prevalence was 13.6% in goats, 7.1% in sheep, and 5.4% in camels with a statistically significant difference ( $X^2 = 24.91$ ,  $P < 0.05$ ) (Table 5). Similarly, the prevalence of brucellosis in small ruminants was found to be higher in the adult animals of both species than in the younger ones and it is statistically significant ( $X^2=10.54$ ,  $P < 0.05$ ) (Table 6). Moreover in small ruminants the association of large flock size and seropositivity is statistically significant ( $X^2 = 10.47$ ,  $P < 0.05$ ) (Table 6). A seroprevalence of 15.8% was observed in adult goats greater than two years old which is statistically significant ( $X^2=10.81$ ,  $P < 0.05$ ); while only 5.7% of sera from the young gave positive results (Table 7). Moreover, the difference in seroprevalence between the sex groups of goats was statistically significant ( $X^2= 6.61$ ,  $P < 0.05$ ). However, the seroprevalence of brucellosis in female and male sheep was 7% ( $n=20$ ) and 8.6% ( $n=3$ ), respectively, but no statistically significant difference ( $X^2 = 1.629$ ,  $P > 0.05$ ) and ( $X^2 = 0.169$ ,  $P > 0.05$ ) was observed in the prevalence of brucellosis between male and female animals and different age groups respectively (Table 8).

Of camels under different age groups, the reactor rates of age  $\leq 4$  years old and  $> 4$  years old were 1.6% (3/187) and 8% (22/274), respectively. As in (Table 9), the Chi-square test for the association of risk factors with seropositivity indicated that herd size ( $X^2=7.024$ ,  $P < 0.05$ ) was significantly associated while sex was not significant  $P > 0.05$ .

**Table 5:** Chi-square test showing the cumulative effect of the three species with brucellosis reactivity

| Variable |          | No. sampled | Complement Fixation Test |            |         |
|----------|----------|-------------|--------------------------|------------|---------|
|          |          |             | Positive (%)             | Chi-square | P-value |
| District | Afambo   | 397         | 43(10.8)                 | 3.37       | 0.338   |
|          | Assayita | 413         | 39(9.4)                  |            |         |
|          | Awura    | 338         | 25(7.4)                  |            |         |
|          | Teru     | 363         | 40(11)                   |            |         |
| Species  | Goat     | 726         | 99(13.6)                 | 24.91      | 0.001   |
|          | Sheep    | 324         | 23(7.1)                  |            |         |
|          | Camel    | 461         | 25(5.4)                  |            |         |
| Sex      | Male     | 255         | 19(7.5)                  | 1.81       | 0.178   |
|          | Female   | 1256        | 128(10.2)                |            |         |



**Table 6:** Association of risk factors with brucellosis reactivity in small ruminants

| Variable   | Category    | No. sampled | Complement Fixation Test |            |         |
|------------|-------------|-------------|--------------------------|------------|---------|
|            |             |             | Positive (%)             | Chi-square | P-value |
| Zone       | 4           | 451         | 49(10.9)                 | 0.44       | 0.508   |
|            | 1           | 599         | 73(12.2)                 |            |         |
|            | Afambo      | 337         | 39(11.6)                 | 4.8        | 0.187   |
|            | Assayita    | 262         | 34(13)                   |            |         |
|            | Awura       | 238         | 19(8)                    |            |         |
|            | Teru        | 213         | 30(14.1)                 |            |         |
| Species    | Goat        | 726         | 99(13.6)                 | 9.32       | 0.002   |
|            | Sheep       | 324         | 23(7.1)                  |            |         |
| Sex        | Male        | 204         | 16(7.8)                  | 3.52       | 0.061   |
|            | Female      | 846         | 106(12.5)                |            |         |
| Age        | ≤2 years    | 200         | 10(5)                    | 10.54      | 0.001   |
|            | >2 years    | 850         | 112(13.2)                |            |         |
| Flock size | ≤30 animals | 462         | 37(8)                    | 10.47      | 0.001   |
|            | >30 animals | 588         | 85(14.5)                 |            |         |

**Table 7:** Association of risk factors with brucellosis reactivity in goats in the study areas

| Variable   | Category    | No. sampled | Complement Fixation Test |            |         |
|------------|-------------|-------------|--------------------------|------------|---------|
|            |             |             | Positive (%)             | Chi-square | P-value |
| Zone       | 1           | 430         | 60(14)                   | 0.90       | 0.764   |
|            | 4           | 296         | 39(13.2)                 |            |         |
| District   | Afambo      | 237         | 30(12.7)                 | 23.43      | 0.054   |
|            | Assayita    | 193         | 30(15.5)                 |            |         |
|            | Awura       | 128         | 15(11.7)                 |            |         |
|            | Teru        | 168         | 24(14.3)                 |            |         |
| Sex        | Male        | 169         | 13(7.7)                  | 6.61       | 0.01    |
|            | Female      | 557         | 86(15.4)                 |            |         |
| Age        | ≤2 years    | 158         | 9(5.7)                   | 10.81      | 0.001   |
|            | >2 years    | 568         | 90(15.8)                 |            |         |
| Flock size | ≤30 animals | 313         | 31(9.9)                  | 6.50       | 0.011   |
|            | >30 animals | 413         | 68(16.5)                 |            |         |

**Table 8:** Association of risk factors with brucellosis reactivity in sheep in the study areas

| Variable   | Category    | No. sampled | Complement Fixation Test |            |         |
|------------|-------------|-------------|--------------------------|------------|---------|
|            |             |             | Positive (%)             | Chi-square | P-value |
| Zone       | 1           | 169         | 13(7.7)                  | 5.377      | 0.146   |
|            | 4           | 155         | 10(6.5)                  |            |         |
| District   | Afambo      | 100         | 9(9)                     |            |         |
|            | Assayita    | 69          | 4(5.8)                   |            |         |
|            | Awura       | 110         | 4(3.6)                   |            |         |
|            | Teru        | 45          | 6(13.3)                  |            |         |
| Sex        | Male        | 35          | 3(8.6)                   | 0.169      | 0.719   |
|            | Female      | 289         | 20(6.9)                  |            |         |
| Age        | ≤2 years    | 42          | 1(2.4)                   | 1.629      | 0.202   |
|            | >2 years    | 282         | 22(7.8)                  |            |         |
| Flock size | ≤30 animals | 149         | 6(4)                     | 3.947      | 0.047   |
|            | >30 animals | 178         | 17(9.7)                  |            |         |

**Table 9:** Association of risk factors with brucellosis reactivity in camel of the study areas.

| Variable  | Category    | No. sampled | Complement Fixation Test |            |         |
|-----------|-------------|-------------|--------------------------|------------|---------|
|           |             |             | Positive (%)             | Chi-square | P-value |
| Zone      | 1           | 211         | 9(4.3)                   | 2.011      | 0.57    |
|           | 4           | 250         | 16(6.4)                  |            |         |
| District  | Afambo      | 60          | 4(6.7)                   |            |         |
|           | Assayita    | 151         | 5(3.3)                   |            |         |
|           | Awura       | 100         | 6(6)                     |            |         |
|           | Teru        | 150         | 10(6.7)                  |            |         |
| Sex       | Male        | 51          | 3(5.9)                   | 0.024      | 0.878   |
|           | Female      | 410         | 22(5.4)                  |            |         |
| Age       | ≤4 years    | 187         | 3(1.6)                   | 8.945      | 0.003   |
|           | >4 years    | 274         | 22(8)                    |            |         |
| Herd size | ≤20 animals | 265         | 8(3)                     | 7.024      | 0.008   |
|           | >20 animals | 196         | 17(8.7)                  |            |         |



## 4.3. Univariate logistic regression analysis of risk factors

### 4.3.1. Effect of risk factors on small ruminants seroprevalence

The univariable logistic regression analysis of the putative risk factors showed statistically significant ( $P < 0.05$ ) difference on brucella reactivity between small ruminants with small and large herd size. Odds of brucellosis reactivity among herds with  $>30$  animals was 1.79 (95% CI: 1.14-2.82) and 2.56 (95% CI: 0.984-6.68) in caprine and ovine, respectively as compared to herds with  $\leq 30$  animals. Furthermore, the univariable logistic regression analysis showed that brucella reactivity was significantly different among the different age-groups of goats. Thus, the reactivity was significantly higher in age-groups within  $>2$  years (OR=3.12, 95% CI: 1.533-6.337;  $P < 0.05$ ) as compared to animals under or two years of age. Moreover, the results of univariable logistic regression showed that geographical location (zone and district) of study animals, and sex of animals had no significant effect on the overall seroprevalence of ovine brucellosis ( $P > 0.05$ ) (Table 10 & 11).

**Table 10:** Effects of risk factors on the individual level sero-prevalence of sheep and goats brucellosis

| Variable   | Category           | Complement Fixation Test |             |         |       |             |         |
|------------|--------------------|--------------------------|-------------|---------|-------|-------------|---------|
|            |                    | Species of animals       |             |         |       |             |         |
|            |                    | Caprine                  |             |         | Ovine |             |         |
|            |                    | OR                       | 95%CI       | P-value | OR    | 95%CI       | P-value |
| Zone       | 4*                 |                          |             |         |       |             |         |
|            | 1                  | 1.069                    | 0.693-1.648 | 0.764   | 1.208 | 0.514-2.841 | 0.664   |
| District   | Teru*              |                          |             |         |       |             |         |
|            | Afambo             | 1.12                     | 0.645-2.049 | 0.64    | 1.56  | 0.52-4.67   | 0.431   |
|            | Assayita           | 1.11                     | 0.62-1.98   | 0.74    | 2.50  | 0.66-9.43   | 0.176   |
|            | Awura              | 1.26                     | 0.641-2.56  | 0.52    | 4.08  | 1.09-15.15  | 0.037   |
| Sex        | Male               |                          |             |         |       |             |         |
|            | Female             | 2.19                     | 1.19-4.04   | 0.12    | 1.26  | 0.35-4.48   | 0.720   |
| Age        | $\leq 2$ years*    |                          |             |         |       |             |         |
|            | $>2$ years         | 3.12                     | 1.533-6.337 | 0.002   | 3.469 | 0.455-26.44 | 0.230   |
| Flock size | $\leq 30$ animals* |                          |             |         |       |             |         |
|            | $>30$ animals      | 1.79                     | 1.14-2.82   | 0.012   | 2.56  | 0.984-6.68  | 0.054   |

\*Reference category

**Table 11:** Effects of risk factors on the overall sero-prevalence of small ruminants' brucellosis

| Risk Factors |           | Complement Fixation Test |           |         |
|--------------|-----------|--------------------------|-----------|---------|
| Variable     | Category  | OR                       | 95%CI     | P-value |
| Zone         | 4*        |                          |           | 0.508   |
|              | 1         | 1.14                     | 0.78-1.67 |         |
| District     | Teru*     |                          |           | 0.194   |
|              | Afambo    | 1.25                     | 0.75-2.09 | 0.387   |
|              | Assayita  | 1.09                     | 0.65-1.87 | 0.725   |
|              | Awura     | 1.89                     | 1.03-3.47 | 0.04    |
| Species      | Sheep*    |                          |           |         |
|              | Goat      | 2.07                     | 1.29-3.32 | 0.003   |
| Sex          | Male*     |                          |           |         |
|              | Female    | 1.68                     | 0.97-2.92 | 0.063   |
| Age          | ≤2 years* |                          |           |         |
|              | >2 years  | 2.88                     | 1.48-5.61 | 0.002   |
| Flock size   | ≤30*      |                          |           |         |
|              | >30       | 1.94                     | 1.29-2.92 | 0.001   |

\*Reference category

#### 4.3.2. Effect of risk factors on camels seroprevalence

The univariable logistic regression analysis of the putative risk factors showed statistically significant (OR= 3.05, 95%CI: 1.289-7.22,  $P < 0.05$ ) difference on seroprevalence of brucellosis between camels from herds > 20 animals than those with less than or equal to 20 heads. In addition the analysis has found that sero-reactivity was significantly different among the different age-groups. Thus, it was significantly higher in age-groups >4 years old (OR=5.354, 95% CI: 1.579-18.158;  $P < 0.05$ ) as compared to animals ≤ 4 years old (Table 12).



**Table 12:** Effects of risk factors on the overall individual level sero-prevalence of camel brucellosis

| Variable  | Category  | Complement Fixation Test |              |         |
|-----------|-----------|--------------------------|--------------|---------|
|           |           | OR                       | 95%CI        | P-value |
| Zone      | 4*        |                          |              |         |
|           | 1         | 1.532                    | 0.664-3.546  | 0.316   |
| District  | Teru*     |                          |              |         |
|           | Afambo    | 1                        | 0.301-3.321  | 1       |
|           | Assayita  | 2.087                    | 0.695-6.25   | 0.19    |
|           | Awura     | 1.12                     | 0.393-3.18   | 0.833   |
| Sex       | Male*     |                          |              |         |
|           | Female    | 1.10                     | 0.318-3.82   | 0.878   |
| Age       | ≤4 years* |                          |              |         |
|           | >4 years  | 5.354                    | 1.579-18.158 | 0.007   |
| Herd size | ≤20*      |                          |              |         |
|           | >20       | 3.05                     | 1.289-7.22   | 0.011   |

\*Reference category

#### 4.4. Multivariable stepwise logistic regression analysis of risk factors for brucella reactivity

The difference for age-group, sex and herd size based infection rates observed in caprine in the initial logistic regression model were also found evident after the control of confounding factors in the stepwise multivariable logistic regression analysis. On the other hand, in ovine only flock size was used while sex and age of animals were excluded from the model indicating that both were not significantly affecting ovine's reaction to brucellosis. But the effect of the variables in small ruminants had different scenario. In camels only sex of the animals was omitted but age and herd size become evident.

Of the different risk factors which appeared significant for the stepwise logistic regression model were age-groups [significantly high reactor rates in caprine >2 years old (OR=3.1, 95% CI: 1.498-6.258; P<0.05) and camel of >4 years old (OR=4.935, 95% CI: 1.45-16.82; P<0.05) as compared to camels of ≤4 years old].

In addition, brucellosis sero-positivity was significantly higher in animals coming from larger herds of small ruminant (OR=1.9, 95%CI: 1.23-3.02); this indicates that age and herd size were significantly affecting animals' reaction to brucellosis test. Table 13 below shows the result of stepwise multivariate logistic regression analysis of risk factors and brucellosis reactivity of sheep, goat and camel in the study area.

**Table 13:** Multivariable stepwise logistic regression analysis of risk factors

| Species                   | Variable*      | Complement Fixation Test |             |         |
|---------------------------|----------------|--------------------------|-------------|---------|
|                           |                | OR                       | 95%CI       | P-value |
| Caprine                   | Female         | 2.075                    | 1.12-3.85   | 0.021   |
|                           | >2 years       | 3.061                    | 1.498-6.258 | 0.002   |
|                           | >30 flock size | 1.91                     | 1.23-3.02   | 0.006   |
| Camel                     | >4 years       | 4.935                    | 1.45-16.82  | 0.011   |
| Caprine and Ovine         | Caprine        | 2.361                    | 1.458-3.825 | 0.000   |
|                           | Female         | 1.785                    | 1.018-3.13  | 0.043   |
|                           | >2 years       | 3.132                    | 1.595-6.149 | 0.001   |
|                           | >30 flock size | 2.036                    | 1.348-3.075 | 0.001   |
| Camel , Caprine and Ovine | Caprine        | 2.754                    | 1.746-4.342 | 0.000   |

\*The Reference categories were omitted to avoid repetition

#### 4.5. Results of questionnaire survey

In the study area above 75% of the interviewed pastoralists own mainly sheep, goats and camel with diverse numbers. Accordingly 61% (74/120), 25.8% (31/120) and 7.5% (9/120) of the respondents keep camels either with both small ruminant species or either of the two or with cattle. In addition the community most of a time keep these species together at browsing, watering, night enclosures and during migration(Annex 8). Very few respondents (30/120= 25%) agreed with the idea that some diseases can be transmitted from animals to humans. A relatively high proportion of the respondents acquired the knowledge about zoonotic diseases from elders 66.7% and from their personal observation 33.3% (10/30). Out of those having awareness about zoonotic diseases, large proportion of respondents (73.3%) indicated meat and milk as a vehicle for disease transmission to humans. Only a few people responded that zoonotic pathogens could be acquired through direct contact and inhalation (Table 14).



Hundred percent of the respondents consume raw milk which contributes to disease transmission. Above three-quarters of the interviewed pastoralists in the study area reported practicing at least one activity considered at risk for transmission of zoonotic diseases. These activities were mainly related to handling animals and their products (assisting delivery, disposal of aborted fetus, pulling of RFM) and consumption of raw animal products (Table 15) (Annex 7).

Specific factors associated with existence of brucellosis were assessed. Out of 120 respondents, 118 reported that they had observed a case of abortion or retained fetal membrane in animals. Moreover history of abortion during the last one year in their herd was reported by 83% of the respondents. Eighty two percent of them leave the aborted materials where they find it and 9% of them give it to dogs or wild scavengers (Table 15).

**Table 14:** Awareness about zoonotic transmission of livestock diseases

| Issues raised                                          | Response Category    | Number of respondents (%) |
|--------------------------------------------------------|----------------------|---------------------------|
| Know how about disease transmission from animal to man | Yes                  | 30(25)                    |
|                                                        | No                   | 90(75)                    |
| Source of Knowledge                                    | Elders               | 20(66.7)                  |
|                                                        | Personal observation | 10(33.3)                  |
| Vehicle for disease transmission                       | Meat and Milk        | 22 (73.3)                 |
|                                                        | Direct contact       | 5(16.7)                   |
|                                                        | Inhalation           | 3(10)                     |
| Knowledge about infectious disease (Brucellosis)       | Yes                  | 103(85.8)                 |
|                                                        | No                   | 17(14.2)                  |
| Encounter Abortion or RFM                              | Yes                  | 120(100)                  |
| Abortion history in the past 1 year                    | Yes                  | 100(83.3)                 |
|                                                        | No                   | 20(16.7)                  |

\*the percentage does not sum to 100% because all responses are not shown.

**Table 15:** Animal products handling and consumption habits

| Issues raised                                                     | Response Category          | Number of respondents (%) |
|-------------------------------------------------------------------|----------------------------|---------------------------|
| Raw milk consumption                                              | Yes                        | 120(100)                  |
| Self protection at handling dystocia and normal delivery          | No                         | 120(100)                  |
| Care for new born                                                 | Cleaning                   | 62(51.7)                  |
|                                                                   | Cutting the umbilical cord | 46(38.3)                  |
|                                                                   | Hand feeding weak calves   | 10(8.3)                   |
| Method of managing an animal frequently abort or fail to conceive | Sell                       | 37(30.8)                  |
|                                                                   | Slaughter                  | 20(16.7)                  |
|                                                                   | Keep                       | 28(23.3)                  |
| Method of managing aborted material                               | Leave in the field         | 98(81.7)                  |
|                                                                   | Proper disposing           | 11(9.17)                  |
|                                                                   | Give to scavengers         | 11(9.17)                  |
| Source of Ram/bull                                                | Raise own ram/Bull         | 70(58.33)                 |
|                                                                   | Village                    | 42(35)                    |
|                                                                   | Own or village             | 8(6.67)                   |
| Selling of breeding female                                        | Yes                        | 74(61.7)                  |
|                                                                   | No                         | 46(38.3)                  |
| Reason for selling breeding female                                | Disease                    | 62(83.8)                  |
|                                                                   | Shortage of money          | 12(16.2)                  |

\*the percentage does not sum to 100% because all responses are not shown.



## 5. DISCUSSION

Livestock brucellosis has significant economic and zoonotic implication for the pastoral communities in consequence of their traditional life styles, feeding habits and management practices. Hence, the present cross-sectional study was conducted in selected four districts of Afar region to determine the prevalence of brucellosis in sheep, goats, and camels and to assess the knowledge of the pastoral community on the zoonotic importance of the disease.

In this study herd level seroprevalence of 51.1% and 24% were found in small ruminants and camels, respectively. In contrast to the present finding Kaoud *et al.* (2010) reported a seroprevalence of 26.7% and 18.9% in herds of sheep and goats in Egypt, respectively. Brucellosis was detected in all the four districts of the two zones and the difference in prevalence among the zones and between the districts in small ruminants and camel was not statistically significant ( $P>0.05$ ). It could be due to the similarity in the agro-ecological conditions and livestock management system in the area.

This study demonstrated that the overall seroprevalence of small-ruminant brucellosis in the study area was 11.62 %, while the proportion in sheep is 7.1% and in goats 13.6% by CFT. The overall seroprevalence in this finding is slightly lower than reports from previous works from other study areas in the same region, by Teshale *et al.* (2006) who reported 16%. The overall epidemiology of the disease at individual and herd level show wider spread of the disease in different species of animals. In Afambo and Assayita districts of zone one, animals are kept in confinement around cultivation fields than the other two districts, being the districts are largely dominated by agricultural irrigation using Awash River. This may be responsible for the high prevalence in zone one as infection is easily transmitted within the entire herd under this management system. Teru and Awura districts are mostly pastoralist settings and are dominated by free range management system. The use of large grazing sites and river banks as communal grazing areas for both camel and small ruminants could favor the spread of brucellosis from small ruminants to camels and from one small ruminant herd to the other.

In sheep, the study is fairly in agreement with different findings; Shehu *et al.* (1999) reported a prevalence of 6.6% in sheep in Nigeria. However, the findings disagree with that of Yesuf *et al.* (2010) who reported a seroprevalence of 1.5% in south Wollo; that of Teshale *et al.* (2006) and Ashenafi *et al.* (2007) who reported seroprevalence of 14.6% and 3.2% in Mille and Dalifage districts of Afar region and in Afar region, respectively. In other countries Okoh (1980) reported a prevalence of 14.5% in Kano as well as Bale *et al.* (2003) reported 15.9% prevalence in a study conducted in Northern Nigeria. The high prevalence of ovine brucellosis obtained in this study may be attributed to the management system prevailing in the region where sheep and goats graze and housed together.

Higher prevalence in goats compared to this finding was reported by Teshale *et al.* (2006) (16.45%), Bale *et al.* (2003) (34.8% %) and Ojo *et al.* (2007) (45.75%) in Afar region of Ethiopia, northern Nigeria and Abeokuta, respectively. However, a lower prevalence compared to this finding was 5.8% and 9.4% reported by Ashenafi *et al.* (2007) and Okewole *et al.* (1988) respectively. The high prevalence and wide distribution are not surprising since small ruminants are not being vaccinated against brucellosis and coupled with the traditional practice of communal grazing in most part of the region.

Statistically significant difference in seroprevalence was observed between sheep and goats with OR=2.066 (CI: 1.286-3.32;  $P < 0.05$ ). Goats were found to be at higher risk (OR = 2.36; CI: 1.458-3.825) than sheep. This finding is in agreement with results of Omer *et al.* (2000) and Radostitis *et al.* (1994). The higher prevalence in goats than in sheep may be in part due to the greater susceptibility of goats to *Brucella* infection than sheep and partly it may be due to the fact that sheep unlike goats do not excrete the *Brucella* organisms for longer periods of time. This can reduce the potential of the spread of the disease among sheep flock (Radostitis *et al.*, 1994).

In this study, the prevalence of the disease in male and female sheep was 8.6% (3/35) and 6.9% (20/189), respectively but the difference was not statistically significant ( $P > 0.05$ ). This may be due to the small number of male sheep sampled because of the pastoralists keep very few numbers of male animals in the herd. Although it is difficult to draw a firm conclusion due to the smaller number of male sheep, the lack of difference between the two sexes observed in this study contradicts the established facts.



Hirsh and Zee (1999) have reported that male animals are less susceptible to *Brucella* infection, due to the absence of erythritol. However, in support of the present findings, Teshale *et al.* (2006) and Ashenafi *et al.* (2007) have also reported no observable difference in the prevalence of brucellosis between male and female sheep and goats.

In the present study, statistically significant difference was observed between age and seropositivity in goats but not in sheep. In the latter the reason may be few number of the young sheep included in the sampling. In goats the overall prevalence of brucella antibodies in the two age categories was 5.7% and 15.8 ( $X^2$ :10.81, CI: 0.02-0.09) for age category of 6 months to 4 years and above 4 years, respectively. This result was in consistent with Ashenafi *et al.* (2007) who reported 1.6% and 5.3% in young and adult small ruminants, respectively.

In addition, this study showed that the prevalence rate of brucellosis in Assayita district was 5.8% and 15.54% in sheep and goats, respectively. The prevalence has increased in the district when it is compared with a previous study done by Ashenafi *et al.* (2007) who found 0% in small ruminants. This increasing may be due to the seasonal migration of livestock from Chifera, Awura, Teru and Golina to assayita and Dubiti districts of the region in search of cotton, maize and sorgume leftover as an animal feed.

Camels are not known to be primary hosts of brucella organisms but they are susceptible to both *B. abortus* and *B. melitensis* (Cooper, 1991). In this study over all seroprevalence of 5.4% (25/461) was observed which was in agreement with the 5.7% seroprevalence in Afar region by Teshome *et al.* (2003); 4.9% in Sudan by Abu-Damir (1984); 6.4% in Kenya by Waghela *et al.* (1978); 5.8% by Moustafa *et al.* (1998). Recently, Musa *et al.* (2008) reported high prevalence of brucellosis (23.8%) from camels kept mixed with ruminant species; spread of brucellosis in camels depends on the *Brucella* species prevalent in other animals sharing their habitat and on the husbandry methods of the different species (Musa *et al.*, 2008). The data presented here suggest that the prevalence of brucellosis in camels of Afar region is generally high. Previous data on brucellosis in camels in the same region in Amibara district found 11.9% by RBPT and 7.6% by CFT (Woldegebrel, 2011).

Brucellosis infection may occur in animals of all age groups but persists commonly in sexually mature animals (Radostits *et al.*, 1994). Younger animals tend to be more resistant to infection and frequently clear infections although few latent infections may occur (Radostits *et al.*, 1994, Walker, 1999). In this study statistically significant difference was observed between age and seropositivity in camels. The overall prevalence of brucella antibodies in the two age categories was 1.6% and 8% for age category of 6 months to 2 years, and above 2 years, respectively. This is in agreement with Agab (1993) and Yagoub *et al.* (1990) who reported that the seroprevalence of brucellosis was three to four folds higher among adult camels than young ones.

In the present study, there were more serologically positive reactors among the female camels (22/410) than males (3/51); however, the difference was not statistically significant. But, this is in contrast with Agab (1993) and Yagoub *et al.* (1990) who reported that the seroprevalence of brucellosis was two-folds higher in females compared to males. This finding also disagrees with the findings of Zaria *et al.* (1990); Egbe-Nwiyi *et al.* (1999); Kudi *et al.* (1997); Adamu *et al.* (1999) and Junaidu *et al.* (2006) all of whom reported higher sero-prevalences of brucellosis among female camels than males.

In the study area above three-fourth of the interviewed pastoralists own mainly sheep, goats and camel with diverse numbers. In addition the community most of a time keep these species together at browsing, watering, night enclosures and during migration which might create opportunity for the inter species transmission of the disease. In agreement with this idea Mohammed *et al.* (2011) reported that seroprevalence of brucellosis in camels in eastern Ethiopia kept without other ruminants, with small ruminants and large ruminants were 1.03%, 4.3% and 5.3% respectively. In consistence, Andreani *et al.* (1982) reported significant difference in Somalia where high chance of brucella transmission from ruminants to camels as they live in free range proximity in the bush and watering points. In addition, Ismaili *et al.* (1988) of Oman and Radwan *et al.* (1992) of Saudi Arabia have recorded that contact between camels and small ruminants were more incriminated for the transmission of brucellosis to the camels. Moreover, Abou-Eisha (2000) observed high seroprevalence in camels with the history of sheep and goats kept together.



Factors that contribute for this high prevalence rate in camels may be related with the management system of livestock in the study area. According to the questioner survey result 61% (74/120), 25.8% (31/120) and 7.5% (9/120) of the respondents keep camels either with both small ruminant species or either of the two or with cattle. Moreover, mixing of the different species during migration, at watering or night enclosures (resting) between camels and small ruminants was recorded. Consequently, Richard, (1980) described that a contributing factor to the spread of camel brucellosis may be the movement of animals for grazing and watering as aggregating the animals around watering point will increase the contact between infected and healthy animals and there by facilitate the spread of the disease.

The questionnaire survey has provided information regarding the knowledge and practices of pastoral communities about zoonotic diseases in the study area. High number of respondents had no detailed and accurate knowledge about zoonotic brucellosis. This low awareness is a limiting factor if control strategies are to be implemented and it also predispose the community for the disease. Elders are their main source of knowledge. This may indicate paucity in health education rendered to the community. The most important practices potentially supporting transmission of zoonotic diseases in the study area were bare hand management of new born, aborted fetus and fetal membrane and consumption of raw milk. Aborted fetuses, though rarely destroyed, are likely to play a role on livestock and human brucellosis. Moreover, the maintenance within the herd and selling of frequently aborting females to others and use of common rams may serve as a source of disease transmission. Since the prevalence of brucellosis was relatively high, surveillance and prevention may be warranted taking into account possible animal genetic improvement programs, unrestricted animal movement in the area and low awareness of the community about zoonoses, which might result in an increased transmission to humans.

## 6. CONCLUSION AND RECOMMENDATIONS

The seroprevalence described in this study shows that brucellosis is a widespread and well-established infection among goats, sheep and camels across the two zones and all the districts of the study area. The most important risk factors identified for individual animal seroprevalence were sex, age, species, and herd size. Seroprevalence of brucellosis was higher in females, older ages, caprine species and animals with in large herd size. Traditional animal husbandry and management practices are thought to support the spread of brucellosis in the area. Therefore it is highly likely that the disease spreads to the unaffected animals and herds given the extensive production system prevailing in the area which may allow contact of animals during grazing and at watering points. Moreover pastoralists are in close contact with their animal, consumption of raw milk and handling of aborted materials is common. Thus, there is a need to design and implement control measures aiming at preventing further spread of the disease in the region through the use of better management practices.

Based on the above conclusions, the following recommendations are forwarded:

- ❖ The pastoral community in general should be made aware of the zoonotic potential of brucellosis through joint intervention of the veterinary and health extension services.
- ❖ There is a need for further study to critically assess the economic impact of the disease, which emanates from its impact on reproductive and production performance of animals
- ❖ Pastoralists in the study areas should be advised regarding the risks of using rams for breeding without proper precaution measures such as having a good knowledge about the health status of breeding rams.
- ❖ Programs and projects involved in humanitarian assistance and livelihood enhancement in the region should work in collaboration so as to implement test and slaughter during livestock purchase and dispatch especially when they are intended for breeding purpose.



- ❖ Further epidemiological studies and isolation and identification of the biotypes of brucella responsible for infection in the region should be carried out.
- ❖ Further detailed epidemiological studies to investigate the link between livestock and human brucellosis and cross infection between different species in the present study area should be conducted.

## 7. REFERENCES

- Abbas, K. and Lichtman H. (2005): Cellular and Molecular Immunology. 5<sup>th</sup> Edition, Elsevier Saunders, Philadelphia.
- Abdel, A. (2004): A preliminary study on brucellosis on camels at Behira province. Assuit University Bulletin Environmental Research, **7**: 39-43.
- Abou-Eisha, M. (2000): Brucellosis in camels and its relation to public health. *Assuit Vet. Med. J.*, **44**: 54-64.
- Abu-Damir, H., Kenyon, J., khalfalla, A. and Idris, O. (1984): Brucella antibodies in Sudanese camels. *Tro. Anim. Hlth. and Pro.*, **16**: 209-212.
- Acha, N. and Szyfers, B. (2001): Zoonosis and Communicable Diseases Common to Man and Animals. Vol. I. Bacteriosis and Mycosis. 3<sup>rd</sup> Edition, Scientific and Technical Publication. No. 580. Washington, D.C, U.S.A, Pp. 40-62.
- Acha, P. and Szyfres, B. (2003): Zoonoses and Communicable Diseases Common to Man and Animals, 3<sup>rd</sup> edition, vol. 1. Pan American Health Organization (PAHO), Washington, DC.
- Adams, G. and Templeton, W. (1998): Genetic resistance to bacterial diseases of animals. *Rev. Sci. Tech. Off. Int. Epiz.*, **17**: 200-219.
- Adams, G., Craford, P., Ficht, A., Sowa, R., Templeton, W., Williams, D. and Wu, M. (1987): New approaches for vaccinating against bovine brucellosis, 40<sup>th</sup> Ann. Brucellosis Conf. Abstract. Chicago.



- Adamu, B. and Ajogi, I. (1999): Serological investigation of camels (*Camelu dromedaries*) slaughtered at Kano municipal Abattoir for Evidence of Brucellosis. *Trop. Vet.*, **18**: 45-48.
- Afar Finance and Economy Bureau (ARFEB) (2007): Regional atlas of Afar region, Semera.
- Agab, H. (1993): Epidemiology of Camel Diseases in Eastern Sudan with Emphasis on Brucellosis: MVSc Thesis, University of Khartoum, Faculty of Veterinary Science, Pp. 184.
- Ahmad, R. and Nemat, Z. (2007): Brucellosis of camels in Iran, Shahid Bahonar University of Kerman. Iran. Priory Lodge Education, priory.com.
- Ahmed, O., Elmeshri, E., Abuzweda, R., Blauo, M., Abouzeed, M., Ibrahim, A., Salem, H., Alzwam, F., Abid, S., Elfahem, A. and Elrais, A. (2010): Seroprevalence of brucellosis in animals and human populations in the western mountains region in Libya, December 2006–January 2008. *Euro Surveill.*, **15**: 196.
- Ajmal, M., Ahmed, D. and Arshad, M. (1989): Serosurveillance of brucellosis. *Pakistan Vet. J.*, **9**: 115-117.
- Al-Ani, K., Al-Sharrifi, M. and Khalil, F. (1998): Serological survey on camel brucellosis in Iraq. *Camel Newslett.*, **14**: 32-33.
- Alton, G., Jones, M. and Pietz, E. (1975): Laboratory Techniques in Brucellosis, World Health Organisation monograph series No. 454, Geneva.
- Alton, G. (1990): *Brucella melitensis*. In: Nielson, K., Duncan, J. R. (ed.): Animal Brucellosis. CRC Press, Boca Raton, FL, Pp. 383-409.

- Amenu, K., Thys, E., Regassa, A. and Marcotty, T. (2010): Brucellosis and Tuberculosis in Arsi-Negele District, Ethiopia, Prevalence in Ruminants and People's Behaviour towards Zoonoses. *J. Trop.*, **28**: 205-210.
- Andreani, E., Prosperi, S., Salim, H. and Arush, M. (1982): Serological and bacteriological investigation on brucellosis in domestic ruminants of the Somali democratic republic. *Rev. Elev. Med. Vet. Pays. Trop.*, **35**: 329-233.
- Arroyo, C., Lopez Rodriguez, I., Sapina, J., Lopez, M., Lafuente, A. and Sacristan, R. (2006): Probable transmission of brucellosis by breast milk. *J. Trop. Pediatr.*, **52**: 380-381.
- Ashagrie, T., Deneke, Y. and Tolosa, T. (2011): Seroprevalence of Caprine brucellosis and associated risk factors in south Omo zone of Southern Ethiopia. *Afri. J. of Microbio. Res.*, **5**: 1682-1476.
- Ashenafi, F., Teshale, S., Ejeta, G., Fikru, R. and Laikemariam, Y. (2007): Distribution of Brucellosis among small ruminants in the pastoral region of Afar, Eastern Ethiopia. *Rev. sci. tech. off. epiz.*, **26**: 731-739.
- Azwai, M., Carter, D., Woldehiwot, Z. and Macmillan, A. (2001): Camel brucellosis; evaluation of field sera by conventional serological tests and ELISA. *J. of Camel Pra. and Res.*, **8**: 185-193.
- Bale, O., Nuru, S. and Addo, B. (2003): Serological study of sheep and goats Brucellosis in Northern Nigeria: *Bull. Anim. Hlth. Pro. Afr.*, **30**: 73-79.
- Barthel, R., Feng, J., Piedrahita, A., McMurray, M., Templeton, W. and Adams, G. (2001): Stable transfection of the bovine *NRAMP1* gene into murine RAW264.7 cells: Effect on *Brucella abortus* survival. *J. Inf. Immun.*, **69**: 3110-3119.



- Beran, W. (1994): Handbook of Zoonoses, 2<sup>nd</sup> edition, CRC press, Boca Raton.
- Bertu, J., Ajogi, I., Bale, O., Kwaga, P., and Ocholi, A. (2010): Sero-epidemiology of brucellosis in small ruminants in Plateau State, Nigeria. *Afri. J. of Microbiol. Res.*, **4**: 1935-1938.
- Blasco, M. (1990): *Brucella ovis*. In: Nielson, K., Duncan, J. R. (ed.): Animal Brucellosis. CRC Press, Boca Raton, Florida, Pp. 351-378.
- Blasco, M. (1997): A review of the use of *B. melitensis* Rev 1 vaccine in adult sheep and goats. *Prev. Vet. Me.*, **31**: 275-283.
- Blasco, M. (2006): Existing and future vaccines against brucellosis in small ruminants. *Small Rum. Res.*, **62**: 33-37.
- Blasco, M. and Molina-Flores, B. (2011): Control and Eradication of Brucellosis. Veterinary Clinics of North America. *J. Food Anim. Med.*, **27**: 95-104.
- Braun, W. (1947): Bacterial dissociation. *Bacteriol. Rev.*, **11**: 75.
- Braun, W., Pomles-Lebron, A. and Stinebring, W. R. (1958): Interactions between mononuclear phagocytes and *Brucella abortus* strains of different virulence. *Proc. Soc. Exp. Biol. Med.*, **97**: 393.
- Brew, D., Perrett, L., Stack, A., MacMillan, P. and Staunton, J. (1999): Human exposure to *Brucella* recovered from a sea mammal. *Vet. Rec.*, **144**: 483.
- Bricker, J., Ewalt, R., Macmillan, P., Foster, G. and Brew, S. (2000): Molecular Characterization of *Brucella* Strains Isolated from Marine Mammals. *J. Clin. Microbiol.*, **38**: 1258-1262.

- Bundle, R., Cherwonogrodzky, W., Caroff, M. and Perry, B. (1987): The lipopolysaccharides of *Brucella abortus* and *B. melitensis*. *Ann. Inst. Pasteur/Microbiol.*, **138**: 92-98.
- Cadmus, B., Ijagbone, F., Oputa, E., Adesokan K. and Stack, A. (2006): Serological survey of brucellosis in livestock animals and workers in Ibadan, Nigeria. *Afric. J. Biomed. Res.*, **9**: 163-168.
- Cameron, S., Hughes, H. and Gregory, W. (1942): Genetic resistance to brucellosis in swine. *J. of Anim. Sci.*, **1**: 106-110.
- Caroff, M., Bundle, R., Perry, B., Cherwonogrodzky, W. and Duncan, R. (1984): Antigenic S-type lipopolysaccharide of *Brucella abortus*. *Infect. Immun.*, **46**: 384-388.
- Celli, J. (2006): Surviving inside a macrophage, the many ways of *Brucella*. *Research in Microbiol.*, **157**: 93-8.
- Central statistical agency (CSA) (2010/11): Agricultural sample survey Report on Livestock and livestock characteristics statistical bulletin 505 Volume 2, Addis Ababa.
- Chain, S., Comerci, J., Tolmasky, E., Larimer, W., Malfatti, A., Vergez, M., Agüero, F., Land, L., Ugalde, A. and Garcia, E. (2005): Whole-genome analyses of speciation events in pathogenic *Brucellae*. *Infect. Immun.*, **73**: 8353-8361.
- Cheers, C. (1984): Pathogenesis and cellular immunity in experimental murine brucellosis. *Developm. in Biolog. Standardiz.*, **56**: 237-46.
- Cherwonogrodsky, W., Perry, B. and Bundle, R. (1987): Identification of the A and M antigens of *Brucella* as the O-polysaccharide of smooth lipopolysaccharides. *Can. J. Microbiol.*, **33**: 979-981.



- Chukwu, C. (1987): Brucellosis in Africa. Part I. The prevalence. *Bull. Anim. Hlth. Prod. Afr.*, **33**: 193-198.
- Cook, E., Williams, S., Thorne, T., Kreeger, J., Stout, G., Bardsley, K., Edwards, H., Schurig, G., Colby, A., Enright, F. and Elzer, H. (2002): *Brucella abortus* strain RB51 vaccination in elk. I. Efficacy of reduced dosage. *J. Wildl. Dis.*, **38**: 18-26.
- Cooper, W. (1991): The epidimilogy of human brucellosis in a well defined urban population in Saudi Arabia. *J. Trop. Med. Hyg.*, **94**: 416-422.
- Corbel, M. (2006): Brucellosis in humans and animals. World Health Organization in collaboration with the Food and Agriculture Organization of the United Nations and the World Organization for Animal Health.
- Corbel, J. (1972): Characterization of antibodies active in the Rose Bengal Plate Test for bovine brucellosis. *Vet. Rec.*, **88**: 447-449.
- Corbel, J. (1988): Brucellosis. In: Fertility and Infertility in Veterinary Practice. Laing, A. (ed.): 4<sup>th</sup> edition, ELBS, Bailliere Tindall, Pp. 189-221.
- Corbel, J. (1990): *Brucella*. In: Parker, T. and Duerden, I. (ed.): Topley and Wilson's Principles of Bacteriology, Virology and Immunology. 8<sup>th</sup> edition, Vol. 2 London: Edward Arnold, Pp. 341-351.
- Corbel, J. (1997): Recent advances in brucellosis. *J. Med. Microbiol.*, **46**: 101-103.
- Crawford, P., Huber, D. and Adams, S. (1990): Epidemiology and Surveillance. In: Nielson, K., Duncan, R. (ed.): Animal Brucellosis. CRC Press, Boca Raton, Florida, Pp. 131-152.

- Dahouk, A., Neubauer, H., Hensel, A., Schoneberg, I., Nockler, K., Alpers, K., Merzenich, H., Stark, K. and Jansen, A. (2007): Changing epidemiology of human brucellosis, Germany, 1962-2005. *Emerg. Infect. Dis.*, **13**: 1895-1900.
- De Massis, F., Di Girolamo, A., Petrini, A., Pizzigallo, E. and Giovannini, A. (2005): Correlation between animal and human brucellosis in Italy during the period 1997-2002. *Clin. Microbiol. Infect.*, **11**: 632-636.
- Del Vecchio, G., Kapatral, V., Redkar, J., Patra, G., Mujer, C., Los, T., Ivanova, N., Anderson, I., Bhattacharyya, A., Lykidis, A., Reznik, G., Jablonski, L., Larsen, N., D'Souza, Bernal, A., Mazur, M., Goltsman, E., Selkov, E., Elzer, H., Hagius, S., O'Callaghan, D., Letesson, J., haselkorn, R., Kypides, N. and Overbeek, R. (2002a): The genome sequence of the facultative intracellular pathogen *Brucella melitensis*. *Proc. Natl. Acad. Sci.*, **99**: 443-448.
- Domenech, J. (1977): Brucellose de dromadaire en Ethiopie. *Revue de` Elevage et Medicine Veterinaire des Pays Tropicaux*, **30**: 141-142.
- Dornand, J., Gross, A., Lafont, V., Liautard, J., Oliaro, J. and Liautard, P. (2002): The innate immune response against *Brucella* in humans. *Vet. Microbiol.*, **90**: 383-394.
- Douglas, T., Rosenberg, Y., Nikaido, D., Verstrete, R. and Winter, J. (1984): Porins of *Brucella* species. *Infect. Immn.*, **44**: 37.
- Egbe-Nwiyi, N., Ameh, A., Zaria, T. and Yita, B. (1999): Sero-prevalence of camel (*Camelus dromedarius*) Brucellosis in semi Arid zones of North of North-Eastern Nigeria. *Trop. Veterinari.*, **17**: 119-123.
- El-Ansary, H., Mohammed, A., Hamad, R., and Karom, G. (2001): Brucellosis among animals and human contacts in Eastern Sudan. *Saudi. Med. J.*, **22**: 577-579.



- El-Boshy, M., Abbas, H., El-Khodery, S. and Osman, S. (2009): Cytokine response and clinicopathological findings in *Brucella* infected camels (*Camelus dromedarius*). *J. Vet. Medi.*, **54**: 25-32.
- Enright, M. (1990): The Pathogenesis and Pathobiology of *Brucella* Infection in Domestic Animals. From: *Animal Brucellosis*, CRC Press, Boca Raton, Pp. 301-320.
- Eze, O., Yuan, L., Crawford, M., Paronavitana, M., Hadfield, L., Bhattacharjee, K., Warren, L., and Hoover, L. (2000): Effects of opsonization and gamma interferon on growth of *Brucella melitensis* 16M in mouse peritoneal macrophages in vitro. *Inf. and Immun.*, **68**: 257-263.
- Franco, P., Mulder, M., Gilman, H. and Smits, L. (2007): Human brucellosis. *The Lancet of Inf. Dis.*, **7**: 775-786.
- Frenchick, J., Markaham, F. and Cochrane, H. (1985): Inhibition of phagosome-lysosome fusion in macrophages by soluble extract of virulent *Brucella abortus*. *J. Vet. Res.*, **46**: 332-35.
- Frost, J., Smith, H., Witt, K. and Keppie, J. (1972): The chemical basis of the virulence of *Brucella abortus*. A surface virulence factor which facilitates intracellular growth of *Brucella abortus* in bovine phagocytes. *Br. J. Exp. Pathol.*, **53**: 587.
- Galanos, D., Luderitz, O., Rietschel, T. and Westphal, O. (1972): Biological activities of lipid A complexed with bovine serum albumin. *Eur. J. Biochem.*, **31**: 230.
- Gall, D. and Nielsen, K. (2004): Serological diagnosis of bovine brucellosis: a review of test performance and cost comparison. *Rev. sci. tech. off. int. Epiz.*, **23**: 989-1002.
- Gameel, E., Mohamed, O., Mustafa, A., and Azwai, M. (1993): Prevalence of camel brucellosis in Libya. *Trop. Anim. Hlth. Prod.*, **25**: 91-93.

- Garin-Bastuji, B., Blasco, M., Grayon, M. and Verger, M. (1998): *Brucella melitensis* infection in sheep, present and future. *Vet. Res.*, **29**: 255-274.
- Ghanem, M., El-Khodery, A., Saad, A., Abdelkadir, H., Heybe, A. and Musse, A. (2009): Seroprevalence of camel brucellosis (*Camelis dromedarius*) in Somaliland. *Trop. Anim. Hlth Prod.*, **41**: 1779-1786.
- Giambartolomei, H., Zwerdling, A., Cassataro, J., Bruno, L., Fossati, A., and Philipp, T. (2004): Lipoproteins, not lipopolysaccharide, are the key mediators of the proinflammatory response elicited by heat-killed *Brucella abortus*. *J. Immunol.*, **173**: 4635-4642.
- González, D, Grilló, J., De Miguel, J., Ali, T., Arce-Gorvel, V., Delrue, M., Conde-Alvarez, R., Muñoz, P., López-Goñi, I., Iriarte, M., Marín, M., Weintraub, A., Widmalm, G., Zygmunt, M., Letesson, J., Gorvel, P., Blasco, M. and Moriyón, I. (2008): Brucellosis vaccines; assessment of *Brucella melitensis* lipopolysaccharide rough mutants defective in core and O-polysaccharide synthesis and export. *Pub. Lib. Sci.*, **3**: 2760.
- Goodlow, J., Tucker, L., Braun, W. and Mika, A. (1952): Effect of the isomeric configuration of the source of nitrogen on changes in population and metabolism in culture of *Brucella*. *J. Bacteriol.*, **63**: 681.
- Hall, H. (1990): Modern Chemotherapy for Brucellosis in Humans. *Revie. of Inf. Dis.*, **12**: 1060-1099.
- Halling, M., Brooke, D., Peterson-Burch, B., Bricker, J., Richard, L., Qing, Z., Li, L., Kapur, V., David, P. and Olsen, C. (2005): Completion of the genome sequence of *Brucella abortus* and comparison to the highly similar genomes of *Brucella melitensis* and *Brucella suis*. *J. Bacteriol.*, **187**: 2715-2726.



- Harmon, G., Adams, G. and Frey, M. (1988): Survival of rough and smooth strains of *Brucella abortus* in bovine mammary gland macrophages. *American J. Vet. Res.*, **49**: 1092-1097.
- Henry, S. (1933): Dissociation in the genus *Brucella*. *J. Infect. Dis.*, **52**: 374.
- Hirsh, C. and Zee, C. (1999): Veterinary microbiology. Blackwell Science, Cambridge, Massachusetts, Pp.196-203.
- Huang, Y., Aliberti, J., Leifer, A., Segal, M., Sher, A., Golenbock, T. and Golding, B. (2003): Heat-killed *Brucella abortus* induces TNF and IL-12p40 by distinct MyD88-dependent pathways: TNF, unlike IL-12, Pp. 40.
- Huddleson, F. and Stahl, H. (1943): Catalase activity of the species of *Brucella* as a criterion of virulence, in studies of Brucellosis. *Agric. Exp. Stn. Tech. Bull.*, **182**: 57.
- Isibasi, A., Ortiz, V., Vargas, M., Paniagua, J., Gonzalez, C., Moreno, J. and Kumate, J. (1988): Protection against *Salmonella typhi* infection in mice after immunization with outer membrane proteins isolated from *Salmonella typhi*. *Infect. Immun.*, **56**: 2953.
- Ismaily, N., Harby, M. and Nicoletti, P. (1988): Prevalence of brucella antibodies in four animal species in the Sultanate of Oman. *Trop. Anim. Health Prod.*, **20**: 269-270.
- Jiang, X. and Baldwin, L. (1993): Effects of cytokines on intracellular growth of *Brucella abortus*. *J. Infect. Immun.*, **61**: 124-134.
- Jones, M. and Winter, J. (1992): Survival of virulent and attenuated strain of *Brucella abortus* in normal and gamma interferon-activated murine peritoneal macrophages. *Infect. and Immun.*, **60**: 3011-3014.

- Junaidu, U., Oboegbulem, I., Sharubutu, H. and Daneji, I. (2006): Brucellosis in camels (*Camelus dromedaries*) slaughtered in Sokoto, northwestern Nigeria. *Anim. Prod. Res. Adv.*, **2**: 11-13.
- Kadohira, M., McDermott, J., Shoukri, M. and Kyule, N. (1997): Variation in the prevalence of antibodies to *Brucella* infection in cattle by farm, area, and district in Kenya. *Epidemiol. Infect.*, **118**: 35-41.
- Kaoud, A., Zaki, M., El-Dahshan, R. and Nasr, A. (2010): Epidemiology of Brucellosis among Farm Animals. *Natu. and Sci.*, **8**: 190-197.
- Kato, Y., Masuda, G., Itoda, I., Imamura, A., Ajisawa, A. and Negishi, M. (2007): Brucellosis in a returned traveler and his wife; probable person-to person transmission of *Brucella melitensis*. *J. Travel Med.*, **14**: 343-345.
- Kattar, M., Zalloua, A., Araj, F., Samaha-Kfoury, J., Shbaklo, H., Kanj, S., Khalife, S. and Deeb, M. (2007): Development and evaluation of real-time polymerase chain reaction assays on whole blood and paraffin-embedded tissues for rapid diagnosis of human brucellosis. *Dia. Microbiol. of Infect. Dis.*, **59**: 23-32.
- Khan, Y., Mah, W. and Memish, A. (2001): Brucellosis in pregnant women. *Clin. Infect. Dis.*, **32**: 1172-1177.
- Kreutzer, L. and Robertson, C. (1979): Surface macromolecules and virulence in intracellular parasitism: comparison of cell envelop components of smooth and rough strains of *Brucella abortus*. *Infect. Immun.*, **23**: 819-828.
- Kudi, C., Kalla, U., Kudi, C. and Kapio, I. (1997): Brucellosis in camels. *J. Arid Environ.*, **37**: 413-417.



- Lory, S. and Tai, C. (1984): Biochemical and genetic aspects of *Pseudomonas aeruginosa* virulence. *Curr. Top. Microbiol. Immunol.*, **118**: 53.
- Mangen, M., Otte, M., Pfeiffer J. and Chilonda, P. (2002): Bovine brucellosis in Sub-Saharan Africa: Estimation of seroprevalence and impact on meat and milk off take potential. Food and Agriculture Organization Livestock Information and Policy Branch, AGAL, December.
- Mantur, G. and Amarnath, K. (2008): Brucellosis in India, a review. *J. Biosci.*, **33**: 539-547.
- Mantur, G., Amarnath, K. and Shinde, S. (2007): Review of clinical and laboratory features of human brucellosis. *Indian J. Med. Microbiol.*, **25**: 188-202.
- Mariana, X., Érica, C., Tatiane, P. and Renato, S. (2009): The genus *Brucella* and clinical manifestations of brucellosis. *Ciência Rural, Santa Maria*, **39**: 2252-2260.
- McCullough, B. (1970): Microbial and host factors in the pathogenesis of brucellosis. In: *Infectious Agents and Host Reactions*, Mudd, S., (ed.): W. B. Saunders, Philadelphia, Pp. 324.
- McDermott, J. and Arimi, S. (2002): Brucellosis in sub-Saharan Africa: epidemiology, control and impact. *Vet. Microbiol.*, **90**: 111-134.
- McDermott, J., Randolph, F., and Staal, J. (1999): The economics of optimal health and productivity in smallholder livestock systems in developing countries. *Rev. Sci. Tech. Off. int. Epiz.*, **18**: 399-424.
- McDonald, L., Jamaludin, R., Mackereth, G., Hansen, M., Humphrey, S., Short, P., Taylor, T., Swinger, J., Dawson, E., Whatmore, M., Stubberfield, E., Perrett, L. and Simmons, G. (2006): Characterization of a *Brucella* species as a marine-mammal type despite isolation from a patient with spinal osteomyelitis in New Zealand. *J. Clin. Microbiol.*, **44**: 4363-70.

- Memish, Z. (2001): Brucellosis control in Saudi Arabia; prospects and challenges. *J. Chemother.*, **13**: 11-17.
- Mert, A., Ozaras, R., Tabak, F., Bilir, M., Yilmaz, M. and Kurt, C. (2003): The sensitivity and specificity of Brucella agglutination tests. *Diag. Microbiol. Infect. Dis.*, **46**: 241-243.
- Ministry of Agriculture and Rural Development (2008): National Guidelines for Livestock Relief Interventions in Pastoralist Areas of Ethiopia. 1<sup>st</sup> edition, Addis Ababa, Ethiopia.
- Mochman, H. and Köhler, W. (1988): 100 years of bacteriology; the history of the discovery of brucellosis. Discovery of the etiology of infectious abortion in cattle by the Danish veterinary pathologist Bernhard Bang and proof of the relation of "*Micrococcus melitensis*" and "*Bacillus abortus*" by the American microbiologist Alice Evans. *Zeits für ärztl. Fort.*, **82**: 381-5.
- Mohammed, O., Megersa, B., Abebe, R., Abera, M., Regassa, A., Abdrehman, Y. and Solomon, M. (2011): Seroprevalence of brucellosis in camels in and around Dire Dawa City, Eastern Ethiopia. *J. Anim. and Vet. Adva.*, **10**: 1177-1183.
- Montaraz, A., Winter, J., Hunter, M., Sowa, A. and Adams, G. (1986): Protection against *Brucella abortus* in mice with O-polysaccharide-specific monoclonal antibodies. *Infect. Immun.*, **51**: 961.
- Moreno, E. (2002): *Brucella* evolution and taxonomy. *Vet. Microbiol.*, **90**: 209-227.
- Moreno, E. and Moriyon, I. (2002): *Brucella melitensis*; A nasty bug with hidden credentials for virulence. *Proc. Natl. Acad. Sci.*, **99**: 1-3.



- Moreno, E., Pitt, W., Jones, G., Schurig, G. and Berman, T. (1979): Purification and characterization of smooth and rough lipopolysaccharides from *Brucella abortus*. *J. Bacteriol.*, **138**: 361.
- Moustafa, T., Omer, E., Basyouni, S. and EL-Badawi, A. (1998): Surveillance of *Brucella* antibodies in camels of the eastern region of Abu Dhabi, United Arab Emirates. In: Proceedings of the third annual meeting for animal production under arid conditions, United Arab Emirates University, **1**: 160-166.
- Muleta, W. (2011): Seroprevalence study of small ruminant brucellosis and its effect on reproduction in Telalak district of Afar region. MSc Thesis, Addis Ababa University, School of Veterinary medicine, Debre-ziet, Ethiopia.
- Musa, T., Eisa, Z., El Sanousi, M., Abdel Wahab, B. and Perrett, L. (2008): Brucellosis in camels (*Camelus dromedarius*) in Darfur, Western Sudan. *J. Comp. Pathol.*, **138**: 151-155.
- Nicoletti, P. (1980): The Epidemiology of bovine brucellosis. *Vet. Sci. Cop. Med.*, **24**: 69-98.
- Nicoletti, P. (1990): Vaccination. In: Nielson, K. and Duncan, R. (ed.): Animal Brucellosis. CRC Press, Boca Raton, Florida, Pp. 283-300.
- Nielsen, K., Heck, F., Wagner, G., Stiller, J., Rosenbaum, B., Pugh, R. and Flores, E. (1984): Comparative assessment of antibody isotypes to *Brucella abortus* by primary and secondary binding assays. *Prev. Vet. Med.*, **2**: 197-204.
- Nielson, K. (2002): Diagnosis of brucellosis by serology. *Vet. Microbiol.*, **90**: 447-459.
- Nielson, K. and Dunkan, R. (1990): Animal brucellosis: In Bovine brucellosis. Manual of standards for diagnostic tests and vaccines, 3<sup>rd</sup> edition. CRC. Pres Inc., Florida, USA, Pp. 252-265.

- Nielson, K., Smith, P., Yu, L., Elmgren, C., Halbert, G., Nicoletti, P., Perez, B., Conde, S., Samartino, L., Nicola, A., Bermudez, R. and Renteria, T. (2008): Validation of a second generation competitive enzyme immunoassay (cELISA) for the diagnosis of brucellosis in various species of domestic animals. *Vet. Immunol and Immunoathlol.*, **125**: 246-250.
- Office International des epizootics (OIE) (2004): Bovine brucellosis. In: Manual of Standards for Diagnostic tests and Vaccine. 5<sup>th</sup> edition. OIE, Paris, Pp. 246-262.
- Ojo, E., Oyekunle, A., Omotainse, O., Ocholi, A., Ogunleye, O. and Bertu, J. (2007): Serological evidence of Brucellosis in a goat flock with recurrent abortion in Abeokuta. *Nigeria. Trop. Vet.*, **25**: 26-33.
- Okewole, A., Eze, N., Okoh, J., Oyetunde, L. and Odeyemi, S. (1988): Small ruminant brucellosis in some parts of Northern Nigeria. *Bull. Anim. Health. Prod. Afr.*, **36**: 251-254.
- Okoh, J. (1980): An investigation of abortion in sheep on Kano LIBC near Kano, Nigeria. *Bull. Anim. Hlth. Prod. Afr.*, **28**: 135-136.
- Oliveira, C. and Splitter, G. (1995): CD8<sup>+</sup> type 1 CD44hi CD45 RBlo T lymphocytes control intracellular *Brucella abortus* infection as demonstrated in major histocompatibility complex class I and class II-deficient mice. *Eur. J. Immunol.*, **25**: 2551-2557.
- Olsen, C. and Stoffregen, S. (2005): Essential role of vaccines in brucellosis control and eradication programs for livestock. *Exp. Rev. Vacc.*, **4**: 915-928.
- Omer, K., Skjerve, E., Holstad, G., Woldehiwet, Z. and MacMillan, P. (2000): Prevalence of antibodies to *Brucella* spp. in cattle, sheep, goats, horses and camels in the State of Eritrea; influence of husbandry systems. *Epidemiol. Infect.*, **125**: 447-53.



- Omer, M., Musa, T., Bakhiet, R. and Perrett, L. (2010): Brucellosis in camels, cattle and humans: associations and evaluation of serological tests used for diagnosis of the disease in certain nomadic localities in Sudan. *Rev. sci. tech. Off. int. Epiz.*, **29**: 663-669.
- Orduña, A., Almaraz, A., Prado, A., Gutierrez, P., Garcia-Pascual, A., Dueñas, A., Cuervo, M., Abad, R., Hernández, B., Lorenzo, B., Bratos, A. and Torres, R. (2000): Evaluation of an immunocapture-agglutination test (Brucellacapt) for serodiagnosis of human brucellosis. *J. Clin. Microbiol.*, **38**: 4000-5.
- Pappas, G., Akritidis, N. and Tsianos, E. (2005): Effective treatments in the management of brucellosis. *Exp. Ops. Pharma.*, **6**: 201-9.
- Pappas, G., Christou, L., Akritidis, N. and Tsianos, V. (2006b): Quinolones for brucellosis; treating old diseases with new drugs. *Clin. Microbio.. Infect.*, **12**: 823-5.
- Pappas, G., Panagopoulou, P., Christou, L. and Akritidis, N., (2006a): Brucella as a biological weapon. *Cell Mol. Life Sci.*, **63**: 2229-2236.
- Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L. and Tsianos, V. (2006c): The new global map of human brucellosis. *Lancet. Infect.*, **6**: 91-99.
- Parascandola, J. (1998): An early woman scientist at NIH. *Public Health Rep.*, **113**: 472-4.
- Park, W., Kim, M., Park, Y., Kim, L., Jang, J., Choi, S., Park, Y., Song, J. and Lee, H. (2007): Fatal systemic infection with multifocal liver and lung nodules caused by *Brucella abortus*. *Am. J. Trop. Medi. Hygi.*, **77**: 1120-3.
- Peery, M. and Belter, F. (1960): Brucellosis and heart disease. II. Fatal brucellosis; a review of the literature and report of new cases. *Am. J. Pathol.*, **36**: 673-697.

- Perry, B., Bundle, R. and Cherwonogrodsky, W. (1986): The structure and serology of the A and M antigens of *Brucella abortus* and *Brucella melitensis* and the structure of the polysaccharide B of *Brucella* species. XIV International Congress of Microbiology. Manchester, England, Pp.7-4.
- Quinn, J., Markey, K., Carte, E., Donnelly, J. and Leonard, C. (2002): *Brucella* species. In: Veterinary Microbiology and Microbial Diseases. London, Blackwell Science Ltd., Pp. 168-172.
- Radostits, M., Blood, C. and Gay, C. (1994): Brucellosis caused by *B. abortus* and *B. melitensis*. In: Veterinary Medicine; Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 8<sup>th</sup> edition. London, Bailliere Tindall, Radwa, Pp. 787-792.
- Radostits, M., Gay, C., Hinchcliff, W. and Constable, D. (2007): Diseases associated with *Brucella* species. Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats. Elsevier Limited.
- Radwan, I., Bekairi, I. and Prasad, V. (1992): Serological and bacteriological study of brucellosis in camels in central Saudi Arabia. *Rev. Sci. Tech.*, **11**: 837-844.
- Refai, M. (2000): Control of brucellosis in animals in Egypt. In: Proc. 2<sup>nd</sup> Intern. Symp. cum-Workshop of the Germany-Egypt-Region Inter-Alumni-Net (GEAR), St. Catherine, Germany.
- Reguera, M., Alarcon, A., Miralles, F., Pachon, J., Juarez, C. and Colmenero, D. (2003): *Brucella* endocarditis: clinical, diagnostic, and therapeutic approach. *Eur. J. Clin. Microbiol. Infect. Dis.*, **22**: 647-650.
- Rice, C. and Boyes, B. (1971): Serum immunoglobulins in bovine brucellosis. *New Zealand Vet. J.*, **19**: 146-154.



- Richard, K. (1980): A seroprevalence study of camel Brucellosis in camel rearing areas of Ethiopia. *Trop. Anim. Hlth and Prod.*, **35**: 381-389.
- Riley, K. and Robertson, C. (1984): Ingestion and intracellular survival of *Brucella abortus* in human and bovine polymorphonuclear leukocytes. *Infect. Immun.*, **46**: 224-230.
- Sadiq, A., Ajogi, I., Bale, O., Mosimabale, B., Tijjani, N. and Kaikabo, A. (2011): Serological Survey of Antibodies against Brucella Organisms in One Humped Camel (*Camelus dromedarius*) Herds in the Lake Chad Area of Borno State, North Eastern Nigeria. *Nigerian Vet. J.*, **32**: 45-48.
- Sanmartino, L. (2005): Brucellosis Vaccines: The 58<sup>th</sup> Brucellosis Conference. Meridia, Yucatan, Mexico, Pp. 31-41.
- Santos, M., Verstrete, R., Perera, Y. and Winter, J. (1984): Outer membrane proteins from rough strains of four *Brucella* species. *Infect. Immun.*, **34**: 1000.
- Schelling, E., Diguimbaye, C., Nicolet, J., Boerlin, P., Tanner, M. and Zinsstag, J. (2003): Brucellosis and Q-fever seroprevalences of nomadic pastoralists and their livestock in Chad. *Pre. Vet. Med.*, **61**: 279-293.
- Schlabritz-Loutsevitch, E., Whatmore, M., Quance, R., Koylass, S., Cummins, B., Dick, J., Snider, L., Cappelli, D., Ebersole, L., Nathanielsz, W. and Hubbard, B. (2009): A novel *Brucella* isolate in association with two cases of stillbirth in non-human primates; first report. *J. Med. Primatol.*, **38**: 70-73.
- Scholz, C., Hubalek, Z., Sedlacek, I., Vergnaud, G., Tomaso, H., Al Dahouk, S., Melzer, F., Kampfer, P., Neubauer, H. and Cloeckert, A. (2008a): *Brucella microti* sp. nov., isolated from the common vole *Microtus arvalis*. *Inte. J. Syst. and Evolut. Microbio.*, **58**: 375-382.

- Schurig, G., Sriranganathan, N. and Corbel, J. (2002): Brucellosis vaccines: past, present and future. *Vet. Microbiol.*, **90**: 479-96.
- Schurig, G., Roop, M., Bagchi, T., Boyle, S., Buhrman, D. and Sriranganathan, N. (1991): Biological properties of RB51, a stable rough strain of *Brucella abortus*. *Vet. Microbiol.*, **28**: 171-88.
- Schwabe, W. (1984): Human Health costs of Animal Diseases. In: Veterinary Medicine and Human Health, Williams and Wilkins, 3<sup>rd</sup> edition. Pp. 33-35.
- Seifert, H. (1996): Brucellosis: In Tropical Animal Health, 2<sup>nd</sup> edition, Dordrecht, Kluwer Academic Publisher group, London, Pp. 356-368.
- Seleem, N., Boyle, M. and Sriranganathan, N. (2009): *Brucella*; a Reemerging Zoonosis. *Vet. Microbiol.* Epublished before print: [www.elsevier.com/locate/vetmic](http://www.elsevier.com/locate/vetmic)
- Sharma, D., Sethi, S., Yadav, P. and Dube, C. (1979): Sero-epidemiologic investigations on brucellosis in the states of Uttar Pradesh and Delhi (India). *Int. J. Zoonoses*, **6**: 75-81.
- Shehu, M., Yusuf, H., Kudi, C. and Kalla, U. (1999): Seroprevalence of Brucellosis in ruminants in Bauchi and Environs. *Nig. Vet. J.*, **20**: 67-74.
- Shurig, G., Sriranganathan, N. and Corbel, M. (2002): Brucellosis vaccines: past, present and future. *Vet. Microbiol.*, **90**: 479-496.
- Sohn, H., Probert, S., Glaser, A., Gupta, N., Bollen, W., Wong, D., Grace, M. and McDonald, C. (2003): Human neurobrucellosis with intracerebral granuloma caused by a marine mammal *Brucella* spp. *Emerg. Infect. Dis.*, **9**: 485-488.



- Solera, J., Espinosa, A., Martínez-Alfaro, E., Sánchez, L., Geijo, P., Navarro, E., Escribano, J. and Fernández, A. (1997): Treatment of human brucellosis with doxycycline and gentamicin. *Antimicrob. Ag. Chemo.*, **41**: 80-4.
- Spink, W. W. (1956): *The Nature of Brucellosis*. Minnesota Press, Minneapolis.
- Tabak, F., Hakko, E., Mete, B., Ozaras, R., Mert, A. and Ozturk, R. (2008): Is family screening necessary in brucellosis? *Infect.*, **36**: 575-577.
- Templeton, W. and Adams, G. (1990): Natural resistance to bovine brucellosis, In: Garry Adams L. (ed.): *Advances in brucellosis research*. Texas A & M University Press, College Station, Texas, Pp. 144-150.
- Teshale, S., Muhie, Y., Dagne, A. and Kidanemariam, A. (2006): Seroprevalence of small ruminant brucellosis in selected districts of Afar and Somali pastoral areas of Eastern Ethiopia and the impact of husbandry practice. *Revue Méd. Vét.*, **157**: 557-563.
- Teshome, H., Molla, B. and Tibbo, M. (2003): A seroprevalence study of camel brucellosis in three camel rearing regions of Ethiopia. *Trop. Anim. Heal. Prod.*, **35**: 381-389.
- The Merck Veterinary Manual (2007): Merck and Company, 7<sup>th</sup> edition, Merck Publishing, Whitehouse Station, NJ, USA.
- Thrusfield, M. (2005): *Veterinary Epidemiology*. 3<sup>rd</sup> edition, Blackwell Science Ltd., London, Pp. 232-242.
- Tizard, R. (2004): *Veterinary Immunology: An introduction*, 7<sup>th</sup> edition, Elsevier, USA, Philadelphia.

- Tsolis, M. (2002): Comparative genome analysis of the alpha-proteobacteria; relationships between plant and animal pathogens and host specificity. *Proceedings of the National Academy of Sciences of the United States of America*, Pp. 12503-12505.
- Vemulapalli, R., He, Y., Sriranganathan, N., Boyle, M. and Shurig, G. (2002): *Brucella abortus* RB51; enhancing vaccine efficacy and developing multivalent vaccines. *Vet. Microbiol.*, **90**: 521-532.
- Verger, M., Grayon, M., Tibor, A., Wansard, V., Letesson, J. and Cloeckeaert, A. (1998): Differentiation of *Brucella melitensis*, *B. ovis* and *B. suis* biovar 2 strains by use of membrane protein or cytoplasmic protein-specific gene probes. *Res. Microbi.*, **149**: 509-517.
- Verger, M., Grimont, F., Grimont, D. and Grayon, M. (1985): *Brucella*, a monospecific genus as shown by deoxyribonucleic acid hybridization. *Int. J. Syst. Bactiol.*, **35**: 292-295.
- Verstrete, R., Creasy, T., Caveney, T., Baldwin, L., Blau, W. and Winter, J. (1982): Outer membrane proteins of *Brucella abortus*: isolation and characterization. *Infect. Immun.*, **56**: 2953.
- Vizcaino, N., Cloeckeaert, A., Verger, M., Grayon, M. and Fernandez-Lago, L. (2000): DNA polymorphism in the genus *Brucella*. *Microbes Infect.*, **2**: 1089-1100.
- Waghela, S., Fazil, A., Gathuma, M. and Kagunya, K. (1978): A serological survey of Brucellosis in camels, north-eastern province of Kenya. *Trop. Anim. Hlth Prod.*, **10**: 28-29.
- Walker, R. (1999): *Brucella*. In: *Veterinary Microbiology*. Dwight, C., Hirsh, A. and Yuan, Z. (ed.): Blackwell Science, Pp. 196-202.



- Wallach, C., Samartino, E., Efron, A. and Baldi, C. (1997): Human infection by *Brucella melitensis*; an outbreak attributed to contact with infected goats. *FEMS Immunol. Med. Microbiol.*, **19**: 315-321.
- Wang, I. and Frash, E. (1984): Development of a *Neisseria meningitidis* group B serotype 2b protein vaccine and evaluation in a mouse model. *Infect. Immun.*, **46**: 408.
- Wattam, R. (2009): Analysis of ten *Brucella* genomes reveal evidence for horizontal gene transfer despite a preferred intracellular lifestyle. *J. Bacterio.*, in press. doi:10.1128/JB.01767-08.
- WHO (1997): The Development of New, Improved Brucellosis Vaccines: Report of the WHO Meeting. Geneva: WHO.
- WHO (2006): The Control of Neglected Zoonotic Diseases. A route to poverty alleviation. World Health Organization, Food and Agriculture Organization and World Organization for Animal Health, Geneva, Switzerland, Pp. 3.
- Winter, J., Howe, E., Duncan, R., Eis, J., Widom, J., Ganem, B. and Morein, B. (1988): Effectiveness of natural and synthetic complexes of porin and O polysaccharide as vaccines against *Brucella abortus* in mice. *Infect. Immun.*, **56**: 3251.
- Woldegebrel, S. (2011): Prevalence and risk factors of camel and human brucellosis in south Afar region, northeast Ethiopia. MSc Thesis, Addis Ababa University, School of Veterinary medicine, Debre-ziet, Ethiopia.
- World Organisation for Animal Health (OIE) (2004): Bovine brucellosis, Section 2.3. In OIE Manual of standards for diagnostic tests and vaccines, 5<sup>th</sup> edition. OIE, Paris.

- World Organization for Animal Health (OIE) (2008a): Bovine brucellosis diagnostics. World Organization for Animal Health Terrestrial Manual, Pp. 624-659.
- World Organization for Animal Health (OIE) (2008b): Caprine and Ovine Brucellosis (excluding *Brucella ovis*). World Organization for Animal Health Terrestrial Manual. World Organization for Animal Health, Paris, France, Pp. 978-982.
- World Organization for Animal Health Organization (OIE) (2008c): List of Tests for International Trade. World Organization for Animal Health Terrestrial Manual. World Organization for Animal Health, Paris, France.
- Wyllie, S., Seu, P. and Goss, A. (2002): The natural resistance-associated macrophage protein 1 Slc11a1 (formerly Nramp1) and iron metabolism in macrophages. *Microbes and Infection*, **4**: 351-359.
- Yagoub, A., Mohamed, A. and Salim, O. (1990): Serological survey of *Brucella abortus* antibody prevalence in the one-humped camel (*Camelus dromedarius*) from Eastern Sudan. *Rev. Elev. Med. Vet. Pays. Trop.*, **43**: 167-171.
- Yesuf, M., Alemu, S., Temesgen, W., Mazengiac, H. and Negussie, H. (2010): Seroprevalence of Ovine Brucellosis in South Wollo, North Eastern Ethiopia. *American-Eurasian J. Agric. Environ. Sci.*, **9**: 288-291.
- Young, J. (1995): An overview of human brucellosis. *Clinical Infectious Diseases*, **21**: 283-290.
- Young, J. (2000): *Brucella* species. In: Mandell, Douglas and Bennet's Principles and Practice of Infectious Disease, 5<sup>th</sup> edition, Mandell, L., Bennett, E., and Dolin, R. (ed.): Churchill Livingstone, Pp. 2386-2393.



- Zaitseva, M., Golding, B., Betts, H., Yamauchi, M., Bloom, T., Butler, E., Stevan, L. and Golding, B. (1995): Human peripheral blood CD4<sup>+</sup> and CD8<sup>+</sup> T cells express Th1-like cytokine mRNA and proteins following *in vitro* stimulation with heat-inactivated *Brucella abortus*. *Infect. Immun.*, **63**: 2720-2728.
- Zaria, T., Egwu, O., Nawathe, R. and Ojeamiren, E. (1990): A preliminary report on the seroprevalence of Brucellosis in camels (*Camelus dromedarius*) in Maiduguri. *Zariya Vet.*, **5**: 64-65.
- Zhan, Y., Yang, J. and Cheers, C. (1993): Cytokine response of T-cell subsets from *Brucella abortus*-infected mice to soluble brucella proteins. *Infect. and Immuno.*, **61**: 2841-2847.
- Zinnstag, J., Roth, F., Orkhon, D., Chimed-Ochir, G., Nansalmaa, M., Kolar, J. and Vounatsou, P. (2005): A model of animal-human brucellosis transmission in Mongolia. *Pre. Vet. Med.*, **69**: 77-95.
- Zowghi, E. and Ebadi, A. (1988): Brucellosis in camels in Iran. *Rev. Sci. Technol.*, **7**: 383-386.
- Zowghi, E., Ebadi, A. and Mohseni, B. (1990): Isolation of *Brucella* organisms from the milk of seronegative cows. *Rev. Sci. Tech.*, **9**: 1175-1178.

## 8. ANNEXES

### Annex 1: Rose Bengal Plate Test (RBPT) procedure

A rapid agglutination slide test for the detection of *Brucella* specific agglutinins

#### Intended use

*Brucella* antigens are bacterial suspensions for use in slide agglutination tests to detect the presence of bacterial agglutinins associated with bacterial infection or previous exposure to a related organism. This slide test is recommended as a screening procedure only to establish the presence or absence of homologous antibody.

#### Principle of the test

In the course of *Brucella* infection with any pathogenic microbiological agent, a variety of antibodies are formed. Among these antibodies are the agglutinins. An agglutinin when combined with homologous antigen (agglutininogen) under the properly controlled conditions is capable of causing agglutination. A suspension of *Brucella* possessing active antigen agglutinates when exposed to homologous *Brucella* antibody. This agglutination forms clumps of bacteria which become macroscopically visible. The Rose Bengal stained *Brucella* antigen is used for the early detection of *Brucella* agglutinins (*Brucella Abortus*, *B. Melitensis* and *B. Suis*).

#### Reagents and serum

- ✓ *Brucella* Antigen, Rose Bengal Stained.
- ✓ Positive Control.
- ✓ Negative Control.
- ✓ White Glass slide (or enamel plate).
- ✓ Stirring Sticks/applicator stick
- ✓ Micropipettes for dispensing 5-100µl serum.

#### Procedure

- 1- Allow reagents and serum samples to reach room temperature for testing.
- 2- Shake the antigen bottle gently to insure a uniform suspension.



- 3- Place 75µl sample serum onto the well of the white enamel plate
- 4- Place one drop (25µl) of the Rose Bengal antigen onto serum sample.
- 5- Mix serum sample with Rose Bengal antigen using Stirring stick.
- 6- Repeat these steps using the positive and negative controls instead of serum sample.
- 7- Gently shake the slide for 2 to 4 minutes (automatic rotator can also be used).
- 8- Observe for agglutination after 2 to 4 minutes from beginning of shaking (this is the optimum time limit).

### Results

Negative: No agglutination

Positive: (Presence of Specific Antibodies): Agglutination

### Interpretation of results

This test is a screening test only for the detection of *Brucella* agglutinins. If result is positive it must be confirmed by CFT or other serological tests for Brucellosis. Reaction was identified as 0, +, ++, +++, according to Nielsen and Dunken (1990).

- ❖ 0 = No agglutination
- ❖ + = barely visible agglutination, using magnified glass
- ❖ ++ = fine agglutination some clearing
- ❖ +++ = Coarse clumping definite clearing

Those samples with no agglutination were recorded as negative, while those +, ++, +++ were recorded as positive.

## Annex 2: Complement Fixation Test (CFT) procedure

### Materials and reagents for CFT

Micro titer plates (U-shaped), multi-channel and single channel micropipettes, pipette tips, universal bottles for preparation of solutions, stirrers (magnetic), Cylinders, weighing balance, thermometers, Incubator, water bath, deep freezer, centrifuge, Antigen, complement, hemolysin (amboceptor's), control sera, SRBC, veronal buffer, Alsever's solutions and washing buffer

#### I. Preparation of SRBC for the hemolytic system:

Ten (10) ml of SRBC in Alsever's was centrifuged at 2500 rpm for 5 minutes.

The supernatant was discarded and replaced by Veronal Buffer Diluents (VBD).

The sRBC were resuspended in diluents completely.

This procedure was repeated three times. Before discarding the supernatant after the last washing, the packed cell volume was measured by placing an identical tube next to the blood containing tube filled up to the level of blood by a measured amount of water.

By addition of calculated amount of water, a 2% sRBC suspension was prepared.

#### II. Amboceptor's (Hemolysin) titration:

A. Prepare 1:500 dilutions up to 1:8000.

5 test tubes were prepared

1ml of VBD was added to test tubes 2-5

10 $\mu$ l amboceptor's was mixed with 4990 $\mu$ l VBD in the first tube

1ml was transferred from the first to the second up to the last tube and 1ml was discarded.

B. Prepare 1:750 amboceptor's and dilute serially up to 1:12000

- 5 test tubes were prepared and 1ml of VBD was added to test tubes 2 to 5

- 10 $\mu$ l amboceptor's was mixed with 7490 $\mu$ l VBD in the first tube



-1ml was transferred from first tube to second up to fifth tube and 1ml was discarded.  
put tubes in order of ascending dilution.

C. 0.5ml was transferred from each of these test tubes to a second set of 10 tubes.

Start with the 1:12,000 dilution

D. 1ml of VBD was added to each of the test tubes

E. 0.5ml of 2% SRBC was added to each of the test tubes and shaken well.

F. The test tubes were left on the bench for 10 minutes.

G. 1ml of complement at working dilution was added.

H. The tubes were incubated for 30 minutes in a water bath at 37 °C

I. Result read and recorded, the last tube showing complete hemolysis (MHD)

The working dilution of amboceptor's is four times the MHD

III. Evaluation of complement.

Freeze dried complement was reconstituted according to its instruction.

A 1:100 complement was prepared

Complement was added into the 9 tubes increasing by 5 µl every time, starting with 10 µl.

- Diluent was added into the 9 tubes in decreasing amount by 5 µl, starting with 40 µl.

- 25 µl of diluents was added into the tubes

- The test tubes were placed at 37 °C water bath for 1 hr.

- 25 µl of 2% sRBC was added in all tubes

- 25 µl of amboceptor at working dilution 1:1,000 was added in all tubes.

The tubes were properly mixed and put again in the water bath at 37 °C for another 30 minutes

The test was read by recording the Minimum Hemolytic Dose of complement (MHD), which was represented by the first tube showing complete hemolysis. The next tube contains the Full Hemolytic Dose (FHD). The complement dilution =  $2\text{FHD}/\text{dilution of complement}$ .

#### V. Titration of antigen

##### Micro titer plate I

- 25  $\mu\text{l}$  of VBD was added in all wells.
- 25  $\mu\text{l}$  prediluted antigen was added to all wells of row A.

By serial doubling (two fold) dilution 25  $\mu\text{l}$  of antigen was transferred from row A to B, and again from row B to C, etc until row G by multi-channel pipette. 25  $\mu\text{l}$  mixture was discarded from row G (row H will only contain the diluent

##### Micro titer plate II

- 50  $\mu\text{l}$  of VBD was added to all wells
- 50  $\mu\text{l}$  of prediluted (1:2.5) in activated positive control serum was added to all wells of column 1.
- 50  $\mu\text{l}$  was serially transferred by two-fold dilution, from column 1 to 2 and again from column 2 to 3 etc. until column 11. 50  $\mu\text{l}$  was discarded from column 11.

##### Mix plate I and II

- 25  $\mu\text{l}$  was transferred from plate II to Plate I.
- 25  $\mu\text{l}$  of complement in 1:40 dilution was added to all wells of plate I
- Plate I was kept in a refrigerator, covered with second empty plate (cold fixation)

The following day, 50  $\mu\text{l}$  of 2% sRBC, amboceptor premature, equal volume, i.e. 25  $\mu\text{l}$  of sRBC and 25  $\mu\text{l}$  of a 1:100 working dilution of amboceptor was added to all cups.

The plate was covered with sealing tape, shaken well and kept in water bath at  $37^{\circ}\text{C}$  for



30 minutes.

The last well with 50% sedimentation was read and recorded. The highest dilution of antigen with 50% sedimentation is the limiting antigen concentration or the right corner value. In this case the corner value was 1:25 dilution and was used throughout the test. The 50% sedimentation was taken as one unit and the working dilution of the antigen was 2units.

The test proper, multiple sera technique:

The sera were prediluted to 1: 2.5 and incubated at  $58^{\circ}\text{C}$  in a water bath for 3 minutes in order to inactivate the native complement.

- 25  $\mu\text{l}$  of diluted test sera were placed in wells of first and second rows of U- bottom plates and
- 25 $\mu\text{l}$  of VBD was added to all except those of the first.
- Serial doubling dilution were then made by transferring 25 $\mu\text{l}$  of serum from the second row onwards continuing for at least four dilutions
- 25 $\mu\text{l}$  of antigen diluted to working dilution excluding those of the anti complementary controls, which received 25 $\mu\text{l}$  VBD instead.
- 25  $\mu\text{l}$  of complement at working dilution (1.25) was added to all wells except control well.

Control wells containing serum+ complement+ diluent, antigen control has antigen+

Complement+ diluent, complement control has complement+ diluent and hemolytic

system has diluent set up to 75 $\mu\text{l}$  total volume in each case before hemolytic system

was added.

- The plate were sealed, incubated at  $37^{\circ}\text{C}$  for 30minutes with agitation
- 25 $\mu\text{l}$  of sRBC suspension was added to each well. The plates were sealed and reincubated at  $37^{\circ}\text{C}$  for 30 minutes with agitation. Before reading the result on the plates were left in the refrigerator at  $+4^{\circ}\text{C}$  for 1-hour in order to allow none lysed cells to settle.

**Figure 2: Titration of Complement**

Plates were taken out from refrigerator and left at room temperature for 10 minutes

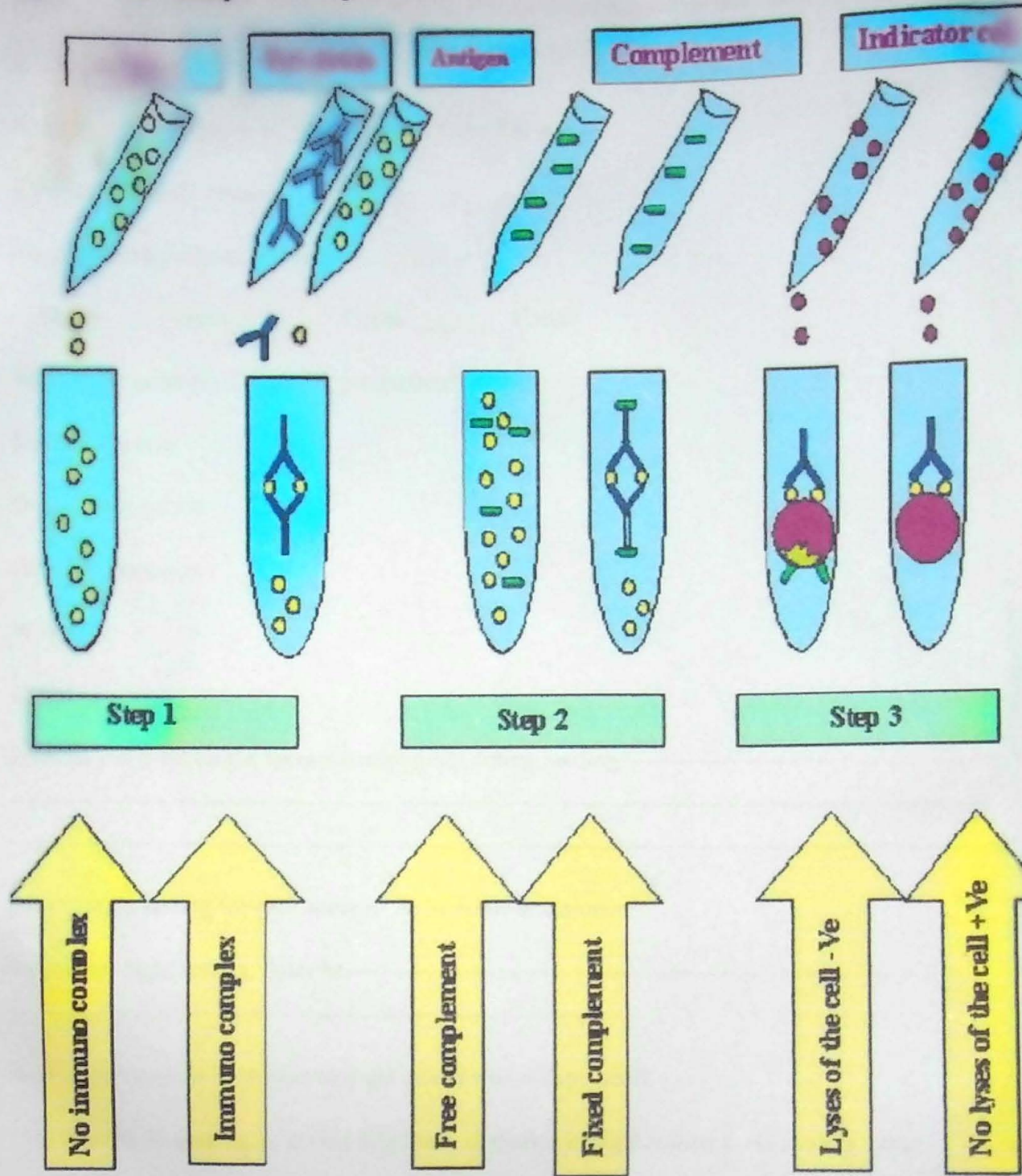
Positive reactions were indicated by sedimentation of sRBC and absence of hemolysis negative reactions by hemolysis of sRBC.

Microplate layout of Lay Out of CFT

|   |              | 1     | 2      | 3      | 4      | 5      | 6       | 7       | 8       | 9        | 10       | 11    | 12     |
|---|--------------|-------|--------|--------|--------|--------|---------|---------|---------|----------|----------|-------|--------|
| A | + ve Control |       |        |        |        |        |         |         |         |          |          |       |        |
| B | - ve Control |       |        |        |        |        |         |         |         |          |          |       |        |
| C | Sample 1     |       |        |        |        |        |         |         |         |          |          |       |        |
| D | Sample 2     |       |        |        |        |        |         |         |         |          |          |       |        |
| E | Sample 3     |       |        |        |        |        |         |         |         |          |          |       |        |
| F | Sample 4     |       |        |        |        |        |         |         |         |          |          |       |        |
| G | Sample 5     |       |        |        |        |        |         |         |         |          |          |       |        |
| H | Sample 6     |       |        |        |        |        |         |         |         |          |          |       |        |
|   |              | (1:5) | (1:10) | (1:20) | (1:40) | (1:80) | (1:160) | (1:320) | (1:640) | (1:1280) | (1:2560) | (1:5) | (1:10) |



Figure 2: The Principle of Complement Fixation Test



**Annex 3: Questionnaire for the assessments of brucellosis in animals and zoonotic risk to human**

**I. General Information**

Region----- Zone----- District----- PA-----

Livestock owner's name \_\_\_\_\_

Livestock composition and number

Sheep \_\_\_\_\_ Goats \_\_\_\_\_ Cattle \_\_\_\_\_ Camel \_\_\_\_\_

What is the purpose of livestock production?

Milk production

Drought mitigation

Herd accumulation

Wealth

Other

How do you position the species composition during herding?-----

-----

-----

How is night resting for each species? A. Separate b. common

If common night resting, describe-----

-----

Where and/or when does your herd get mixed with village herd?

a. watering b. grazing, c. during migration, d. market e. night resting f. vet clinic g. other



## II. Public Health importance

1. Do you know a disease transmitted from animal to man? Yes/NO
2. If yes, How do you acquired the knowledge about zoonotic diseases.
  - a. elders b. personal observation c. health extension d. other
3. What do you think the vehicle for diseases transmission to humans??
  - a. meat b. milk c. direct contact d. inhalation e. others
4. Mention some of the zoonotic diseases you know? -----  
-----
5. Have you ever noticed a person with any one of the above mentioned zoonotic disease?  
Yes/no,  
What were the symptoms?-----  
-----
6. Have you ever assisted livestock delivery? Yes /No  
If yes describe? a. bare Hand b. safe and protected c. others-----  
-----
7. Have you ever assisted calf birth with bare hand? Yes/No
8. If yes, what was the nature of the delivery?
  - a. normal b. stillbirth
9. Who assisted calf birth most? Males/Females
10. What do you use to protect yourself when you encounter/handle aborted material? -----  
-----
11. What do think that cause abortion in animals?-----
12. How do you take care for new born?

a. Cleaning newborn b. Hand feeding of weak calves' c. Carrying newborn to home

13. Have you ever practiced any one of the following activities?

a. Milking b. slaughtering c. skinning c. chopping meat d. consuming raw milk e. meat

14. Do you know what **brucellosis** is?

Yes \_\_\_\_\_ No \_\_\_\_\_

15. If yes, which species of animals frequently abort?-----

### III Reproductive disease events in the herd (indicators of brucellosis)

16. Have you ever encountered a case of abortion or RFM in animals? Yes/ No

17. If yes, what month of pregnancy and which species? -----

18. During last one year, was there any record of abortion in your herd?

19. If yes, what species \_\_\_\_\_

20. What do you do with an animal that frequently abort? a. Sell, b. slaughter, c. keeping d. other

21. How do you manage aborted fetus/ fetal membrane? a. Leave in the field b. disposing c. Give to dog d. others-----

22. What do you do with female that doesn't conceive? a. Sell, b. slaughter, c. keeping d. other---

23. What is the source of bull/ram? a. From own herd b. village bull c. others-----

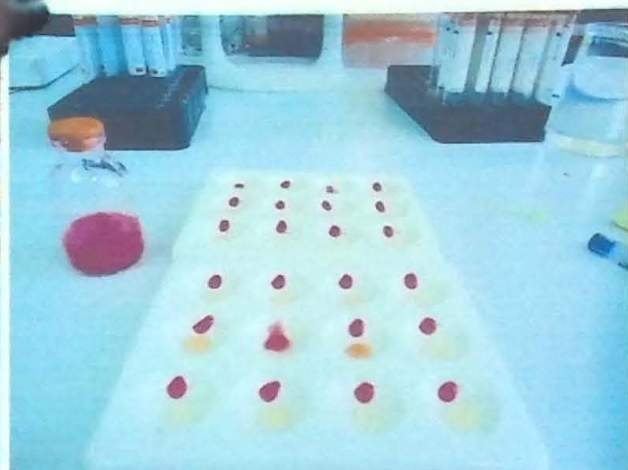
24. Have you ever sold breeding females? (Yes/No), which spp??

25. If yes what was the reason of selling? a. Disease b. Infertility c. Shortage of money d. Others-----

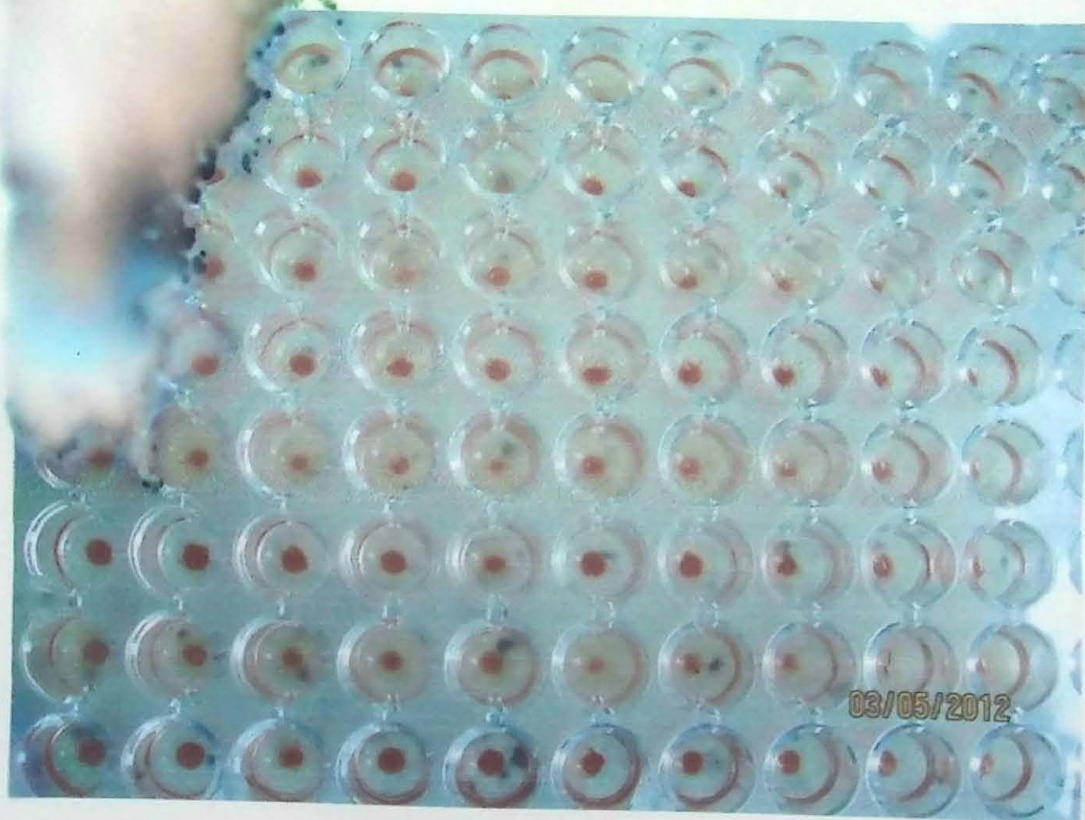


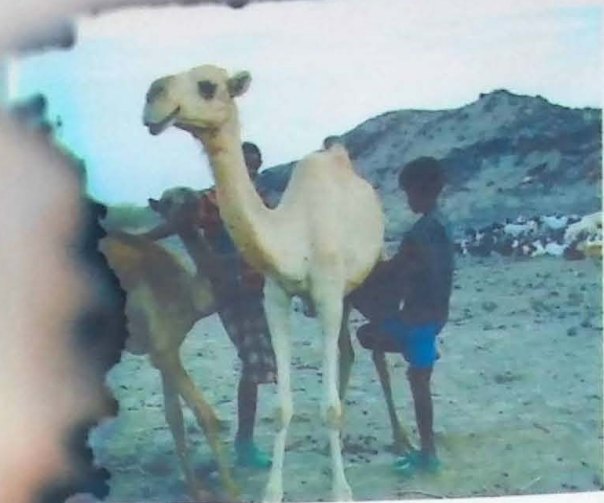
**Annex 4: Figure showing sample collection**









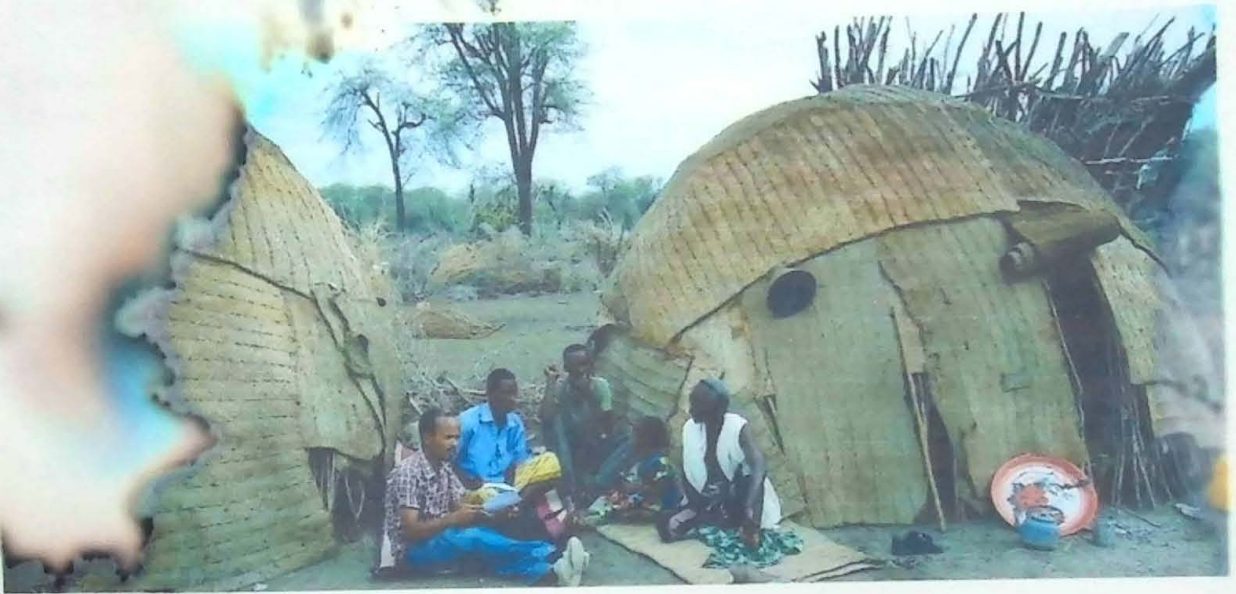












## 9. CURRICULUM VITAE

### 1. PERSONAL DETAILS

Name                      Wesinew Adugna Bekele  
Sex                        Male  
Date of Birth            April 2/1983  
Place of Birth           Addis Ababa  
Marital Status        Married  
Nationality            Ethiopian  
Current Address      Mobil: +251911796753, +251910022650  
Email:                  wesinewa@gmail.com  
                             wesinewa@yahoo.com  
Fax:                      +251336660099

### LANGUAGES

Amharic (mother tongue)

English: spoken good; written good; reading good

### Computer skill

Diploma from DOT NET Computer technology

### 2. EDUCATION/QUALIFICATIONS

**2001 to 2005: Veterinary Faculty, Addis Ababa University**

Award: Degree of Veterinary Medicine (DVM)

**1995 to 1999: Yehiberet Fire Senior secondary school, South Western Shewa, Tullu Bollo town, Ethiopia.** Award: Ethiopian Leaving Certificate (ESLCE)

### 3. PROFESSIONAL EXPERIENCE

**July, 2007 to Present: Semera Veterinary Laboratory Afar Region, Ethiopia**

**Position:** Initially Serology section team leader then, from 2009, Microbiology Department Head.

**Sept, 2005 to Jun, 2007: Aballa District, Agriculture, Natural Resource and Environment Protection office Northeastern Afar, Ethiopia**

**Position:** Veterinary Officer and then Veterinary team leader



#### 4. SHORT TRAINING COURSES AND WORKSHOPS ATTENDED

| <u>Date</u>                 | <u>Topic, organizer and Place</u>                                                                                                                                                                                                  |
|-----------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Feb.28-Mar.2/2011           | The livestock Emergency Guidelines and Standards (LEGS)<br>Care Ethiopia, Awash Sebat Kilo, Afar                                                                                                                                   |
| Dec, 22/08-Jan,15/09        | Sample collection and handling Bacterial Isolation & Identification<br>Bacterial and viral serology, Pathology, Acarology<br>National Animal Health Diagnostic and Investigation Center,<br>Sebeta                                 |
| Nov, 20- 30/07              | Epidemiology of Camel diseases, surveillance, principles of<br>common diagnostic Methods, proper sample collection, handling,<br>transport and submission to diagnostic Laboratory.<br>FAO, Afar livestock Recovery project, Awash |
| Sep,19 to Oct<br>19/06      | Practical aspect of Serology, Bacteriology, Virology, and Laboratory<br>animal management,<br>FAO and National Veterinary Institute, Debere zeit                                                                                   |
| July 24-26/2006,            | Early warning and Food security Concepts.<br>Save the children (UK), Mekele                                                                                                                                                        |
| July 1-15/2006,<br>workers. | Trainer's course for training Community based animal health<br>workers.<br>FAO-ALRP, Adama                                                                                                                                         |
| March 29-3/2005,            | ELISA and Hemagglutination Inhibition Technique.<br>Ethio-France Project and NARCH,Diredewa                                                                                                                                        |

#### PUBLICATIONS

- Seminar on: Immunopathogenesis of Mycobacterium Infection
- A seminar on: Autoimmunity in domestic animals and man.
- A DVM thesis on: Bovine Tuberculosis an Epidemiological survey in specialized and smallholder dairy farms.

## 10. SIGNED STATEMENT OF DECLARATION

I, the undersigned, declare that the thesis is my original work that has not been presented for a degree in any other university, and that all sources of materials used for the thesis have been dully acknowledged.

Name:       Wesinew Adugna

Signature       \_\_\_\_\_

Date of submission June 2012

The thesis has been submitted for examination with my approval as University advisor

Advisor

Signature

Dr. Tesfaye Sisay

\_\_\_\_\_



## 10. SIGNED STATEMENT OF DECLARATION

I, the undersigned, declare that the thesis is my original work that has not been presented for a degree in any other university, and that all sources of materials used for the thesis have been duly acknowledged.

Name: Wesinew Adugna

Signature \_\_\_\_\_

Date of submission June 2012

The thesis has been submitted for examination with my approval as University advisor

Advisor

Signature

Dr. Tesfaye Sisay

\_\_\_\_\_