

ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

SERO-PREVALENCE STUDY OF BRUCELLOSIS IN CATTLE AND HUMANS IN
DIBATE AND WEMBERA DISTRICTS, METEKELE ZONE, BENISHANGUL GUMUZ
REGION

BY

KASSAYE ERKIHUN ADUGNA

JUNE, 2008
DEBRE ZEIT, ETHIOPIA

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A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS
ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF MASTERS IN TROPICAL VETERINARY PUBLIC HEALTH.

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ABBREBATION

AAU	Addis Ababa University
AI	Artificial Insemination
APHIS	Animal and Plant Health Inspection Service
BBAT	Buffered Brucella Antigen Tests
BPAT	Buffered Plate Agglutination Test
CFT	Complement Fixation Test
CSA	Central Statistic Authority
DVM	Doctor of Veterinary Medicine
ELISA	Enzyme Linked Immunosorbent Assay.
FAO	Food and Agriculture Organization
FPA	Fluorescence Polarization Assay
FPSR	False positive serological reaction
IFA	Immunofluorescent Antibody
IFAT	Immuno fluorescent Assay Test
Ig	Immunoglobulin
IU	International Unit
masl	meter above sea level
MRT	Milk Ring Test
MZN	Modified Zeihl-Neelson
OIE	Office International des Epizooties
OPS	O-Chain Polysaccharide
PA	Peasant Association
PAHO	Pan American Health Organization
RBPT	Rose Bengal Plate Test
SAT	Serum Agglutination Test
SDA	Serum Dextrose Agar
S-LPS	Smooth Lipopolysaccharide
S-RB51	Strain Rough <i>Brucella</i> 51
TSA	Tryptone Soy Agar.
USDA	United States Department of Agriculture
WHO	World health organization United Nation

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ACKNOWLEDGMENTS

My heartfelt thanks goes to my advisors Dr. Girma Zewde and Dr Getahun Ejeta for their guidance, valuable advice, constructive suggestions and reviewing the manuscript.

I am indebted to Benishangul Gumuze Regional State bureau of agriculture and rural development for allowing me to pursue my Msc course.

Special thanks are due to Dr Anteneh Yenehun and Dr Tadese Kebede who had been with me during blood sample collection in the field

I acknowledge with gratitude the Assosa regional veterinary laboratory and its staffs for providing material and moral support in the course of my field and laboratory work

My great goes to Ato K/Mikael Mekuria and W/ro Almaz Sharew who helped me during CFT testing at NVI.

I owe a great deal to FVM postgraduate program for creating conducive environment to accomplish my study.

It is my wish to thank members of the board of examiners for their incalculable time devotion in evaluating this paper and sharing their experience.

ABSTRACT

A cross-sectional study was conducted in Debate and Wembera districts of Metekele Administrative Zone, Benishangul Gumuze Regional State, between October 2007 and March 2008, to determine the sero-prevalence of brucellosis in cattle and humans as well as to identify the likely potential risk factors. Two-stage cluster sampling technique was employed, by which 12 peasant associations were identified as primary units and 164 individual cattle owners as secondary units. Accordingly, 1152 cattle (164 herds) were sampled. In the sampling, all cattle in the herd above 6 months of age were included. Human risk groups were sampled using purposive sampling method. The study methods involved collection of serum sample, questionnaire surveys and serological tests.

Tests used to detect the presence of *Brucella* antibodies were Rose Bengal Plate Test (RBPT) as screening test, and Complement Fixation Test (CFT) as confirmatory test. Results of the two tests were interpreted serially. A herd was said to be positive if at least one animal reacts positively by both RBPT and CFT.

The overall individual cattle and herd sero-prevalence of bovine brucellosis the study area were 1% (11/1152) and 4.9 % (n= 8/164), respectively. Within herd sero-prevalence ranged between 0% and 15.4%, While considering only positive herds (n=8), it varied from 5.3%-15.4%.

Both univariable logistic regression and multivariable logistic regression analyses showed that the risk of sero-positivity was increased by increases herd size of the animal ($p=0.000$, $OR=1.34$). Brucellosis sero-positivity was statistically different among cattle, which experienced abortion than those, which did not ($OR= 6.6$, $P = 0.013$).

The reproductive status (lactation, pregnancy, dry cow and heifer) did not show significant difference in the rate of sero-prevalence among the four reproductive statuses. Multivariable logistic regression analysis showed that, the age of cattle was not significantly associated with sero-positivity ($P=0.205$). With univariable, logistic regression analysis sex was not found to be significantly associated with sero-positivity of cattle in the study area ($p=0.994$), even though all positive cattle were females.

All sampled human serums were found negative for *Brucella* antibodies.

Risk factors are discussed; conclusions are drawn and pertinent recommendations are forwarded.

Key words: *Brucellosis*, Cattle, CFT, Humans, RBPT, Sero-prevalence, Zoonosis

1. INTRODUCTION

Ethiopia is the third most populous countries in Africa next to Nigeria and Egypt, having an estimated population of about 67.2 million with annual growth rate of 2.9%. The total cattle population of Ethiopia is estimated to be 41,527,142; with the proportion of male to female of about 50.2% and 49.8%, respectively. Cattle population of Benishangul Gumuz Regional State is estimated to be 309627, of which 100589 were found in study area (Dibate and Wembera) (CSA, 2003). Livestock in Ethiopia contributes about 30% of the agricultural GDP and 19% to the export earnings. Besides, about 6 million oxen provide draught power for the cultivation of grain crops (Tegegne and Gebre Wold, 1997). The comparative huge resource that the country possesses and the economic return gained from this sub-sector does not seem to coincide with the number of cattle. The reasons that are attributed to these are animal diseases, under nutrition, low productivity and age-old traditional management. One of the infectious diseases, which is major constraint for animal productivity, is brucellosis (Tamirat, 1985).

Brucellosis is one of the oldest zoonotic diseases tracing back to the date of Hippocrates 450 A.D. It is a major problem confronting food production in tropical and sub-tropical regions of the world (Nicoletti, 1984). David Bruce, after whom the genus *Brucella* is named, isolated the causative organism in 1887, from the spleens of five fatal cases and placed it with in the genus *Micrococcus*. Fredrick Bang of Copenhagen discovered *Brucella abortus* in 1897 (Corbel, 1990).

Bovine brucellosis (contagious abortion, Bang's disease) is a disease of cattle usually caused by *Brucella abortus*, less frequently by *Brucella melitensis* and rarely by *Brucella suis*. The disease is characterized by abortion in the last trimester or birth of unthrifty newborn in the female and orchitis and epididymitis with frequent sterility in the male (Radostitis *et al.*, 2000; OIE, 2004) Occasionally, other species of animals such as goat, sheep, pig, dog and horse may be infected by *Brucella abortus* (Nicoletti, 1998).

Although brucellosis has been, or close to being eradicated from a number of developed countries, it continues to be a major public and animal health problem in many regions of the world, particularly where livestock are a major source of food and income. There are many

reasons, why brucellosis remains endemic. These include expansion of livestock herds and flocks, with associated uncontrolled movements; lack of veterinary services and vaccines; and husbandry practices favoring the spread of infection (FAO, 2003). Sources of infection for the transmission of bovine brucellosis are aborted fetuses, fetal membranes, vaginal discharges and milk from infected cows (Acha and Szyfres, 2001; Radostits *et al.*, 2000). The most common route of transmission is the gastrointestinal tract following ingestion of pasture, feed, fodder, water, contaminated with infected after birth, fetuses, and newborn calves, all of which may contain a large number of the organisms and constitute a very important sources infection (Acha and Szyfres, 2001). Abortion after the 5th months of pregnancy in highly susceptible non-vaccinated pregnant cattle is the typical feature of the disease. In subsequent pregnancies, the fetus is usually carried to full term, although second or even third abortions may occur in the same cow. Retention of the placenta and metritis are common sequel to abortion (Radostits *et al.*, 2000).

Brucellosis is considered as one of the most widely spread zoonosis (Schelling *et al.*, 2003). The incidence of brucellosis in humans is clearly correlated with the degree of incidence in the domestic animals (Weidmann, 1991). Human cases continue to occur following international travel, traditional use of raw milk products and following close contact with infected animals. Human brucellosis can be a very debilitating disease, although the case fatality rate is generally low; it often becomes sub-clinical or chronic, especially if not recognized early and treated promptly. All age groups are susceptible, and even congenital cases have been recorded (FAO, 2003). Brucellosis is an important zoonosis causing undulant fever to man. The possibility of infection by drinking of contaminated milk necessitates the pasteurization of milk. However, most cases in humans are occupational and occur in farmers, veterinarians and butchers (Radostits *et al.*, 2000).

Brucellosis is an economically important reproductive disease of livestock, and is prevalent in most of the developing countries. The disease induces infertility, delayed heat, loss of calves, reduced meat, and milk production, and is of zoonotic importance in developing countries (Renukaradhya *et al.*, 2002). Indirect losses have not been estimated in sub-Saharan Africa particularly in those countries that want to attain brucellosis free status to access regional or international livestock markets, but could be a considerable constraint to future trade. The impact of brucellosis and other zoonoses affecting livestock production are

considerably magnified by their consequence in humans. For brucellosis, years of life lived with disability could be the major impact. Additionally, brucellosis incurs economic losses in the health sector while it is a chronic and relatively difficult infection to treat (McDermott and Arimi, 2002). Chukwu (1987) summarized the economic losses: 1) abortion in the affected animal population. 2) diminished milk production, mastitis and contamination of milk; 3) culling and condemnation of infected animals due to breeding failure; 4) banning of animal export; 5) human brucellosis causing reduced work capacity through sickness of the affected people 6) government costs on research and eradication schemes and;7) losses of financial investment.

The approach to control, prevention or eradication of brucellosis in a country or region depends on many factors, such as the level of infection in the herds or flocks, type of husbandry, economic resources, public health impacts and potential international trade implications. Decision-making by those charged with policymaking is likely to be intuitive unless accurate and current epidemiological information is available (FAO, 2003)

Sero-prevalence of bovine brucellosis has been reported in different parts of Ethiopia ,18.4% by Kibru (1985), 7.6% by Bayleyegn (1989), 38.7% by Muktar (1993), 22% by Tariku (1994), 8.1% by Yilkal *et al.* (1998), 4.9% by Abay *et al.* (2000), 2.46% in intensive and 1.66% in extensive by Kassahun (2004), 0.77% in intensive and 0.2% in extensive cattle production system by Tolossa (2004) and 11% in smallholder dairy farms by Kebede *et al.*(2008). Much of these studies had concentrated in the state dairy farms in the central part of Ethiopia However; sero-prevalence of bovine brucellosis was not studied in Benishangul Gumuz Regional State. To overcome the scarcity of food of animal origin and attain food self-sufficiency, the major animal diseases including brucellosis should be studied, controlled and eradicated. This will increase the productivity of the animals and reduce the disease incidence in humans. Gathering information on the occurrence of the disease is of paramount important in` this regard. Therefore, the objectives of this work were:

To determine the prevalence of bovine and human brucellosis in the two districts of Benishangul Regional State and

To investigate potential risk factors associated with brucellosis

2. LITERATURE REVIEW

2.1. Etiology

Brucellae are coccobacilli or short rods measuring from 0.6 to 1.5µm long and from 0.5 to 0.7 µm wide. They are usually arranged singly, and less frequently in pairs or small groups. The morphology of *Brucella* is constant, except in old cultures where pleomorphic forms may be evident. *Brucellae* are non-motile. They do not form spores, and flagella, Pili or true capsules are not produced. *Brucellae* are Gram negative and usually do not show bipolar staining. They are not truly acid-fast, but are resistant to decolorization by weak acids and thus stain red by the Stamp's modification of the Ziehl Neelsen Method (OIE, 2004).

Six species are presently known in the genus *Brucella*: *B. abortus*, *B. melitensis*, *B. suis*, *B. neotomae*, *B. ovis* and *B. canis*. The first three species (called "classical *Brucella*") have been sub-divided into biovars that are distinguished by their different biochemical and /or reactions to the mono-specific A (*abortus*) and M (*melitensis*) sera (Acha and Szyfres, 2001). Each *Brucella* species has preferred natural host that serves as a reservoir of infection. Secondary hosts play only a very small part, if any, in the maintenance or spread of a particular *Brucella* species (Quinn *et al.*, 1999). Bovine brucellosis (contagious abortion, Bang's disease) is a disease of cattle usually caused by *B. abortus*, less frequently by *B. melitensis* and rarely by *B. suis*. *B. suis* does not appear to be contagious from cow to cow (Nicoletti, 1998). *B. abortus* has seven biovars (1, 2, 3, 4, 5, 6, and 9); biovars 7 and 8 are no longer regarded as valid (Corbel, 1990; OIE, 2004).

B. abortus is mainly infective in cattle, but occasionally other species of animals such as sheep, swine, dogs, and horses may be infected. The infections in cattle caused by heterologous species of *Brucella* are more transient than that caused by *B. abortus* (Acha and Szyfres, 2001). In horses suppurative bursitis, most commonly recognized as "fistulous wither" or "poll evil" is most common condition associated with brucellosis. It is unlikely that infected horses are a source of the disease for other horses, other animal species, or man (Nicoletti, 1998).

2.1.1. Resistance and Survival Properties

Under appropriate conditions, *Brucella* organisms can survive in the environment for a very long period. Their ability to withstand inactivation under natural conditions relatively high compared with most other groups of non-sporing pathogenic bacteria (WHO, 1986). *B. abortus* is sensitive to pasteurization temperatures and its survival outside the host is largely dependent on environmental conditions. The pathogen may survive in aborted fetus in shade for up to eight months, three to four months in feces, in wet soil for two to three months, one to two months in dry soil and eight months in liquid manure tanks (Bishop *et al.*, 1994; Walker, 1999). Survival is prolonged at low temperatures and organisms will remain viable for many years in frozen tissues. *Brucellae* in aqueous suspensions are readily killed by most disinfectants. A 10 g/l solution of phenol will kill *Brucellae* in water in less than 15 minutes exposure at 37°C. Formaldehyde solution is the most effective of the commonly available disinfectants if the ambient temperature is above 15°C (WHO, 1986).

2.2. Epidemiology

2.2.1. Occurrence

Brucellosis occur world wide, 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, 2002). The Mediterranean countries of Europe, Northern and Eastern Africa, Near East countries, India, Central Asia, Mexico and Central and South America are especially affected (FAO, 2003).

The occurrence of brucellosis is increasing in developing countries in an even aggravating epizootiological situation. This depends on the policy of many developing countries of importing exotic breeds without having the required veterinary infrastructure and the appropriate level of development of the socio-economic situation of the animal holder. Further more, the increasing international animal trade, with increasing movement of animals and the trends towards intensification of animal production have facilitated the spread of

brucellosis (Seifert, 1996). In India, the long-term serological studies have indicated that 5% of cattle and 3% of buffaloes are infected with brucellosis (Renukaradhya *et al.*, 2002). The disease is prevalent in many countries of Africa (Kagumba and Nandokha, 1978; Nicoletti, 1984; Chukwu, 1987; 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). Surveys on the prevalence of bovine brucellosis have been carried out in four West African countries. Highest prevalence was found in Guinea Bissau with 15.1% followed by Guinea with 7.2%. Considerable lower numbers of cattle reacted to serological testes in Senegal and the Gambia with 0.6% and 1.1%, respectively. In addition, the epidemiological situation differed clearly between districts within the countries (Unger *et al.*, 2003). The occurrence of brucellosis, like other infectious diseases of animals in sub-Saharan Africa, incidence is poorly estimated (McDermott and Arimi, 2002). In pastoral systems and livestock-subsistence crop systems in semi-arid areas of sub-Saharan Africa, serological prevalence is almost always greater than 5% (range 4.8 – 41) (Jiwa *et al.*, 1996; Kadohira *et al.*, 1997; Domingo, 2000). Differences in the sensitivity and specificity of the different serological tests used are one important factor that contributes to this variation. Other factors associated with the high and variable prevalence in these extensive livestock production systems are large herd sizes, extensive movement of cattle, and common grazing and watering points (Kadohira *et al.*, 1997). For crop-livestock systems in both the sub-humid and high land zones, prevalence tends to be lower than in pastoralist area (Muriuki *et al.*, 1997; Baba *et al.*, 2001). However, prevalence varies even more widely than in pastoralist systems ranging from high (25%) in large intensively managed herds (Mohan *et al.*, 1996) to lower non-existent in zero-grazing smallholdings in east African highlands (Bekele *et al.*, 1989; Bedard *et al.*, 1993; Kadohira *et al.*, 1997). The prevalence of bovine brucellosis in some African countries is summarized in Table-1.

Table 1: Sero-prevalence of bovine brucellosis in Africa

Country	Sero-prevalence (%)	Authors and years
Guinea	6.9%	Chukwu, 1985
Kenya	10%	Kagumba and Nandokha, 1978
Kenya	7.73%	Chukwu, 1985
Kenya	9.2%	Chukwu, 1985
Kenya	3.74%	Chukwu, 1985
Nigeria	1.5%-14.3% private 79.7% government ranch	Esuruoso, 1980
Somalia	9.5%	Wernery <i>et al.</i> , 1979
Somalia	2.8% farm 11.9% nomadic	Hussein <i>et al.</i> , 1978
South Africa	2.1%	Bakunzi <i>et al.</i> , 1993
Sudan	6.5% - 22.5%	Hellmann <i>et al.</i> , 1984
Djibouti	4%	Chntel <i>et al.</i> , 1994
Tanzania	4.3% Local management 6.3% dairy farm 15.8% ranch	Jiwa <i>et al.</i> , 1996
Eritrea	8.5%	Omer <i>et al.</i> , 2000
Ghana	6.6%	Kubuafor <i>et al.</i> , 2000
Senegal	0.6%	Unger <i>et al.</i> , 2003
Gambia	1.1%	Unger <i>et al.</i> , 2003
Guinea Bissau	15.1%	Unger <i>et al.</i> , 2003
Guinea	7.2%	Unger <i>et al.</i> , 2003

Different studies showed that bovine brucellosis is prevalent in various regions of Ethiopia. Yilkal *et al.* (1998) found an overall prevalence of 8.1% of the 1,114 dairy animals tested in and around Addis Ababa. Bekele *et al.* (2000) in their study in southwestern Ethiopia recorded a sero-positivity of 7.3%. Sero-prevalence study of bovine brucellosis in dairy cattle kept under intensive and semi-intensive management system in Addis Ababa, Ethiopia 10%

prevalence was reported (Eshetu *et al.*, 2005). Kebede *et al.* (2008) found an over all prevalence of 11% of the 1136 cattle tested in smallholder farms in central Ethiopia (Wuchale-Jida district). The surveys have showed that bovine brucellosis is an endemic and widespread disease with high prevalence in organized farms than extensively managed herds

Table 2: Sero-prevalence of bovine brucellosis in Ethiopia

No	Location	No of Animals examined	Breed	Test used	Prevalence	Reference
2	Arsi Region	2178	Exotic, Cross, Zebu	SAT	7.62	Bayleyegn (1989)
3	Central Ethiopia	1609	Zebu	RBPT	4.2	Bekele <i>et al.</i> (1989)
4	Around Bahirdar	1855	Cross, Zebu	SAT	12.34	Asegid(1994)
5	In and around Addis Ababa	950	Exotic, Cross	CFT	8.11	Yilkal <i>et al.</i> (1998)
6	South East Ethiopia	4243	Cross, Zebu	CFT	4.9	Bekele <i>et al.</i> (2000)
7	North Gondar	1447	Zebu, Cross	CFT	0.14	Tadese (2003)
8	Jima Zone	1813	Zebu, cross	CFT	0.61	Tolosa (2004)
9	Addis Ababa	540	Dairy cattle	CFT	10	Eshetu <i>et al.</i> (2005)
10	Wuchale-Jida district	1136	Zebu, Cross, Holstein	RBPT/CFT	11	Kebede <i>et al.</i> (2008)

2.3. Transmission

2.3.1. Source of infection

The concentration of *Brucella* organism is highest in pregnant uterus, the fetus and the fetal membranes, and these are considered as a major source of infection. (Radostits *et al.*, 2000). Aborted fetuses, placental membranes or fluids, and other vaginal discharges are all highly contaminated with infectious *Brucella* organisms (USDA-APHIS, 2003). Infected animals also shed organisms in the milk; therefore raw milk or raw milk products of bovine origin are sources for infection in humans (Walker, 1999).

3.2.2. Mode of transmission and route of infection

Brucellosis spreads within the herd primarily by ingestion of contaminated material. Congenital (*in utero*) or perinatal infections may also occur with the ensuing development of latent infections. Venereal infections can also occur, but this is mainly seen with *B.suis* infection. Grazing on infected pasture or ingestion of other feedstuffs and water supplies contaminated by discharges and fetal membranes from infected cows and contact with aborted fetuses and infected newborn calves are the most common methods of spreads (Radostits *et al.*, 2000).

The most common route of transmission is the gastro-intestinal tract following ingestion of contaminated pasture, feed, fodder, and water. Moreover, cows customarily lick after birth, fetuses, and newborn calves, all of which may contain a large number of the organisms and constitutes a very important source of infection. Bulls do not usually transmit infection from infected cows to non-infected mechanically. The use of infected bulls for AI constitutes an important risk, since the infection can be spread to many herds (Acha and Szyfres, 2001)

2.4. Risk factors

2.4.1. Animal risk factors

Age, sex, breed, and reproductive status of the individual animal influence susceptibility of cattle to *B. abortus* infection. Infection occurs in cattle of all ages but persists most commonly in sexually mature animals. Natural exposure to field strains occurs primarily at the time of parturition of infected cows. The greater the number of infected cows that abort or calve, the greater the exposure risk to the other cattle in the herd. Young cattle are less susceptible to *B. abortus* than older sexually mature cattle (Radostis *et al.*, 2000). Susceptibility increases with pregnancy and as stage of gestation increases (Bishop *et al.*, 1994)

Age

It is widely accepted that susceptibility increases with sexual development and pregnancy (Nicoletti, 1980; Morgan and McKinnon, 1979). Young sexually immature cattle generally do not become infected following exposure or recover quickly (Radostitis *et al.*, 2000). Numerous reports now confirm that a small but important percentage of heifer calves, which are infected in early life, are negative to serologic tests, and abort or have an infected calving during the first pregnancy. It is estimated that 2.52% of heifer calves born to serologically positive dams reacted in early adulthood and constitute a risk to the herd. Certain that latent infections are more frequent than previously believed and present serious difficulties in elimination of brucellosis in cattle herds (Nicoletti, 1980).

Sex

No control studies have been conducted on the relative susceptibility of female and male cattle to brucellosis; nonetheless, based on reactor rates it is probable that bulls are more resistant than sexually mature heifers and cows, and less resistant than sexually immature heifers. There is a tendency for bulls to become infected at younger age than females and they may acquire infection during calf hood and retain it into adult life (Nicoletti, 1980)

Breed

All breeds of cattle appear to be comparable in susceptibility to brucellosis and apparently, no specific breed resistance to brucellosis is known (Radostitis *et al.*, 2000). Susceptibility between breeds has not been reported (Madsen, 1989). Rather there are varying degrees of individual resistance to infection, which are dependent up on gestation, exposure dose, age, vaccination and unknown host-resistance factors (Nicoletti, 1980).

2.4.2. Management risk factors

The spread of the disease from one herd to another and from one area to another is usually due to the movement of an infected animal from an infected herd in to a non-infected susceptible herd. The unregulated movement of cattle from infected herds or areas to brucellosis free herds or areas is the major cause of breakdowns in brucellosis eradication programs (Radostits *et al.*, 2000). Herd size, population density, method of housing and use of maternity pen influence the probability of exposure to infection (Crawford *et al.*, 1990).

There is a close correlation between the kind and intensity of the husbandry system and the rate of infection. In general, increased animal concentration and contacts favor the spread of brucellosis (Weidmann, 1991). Management factors for high incidence of infection are high stocking densities, unhygienic housing and calving, lack of sanitary measures (cleaning and disinfection) and large herd size (Crawford *et al.*, 1990; Nicoletti, 1980; Morgan and McKinnon, 1979). Frequent farm disinfection is an effective method of control of brucellosis because of low resistance of *Brucella* (FAO/WHO, 1986).

2.4.3. Pathogen risk factors

B.abortus is a facultative intracellular pathogen, which is capable of multiplication and survival with in host phagocytes (WHO, 1997).The organisms are phagocytosed by polymorph nuclear leukocytes in which some survive and multiply. These are then transported to lymphoid tissues and fetal placenta. The inability of the leukocytes effectively to kill virulent B. abortus at the primary site of infection is a key factor in the dissemination to regional lymph nodes, other sites such as the reticuloendothelial system, and organs such as

the uterus and udder. The organism is able to survive within macrophages because it has the ability to survive phagolysosome. *Brucella* is able to survive within host leukocytes and may utilize both neutrophils and macrophages for protection from humoral and cellular bactericidal mechanisms during the periods of hematogenous spread (Radostits et al., 2000).

Brucella are facultative intracellular bacteria, hence has protection from the innate host defense and from therapeutics; in quiescent state does not cause formation of humoral antibodies; it is unknown if aberrant forms of the bacteria are produced and play a role in the host parasite relationship. Nevertheless, this cytoplasmal survival results in many epidemiological problems (Nicoletti, 1980).

It is generally accepted that the organism does not multiply in the environment, but merely persist and its viability outside the host is influenced by the existing environmental conditions temperature, humidity, presence of other microorganisms, nutrition, autolytic enzymes and pH of the environment influence the survival of *Brucella* (Radostitis *et al.*, 2000). The organism is sensitive to direct sunlight, disinfectant and pasteurization (FAO/WHO, 1986; Seifert, 1996). *Brucella* survive for up to 4 months in aborted fetuses and placenta, 22 weeks in humid feces, 44 days in dust of street, 30 days in tap water, 51 days in sterile water, 2 months in desert soil, up to 2 years in frozen soil. *Brucella* survive up to 6 months in soft cheese, up to 4 months in butter, up to 6 weeks in milk, for 14 days in cooled meat and in ice cream up to 30 days (Weidmann, 1991). Disinfectants like caustic soda, formalin 2% and lysol 1% destroy *Brucella* (Seifert, 1996). Exposure to sunlight kills the organisms within a few hours (Nicolletti, 1998).

2.5. Pathogenesis

The key aspect of *Brucella* virulence is its ability to survive and multiply within macrophage. Recent studies in the cell biology of *Brucella* cell interaction have shown that it uses unique strategy by way of modifying the process of phagosome maturation. Virulence factors that have been produced by the organism to neutralize acidification of the phagosome protect the organism from hydrolytic enzymes (Boschioli *et al.*, 2001). *Brucellae* entering the animal body multiply first in the regional lymph nodes and are later carried by lymph and blood to different organs. *Brucella* organisms are most commonly found in the lymph nodes, uterus,

udder, spleen, liver and in bulls the genital organs. Large quantities of erythritol, a carbohydrate that stimulates the multiplication of brucella have been found in cow placenta. This could explain the high susceptibility of bovine fetal tissue (Acha and Szyfres, 2001). Multiplication of the organisms may last for several months, resolve itself, or be recurrent for at least two years in 5-10% of animals. Recurrence occurs particularly at the time of parturition. During the bacterimia, organisms are carried intracellularly in neutrophils and macrophages or free in the plasma and localize in various organs, especially the gravid uterus, udder, and supramammary lymphnodes. Localization may also occur in other lymphnodes, spleen, testes, and male accessory sex glands. Occasionally, bacterial localization occurs in synovial fluids causing a purulent tendovaginitis, arthritis or bursitis (Bishop *et al.*, 1994).

In pregnant animals, the uterus is a preferred site of infection where it leads to a necrotizing placentitis. In non-pregnant animals, the first infection often occurs in the udder followed by the infection of the uterus later after the onset of pregnancy (Seifert, 1996). Infected udders are clinically normal, but they are important as a source of reinfection for calves or humans drinking the milk. Invasion of the gravid uterus results in a severe ulcerative endometritis of the intercotyledonary spaces. The allanto-chorion, fetal fluids and placental cotyledons are invaded and the villi destroyed. In acute infections pregnant cows, up to 85% of the bacteria are in cotyledons, placental membranes and allantoic fluid. Abortion occurs principally in the last three months of pregnancy, the incubation period being inversely proportional to the stage of development of the fetus at the time of infection (Radostits *et al.*, 2000). Following entry into the host, *Brucella* is free either in the extracellular environment or in phagocytes localized to the regional lymph nodes (Quinn *et al.*, 1999). In the phagocytes, the organism is capable of surviving and multiplying. Intracellular survival in the macrophages and to lesser extent neutrophils is enhanced by suppression of the myeloperoxidase H_2O_2 halide system and production of super oxidase dismutase and catalase against oxidative killing (Walker, 1999). Seronegative infections prior to sexual maturity and first parturition (latency) perpetuate the disease (Nicoletti, 1998). The predilection site for *Brucella* is the reproductive tract of males and females, especially pregnant uterus and reticuloendothelial system (Morgan and McKinnon, 1979; Quinn *et al.*, 1999). Allantoic factors, erythritol and steroid hormones which are present in the placenta and male genital tract of cattle, sheep, goats and swine, but not human, stimulate growth of *Brucella* (Quinn *et al.*, 1999). *Brucella* possesses an endotoxin that contributes to the pathogenesis (Walker, 1999). The biggest problem of

Brucella infection is the uncertain incubation period, which an infected animal can conceal under certain conditions for months and years (Weidmann, 1991).

2.6. Clinical findings

In natural infection, it is difficult to know the duration of the incubation period (from the time of infection to abortion or premature birth), since it is not possible to determine when infection has occurred. Experiments have shown that the incubation period varies considerably and is inversely proportional to fetal development. The more advanced the pregnancy the shorter the incubation period. The period of serologic incubation “(from the time of infection to the appearance of antibodies)” lasts several weeks to several months (Acha and Szyfres, 2001). The period varies considerably and is affected by factors such as gestation, exposure dose, age, vaccination, and other unknown host-resistance influences (Nicoletti, 1980).

There is no effective way to detect infected animals by their appearance. The primary clinical manifestations of brucellosis are related to the reproductive tract. Abortion that occurs in the last trimester is the most obvious manifestation. The quantity of *Brucella* excreted during an abortion could be as much as 10^{12} - 10^{13} capable of infecting 60,000- 600,000 pregnant heifers (Nicoletti, 1980; Fensterbank, 1986). Milk production may be reduced from changes in the normal lactation caused by abortion and delayed conceptions. Not all infected cows abort, but those that do usually abort between the fifth and seventh month of pregnancy. Infected cows usually abort once, but a percentage will abort during additional pregnancies and calves born from later pregnancies may be weak and unhealthy (USDA-APHIS, 2003). Other signs of brucellosis include an apparent lowering of fertility with poor conception rates, retained after births with resulting uterine infection and (occasionally) enlarged arthritic joints. Infection may cause stillbirth or weak calves, retained fetal membrane. Seminal vesicles, ampullae, testicles, and epididymis may be infected in bulls. Testicular abscesses may occur; long-standing infections may result in arthritic joints and hygroma in some cattle (Nicoletti, 1998; Seifert, 1996). In male animal, the epididymides are usually affected, but also the accessory sexual glands reveal painful necrotic tissue degeneration and a decrease in semen quality (Weidmann, 1991).

2.7. Diagnosis

The clinical picture is not pathognomonic, although the herd history may be helpful. Unequivocal diagnosis of *Brucella* infections can be made only by the isolation and identification of *Brucella*, but in situations, where bacteriological examination is not practicable, and diagnosis must be based on serological methods. There is no single test by which a bacterium can be identified as *Brucella*. A combination of growth characteristics, serological and bacteriological methods are usually needed (OIE, 2004). Diagnosis of brucellosis is based on the disease history of the herd, epidemiological observations, serological examinations and demonstration of causal agent considering all these methods must be considered in diagnosis of brucellosis (Sharma *et al.*, 1996). Diagnosis of brucellosis is made possible by direct demonstration of the causal organism using staining, immunofluorescent antibody, culture, animal inoculation and polymerase chain reaction (PCR) and indirectly by demonstration of antibodies using serological techniques (Alton *et al.*, 1975; Weidmann, 1991; Corbel, 1997; Quinn *et al.*, 1999).

2.7.1. Microscopic examination

Gram staining of fetal stomach contents from aborted fetus, placenta, vaginal discharge, and semen reveal gram-negative cocci, coccobacilli and small rods. In the modified Ziehl-Neelsen method, smears are made from fetal membranes, fetal stomach contents, vaginal swabs and semen. The smear is dried and fixed over a flame, stained with carbol fuchsin for 10 minutes, washed in tap water, flooded with 0.5 % acetic acid for 30 seconds, washed thoroughly and counter stained using 1% methylene blue for 20 second, *Brucella* stain red with a blue background (Alton *et al.*, 1975). Care must be taken in the interpretation of result as other infectious agents with similar morphology such as *Coxiella burnetii* and *Chlamydia psittaci* may pose confusion (Quinn *et al.*, 1994).

2.7.2. Bacterial culture

Cultural examinations are very important in the epidemiology of bovine brucellosis, in which positive results are conclusive and should be the basis of evaluation of all other diagnostic methods. Biotype identification is sometimes useful for investigation on possible sources of

infection (Nicolleti, 1980). Identification of species is carried out by the cultural growth requirements, colony morphology, staining character, agglutination with specific sera and phage typing (Quinn *et al.*, 1994; Sharma *et al.*, 1996). Materials for laboratory examination should be cooled immediately or frozen and transported to the laboratory as quickly as possible in leak-proof containers (Alton *et al.*, 1975). Appropriate samples for culture are stomach contents, lung, spleen, and meconium of aborted fetus, fetal membrane, vaginal swabs, blood, milk and lymph nodes (Supramammary, submaxillary, internal iliac). Isolation and identification of the *Brucella* is the only diagnostic procedure not subject to equivocation; however, negative culture result is not sufficient to rule out infection (Weidmann, 1991). Culture media are classified into primary media and selective media; primary media in turn are divided into solid and liquid media (FAO/WHO, 1986). Solid Media include nutrient agar, serum dextrose blood agar, commercial media (tryptose agar, trypticase Soya). liquid media either trypticase Soya broth, tryptose broth, or other commercial selective media prepared by addition of antibiotics such as cycloheximide, bacitracin, polymixin B, and chemical ethyl violet on serum-dextrose or serum-potato agar or any of the basal media (Alton *et al.*, 1975; FAO/WHO, 1986; Corbel, 1990).

B. abortus requires supplementation of 5-10% CO_2 for growth, but *B. melitensis* does not require supplementation of CO_2 (Quinn *et al.*, 1999). Optimum temperature and pH for growth of *Brucella* are 37°C and 6.6-7.4, respectively, but growth can occur between 20°C – 40°C ; after 3-5 days incubation on selective serum agar colonies are pinpoint, smooth, glistening, bluish, translucent about 3-4mm in diameter (FAO/WHO, 1986; Quinn *et al.*, 1999).

2.7.3. Immuno-fluorescent antibody method

An anti-*Brucella* serum conjugated with two-resicin isothiocyanate fluorescent antibody is used. This is then applied on a smear on a thin microscope slide, which is fixed by acetone for 10 minutes. The preparation is allowed to react for 30 minutes to 1 hour at 37°C in closed humidity container. If *Brucella* is present in the smear, a green-yellow fluorescent appears (Alton *et al.*, 1975)

2.7.4. Biochemical method

The organism is catalase and oxidase positive, (*Brucella ovis* and *Brucella neotomae* are oxidase negative) reduce nitrate to nitrite (except *Brucella ovis*), rapid urease activity, some strains *Brucella abortus* produce H₂S while others do not. *Brucella melitensis* does not produce H₂S, *Brucella* does not cause haemolysis on blood agar, does not produce acid on agar containing glucose, and does not ferment lactose; usually grow in the presence of basic fuchsin and thionin at standard concentration (Corbel and Morgan, 1984; Quinn *et al.*, 1999). Generally, *Brucella* usually hydrolyze urea but some strains may not, after three to four days of cultivation, examination of the media /culture/ for growth is required; since *Brucella* is fastidious, most recovery is made within 7-14 days and the media should be kept at least for 35 days before discarding as negative (FAO/WHO, 1986; Quinn *et al.*, 1999).

2.7.5. Animal inoculation

Animal inoculation is the most sensitive method for detection of *Brucella* and is sometimes necessary when very low number of organisms is present. Guinea pigs are the most sensitive laboratory animals, two guinea pigs are inoculated IM 0.5-1.0 ml of suspected tissue homogenate and are sacrificed at 3 and 6 weeks post inoculation and serum is taken along with spleen and other abnormal tissue for serology and bacteriological examination, respectively (Walker, 1999).

2.7.6. Serology

Diagnosis of brucellosis in cattle is frequently difficult to achieve because the serologic response in cattle infected with *Brucella abortus* is influenced by several factors, including long and variable incubation period, cattle vaccination status, type of exposure (Vaccinated, infected or challenged) and stage of gestation at the time of exposure to infection (Lord *et al.*, 1989).

The perfect serological test is the one, which would detect infection early in the incubation stage of the disease, discriminate antibodies due to vaccination and field infection and not subject to cross reaction due to non specific antibodies. However, such an assay is not available at present, although multiple tests increase the confidence in the diagnosis, and sequential testes over time provide a better insight than single test (MacMillan, 1990).

The diagnosis of brucellosis is best established, when the causative organism is isolated from blood and other body fluids in suspected cases. However, the tedious process of bacteriology, fastidiousness, growth requirements, less viability of organisms in the sample during transportation and prolonged incubation are limiting factors to examine a large population as in surveys. Hence, indirect diagnosis through detection of *Brucella* antibodies in the serum is simpler and indicative that the animal has been in contacted (Sharma *et al*, 1996).). A battery of serological tests has been developed for the diagnosis of *Brucella*. Combinations of tests show a degree of sensitivity, which appear to be sufficient to detect infected animals. Frequently, highly sensitive but less specific tests are used for screening purposes and this then are followed by more specific tests for confirmation (Walker, 1999; Garrido-Abellan *et al.*, 2001). The commonly used serological tests are the RBPT, SAT, CFT, ELISA (Quinn *et al.*, 1999).

For the control of brucellosis at the national or local levels the buffered brucella antigen tests (BBATS) i.e. the rose Bengal test (RBT) and the buffered plate agglutination test (BPAT) as well as the ELISA and the fluorescence polarisation assay (FPA) are suitable screening tests. Positive reaction should be retested using a suitable confirmatory strategy. Complement fixation test (CFT) is widely used and accepted confirmatory test although it is complex to perform, requiring good laboratory facilities and adequately trained staff to accurately titrate and maintain the regents. The CFT is very specific. However, like all other serological tests, it could some times give a positive result due to S19 vaccinations or due to false positive serological reaction (OIE, 2004).

Although successful eradication programs have been carried out using SAT or CFT, there has been a concern that these assays may only measure some of the isotopes of antibody. This led to the adoption of the RBT (which has a high sensitivity but a low specificity) as a screening test followed by a confirmatory test such as the CFT with a high specificity but lower

sensitivity (Nielsen *et al.*, 1988). Various authors have compared conventional serological tests used in the diagnosis of *B. abortus* infections to each other or compared the performance of the conventional tests with ELISA (Dohoo *et al.*, 1986).

Rose Bengal plate test

The RBPT is very sensitive test; however, like all other serological tests it could sometimes give a positive result due to S19 vaccination or due to false-positive serological reactions (FPSR). Therefore, positive reactions should be investigated using suitable confirmatory strategies and epidemiological investigation (OIE, 2004).

The Rose Bengal plate test has a wide application as a screening test for individual diagnosis in herds of cattle. It is generally considered oversensitive, especially in cattle immunized with strain -19. For these reasons sera positive in the Rose Bengal test are usually retested by a definitive test, such as the complement fixation test (FAO/WHO, 1986). False-negative reactions occur rarely, mostly due to prozoning and can be avoided by diluting the serum sample or retesting after a given time. Nevertheless, RBPT appears to be adequate as a screening test for detecting infected herds or to guarantee the absence of infection in brucellosis free herds (OIE, 2004).

The antigen consists of *Brucella* stained with Rose Bengal and suspended in buffer at pH 3.65 ± 0.05 . The test is conducted on ruled enamel strips, on a glass or ceramic tile or WHO hemagglutination plate. Equal amounts of serum and antigen (0.03ml) are put on white enamel or plastic trays, mixed, and read after a 4-minutes rocking (Alton *et al.*, 1975; MacMillan, 1990). The test is usually interpreted as positive if any agglutination is apparent, and the reaction may be graded according to its rapidity and appearance (MacMillan, 1990). The RBPT is economical, simple and rapid, and gives few false negative and false positive results (Fensterbank, 1986).

Complement-fixation test

The Complement-fixation test is recognized as the most reliable diagnostic test now in routine use for individual animals (FAO/WHO, 1986). CFT is a widely used and accepted confirmatory test although it is complex to perform, requiring good laboratory facilities and

adequately trained staff to accurately titrate and maintain the reagents (OIE, 2004). The workload resulting from the technical complexity of the complement-fixation test can be greatly reduced by using it only as a definitive test on samples that have been found positive in a preliminary screening test (FAO/WHO, 1986). CFT was found to be 92.9% sensitive and 100% specific in non-vaccinated populations (Dohoo *et al.*, 1986). The CFT detects IgG1 and IgM antibody classes (Fensterbank, 1986).

There are numerous variations of the CFT in use, but this test is most conveniently carried out in a microtitre format (OIE, 2004). Either warm or cold fixation may be used for the incubation of serum, antigen and complement either 37°C for 30 minutes or 4°C for 14–18 hours). A number of factors affect the choice of the method: anti-complementary activity in serum samples of poor quality is more evident with cold fixation, while fixation at 37°C increases the frequency and intensity of prozones and a number of dilutions must be tested for each sample (OIE, 2004).

The CFT is very specific. However, like all other serological tests, it could sometimes give a positive result due to S19 vaccination or due to FPSR. Therefore, positive reactions should be investigated using suitable confirmatory strategies. The CFT is also particularly useful to differentiate between young hood vaccination and infection, as antibodies produced by vaccination react negative six months post vaccination (Morgan and McKinnon, 1979). The EU considers any serum containing 20 ICFTU, or more as positive (Macmillan, 1990; OIE, 2004)

Serum agglutination test

Serum agglutination test, which is the ancestor of all serological tests, is still widely used often in conjunction with the CFT and ring tests (Fensterbank, 1986). While not recognized as a prescribed or alternative test, the SAT has been used with success for many years in surveillance and control programmes for bovine brucellosis (OIE, 2004).

The antigen represents a bacterial suspension in phenol saline (NaCl 0.85 % [w/v] and phenol at 0.5 % [v/v]). Formaldehyde must not be used; EDTA may be added to the antigen suspension to 5 m M final test dilution to reduce the level of false-positive results. Subsequently the pH of 7.2 must be readjusted in the antigen (OIE, 2004). Normally

conducted by making doubling dilution of the serum in phenol saline in a round-bottomed tube and adding an equal amount of (volume) of standard antigen. After mixing, the tubes are incubated overnight at 37°C, and the degree of agglutination is read by comparing the opacity against standards representing various degrees of agglutination (Alton *et al.*, 1975; Macmillan, 1990). The OIEISS contains 1000 IUs of agglutination. The mixture of antigen and serum dilutions should be incubated for 16–24 hours at 37°C. If the test is carried out in microplates, the incubation time can be shortened to 6 hours. At least three dilutions must be prepared for each serum in order to refute prozone negative responders (OIE, 2004).

Although the SAT is widely used, it has the following limitations, detects non-specific antibodies, and does not reach diagnostically significant level during the incubation stage of the disease, inability to detect chronic stage of the disease and inability to differentiate antibodies resulting from natural infection and vaccination (Radostitis *et al.*, 2000). SAT detects antibodies of the classes IgG2 and IgM (Fensterbank, 1986).

The EC antibody level of less than 30 IU in brucellosis-free herds and less than 80 IU in vaccinated are classified as negative. Whereas in the US for vaccinated herds antibody level of 200 IU or more is positive and less than 50 IU negative, for non-vaccinated 100 IU or more is positive and less than 25 IU considered negative (MacMillan, 1990; OIE, 2004). It should be stressed that the serum agglutination test (SAT) is generally regarded as being unsatisfactory for the purposes of international trade (OIE, 2004)

Enzyme linked immuno-sorbent assay

The ELISA test has gained wide acceptance for serological diagnosis of bovine brucellosis because of its ability to detect antibody of all isotypes unlike other conventional testes (Nielsen *et al.*, 1988). The ELISA test can be useful in conjunction with the CFT during the latter stages of an eradication program when it is important to reduce the number of false negative serological reaction (Sutherland *et al.*, 1986). It is a useful test during an eradication program after vaccination has ceased for screening or as a supplementary test to the CFT (Radostitis *et al.*, 2000)

The large majority of ELISAs in use in brucellosis diagnosis are indirect ELISA (iELISA). Antigen is bound to polystyrene micro titer plate so that antibody, if present in a sample,

binds to the antigen and may be detected by anti-globulin-enzyme conjugate combined with chromatogenic substrate giving a color reaction indicative of the presence of antibody in sample (Garrido-Abellan *et al.*, 2001). I-ELISA provides a rapid, simple, highly sensitive (100%) and specific (99.8%) diagnostic system for large-scale detection of antibodies against *B abortus* (Paweska *et al.*, 2002).

The indirect ELISA, while more sensitive than the conventional tests, has been less specific, even using highly specific monoclonal antibodies to bovine immunoglobulin's as detection reagents. Similarly, the indirect ELISA could not distinguish antibody arising either from vaccines or infection and the cost per test is considerably high (Nielsen *et al.*, 1996).

A competitive ELISA more specific than the indirect ELISA and which could discriminate vaccinal antibody from antibody induced by infection is also available. This assay use either O-polysaccharide prepared from lipopolysaccharide or lipopolysaccharide from *B. abortus* as the antigen and a monoclonal antibody specific for an O-polysaccharide epitope for competition. Lipopolysaccharide, because of the hydrophobic lipid a portion of the core region, attaches readily to polystyrene (Nielsen *et al.*, 1996).

Milk ring test (MRT)

An efficient means of screening dairy herds is by testing milk from the bulk tank. Milk from these sources can be obtained cheaply and more frequently than blood. When positive test result is obtained, all cows contributing milk should be blood tested. If necessary samples could be pretreated with preservative (0.1% formalin or 0.02% bronopol) for, 2-3 days at 4°C prior to be tested (OIE, 2004).

The test is carried out by adding one drop (0.03ml) of antigen, (haematoxylin or tetrazolium stained) to 1ml of milk (Alton *et al.*, 1975). The milk/ antigen mixtures are normally incubated at 37°C for one hour, together with positive and negative working standards. Overnight incubation at 4°C increases the sensitivity of the test and allows for easier reading (OIE, 2004). A strongly positive reaction is indicated by formation of a dark blue ring above a white milk column. Any blue layer at the interface of milk and cream should be considered positive.

The milk is negative if the color of the underlying milk exceeds that of cream layer (OIE, 2004).

In large herds (>100 lactating cows), the sensitivity of the test becomes less reliable. False-positive reactions may occur in cattle vaccinated less than 4 months prior to testing, in samples containing abnormal milk (such as colostrums) or in cases of mastitis. Therefore, it is not recommended to use this test in very small farms where these problems have a greater impact on the test results (MacMillan, 1990; OIE, 2004).

2.7.7. Allergic test

An alternative immunological test, the brucellin skin test, can be used for screening unvaccinated herds. Brucellin preparations should not contain smooth lipo-polysaccharide antigen to avoid nonspecific inflammatory reaction or interfere with subsequent serological tests and be standardized, one such is bruellin INRA prepared from a rough strain of *B. melitensis* and standardized antigen preparation (brucellin INRA) is used (OIE, 2004).

The delayed type hypersensitivity reaction, which is mediated by sensitized T-lymphocytes, is more widely used in the diagnosis of brucellosis in sheep and goats than cattle (Morgan and McKinnon, 1979). The technique involves inoculation of 0.1 or 0.2 ml of *Brucella* allergen into the caudal fold or dorsal surface of the ear of goat intradermally. It can also be given at a dose of 0.5 ml subcutaneous or 0.1 ml intradermally in to the upper or lower eyelid (Alton *et al.*, 1975).

The test is well suited to large scale testing and avoids collection of numerous blood samples and it may aid in the interpretation of serological reactions thought to be due to infection with other cross-reacting bacteria and hence highly specific (Alton *et al.*, 1975; Fensterbank, 1986). The allergic test seems to give positive results earlier than serological tests; also, serum antibodies increase titer after the test (FAO/WHO, 1986; Alton *et al.*, 1975).

The drawbacks of allergic test is that it does not differentiate infection from vaccination; animals recovered from infection may be detected as reactor, animals shedding the organism

in the milk or genital secretions may be negative, hence less sensitive (Alton *et al.*, 1975; Morgan and McKinnon, 1979; FAO/WHO, 1986)

The use of dermal hypersensitivity test in brucellosis has been suggested for several reasons including clarification of serological results, earlier detection of infection and for survey (Nicoletti, 1983). The skin test gave valuable information, in combination with the serological tests, in both acute and chronic brucellosis. The skin test discriminated brucellosis clearly from false positive serological reactions due to infections with *Yersinia enterocolitica* O9 (Saegerman *et al.*, 1999; OIE, 2004).

The allergic test is recommended in France for diagnosing ovine brucellosis as part of official prevention policy; three countries Cyprus, Switzerland and the former USSR used the test in their control strategy (Fensterbank, 1986; FAO/WHO, 1986).

2.7.8. Molecular diagnosis

Because of their potential to detect very small numbers of organisms, PCR-based assays have been applied recently to diagnose many infectious diseases (Al-Attas *et al.*, 2000). Direct proof of *Brucella* infection is now done by identification of bacteria with well-established PCR (polymerase chain reaction). The high sensitivity and specificity of PCR-ELISA, together with its speed, versatility in sample handling, and risk reduction for laboratory personnel, makes this technique a very useful tool for the diagnosis of brucellosis (Morata *et al.*, 2003).

2.8. Immunity

Brucella is a facultative intracellular organism that survives and replicate in both phagocytes and non-phagocyte cells. The antibody response following infection depends on whether or not the animal is pregnant and on the stage of gestation. Agglutinin and complement fixation antibodies become positive 4 weeks following experimental infection (Radostits *et al.*, 2000). An important problem is the immunity that develops after recovery from an acute infection. The animal mostly will remain with unsterile immunity in females and thus the animals may

remain life long carrier and can excrete the pathogen. This is especially true of those animals, which have developed chronic process of hygroma (Seifert, 1996).

Brucella has both humoral and cellular immune response. Phagocytes play a key role in initiating T-cell response by processing and presenting antigens. T-cell plays a major role in the acquired specific resistance to intracellular bacteria determining the resolution of infection (Tizard, 1996). The S-LPS are the major surface antigen for humeral immune response in smooth strains of *B. abortus*, *B. melitensis*, and *B. suis* (Nielsen, 2002)

2.9. Economic and public health significance

2.9.1. Economic significance

It is estimated that brucellosis causes heavy economic losses in the livestock sector. The economic losses stem from abortion, diminished milk production, cull and condemnation of animals due to breeding failure, endangering animal export trade of a nation particularly when other countries purchase only *Brucella* free animals as West Germany does. EU regulations require that all animals above six months of age be tested. human brucellosis causing loss of man hours and medical costs and government costs on research and eradication schemes (Chukwu, 1987; Mustofa and Nicoletti, 1993) Chukwu (1987) summarized the economic losses of brucellosis to be: 1) Losses due to abortion in the affected animal population. 2) Diminished milk production, *Brucella* mastitis and contamination of milk; 3) Cull and condemnation of infected animals due to breeding failure; 4) Endangering animal export trade of a nation; 5) Human brucellosis causing reduced work capacity through sickness of the affected people 6) Government costs on research and eradication schemes;7) Losses of financial investment

Official estimates put annual losses from bovine brucellosis in Latin America at approximately US \$ 600 million (Acha and Szyfres, 2001). The New Zealand brucellosis eradication scheme found a 10.3% internal rate of return as a result of increased milk yield decreased culling rate and market shares after successful eradication of the disease (FAO, 1998).

Unfortunately, only few controlled studies have been conducted to evaluate the effect of brucellosis on reproductive efficacy and the cost of various control methods. The inflammatory changes in the infected mammary gland reduce milk production by an estimated 10% (FAO/WHO, 1986).

Brucellosis creates a serious economic problem for intensive and extensive animal production systems in the tropics (Seifert, 1996). Losses in animal production due to bovine brucellosis can be of major importance primarily, because of decreased milk production in aborted cows. The common sequel of infertility increases the period between lactation and in an infected herd, the average inter calving period may be prolonged by several months. In addition to the loss of production, there is a loss of calves and interference with the breeding program due to culling of valuable cows. This is of greater importance in beef herds where calves represent the sole source of income high incidence of temporary and permanent infertility results in heavy culling, because of acute metritis, following retention of the placenta (Radostits *et al*, 2000).

At the time when the American bovine brucellosis eradication program began in 1934, 11.5% of adult cattle were infected with brucellosis and the estimated annual economic loss was US \$ 100 million. Similarly, in Sweden prior to the eradication of brucellosis in 1957 the annual economic loss was estimated well above US \$ 8.8 million (Schwabe, 1984). Official estimates put annual economic losses due to bovine brucellosis in Latin America at approximately US \$ 600 million (Acha and Szyfres, 2001).

The disease has considerable impact on the economy through reduced milk and meat production, restriction in international live stock trade, and diminishes the animal's working power (Unger *et al.*, 2003). In America, annual losses from lowered milk production, aborted calves and pigs, and reduced breeding efficiency have decreased from more than \$ 400 million in 1952 to less than \$ 1 million today. In America, studies have shown that if brucellosis eradication program efforts were stopped the costs of producing beef and milk would increase by an estimated \$ 80 million annually in less than 10 years (USDA-APHIS, 2003). In intensive dairy production, system of the tropics incidence of infection up to 80% has been reported. The annual economic loss due to bovine brucellosis was 150 million

French Franc, in cote d'Ivoire, 233.88 million US \$ in Nigeria and 33.4 million US \$ in Kenya, Tanzania and Uganda (Chukwu, 1987; Seifert, 1996).

2.9.2. Public health significance

Occurrence in humans

Zoonoses are of public health importance and they are acquired through direct contact or through consumption of contaminated animal products & are an occupational hazard. With increasing demand for milk and derived products, the possible spread of milk borne diseases also increases. This is particularly the case for brucellosis, causing severe disease symptoms in humans.). The significance of bovine brucellosis as a zoonosis has even increased in recent times. Some of the reasons for these are the expansion of international commerce in animal and animal products, increasing urbanization with growing numbers of animals in close proximity to people, increasing tourism (consumption animal products), and new methods of cattle production involving higher animal concentration, which is a main factor in the spread of the disease (Weidmann, 1991; Seifert, 1996).

Human brucellosis is widely distributed all over the world, with high endemicity in the Mediterranean, Middle East, Latin America, and parts of Asia (Corbel, 1997). The true incidence of human brucellosis is unknown (Garrido-Abellan *et al.*, 2001). More than 500,000 new cases are reported each year, and according to the World Health Organization, this figure underestimates the magnitude of the problem (WHO, 1997). In Saudi Arabia 7,893 human cases of brucellosis were recorded 74 per 100, 000 inhabitants (Acha and Szyfres, 2001). In Iran 71,051 cases, 13 per 100,000 were recorded in 1988, and it is estimated that 80,000 cases have occurred each year since 1989. In Turkey 5,003 cases 9 per 100,000 were recorded in 1990 (Acha and Szyfres, 2001). The incidence in humans ranges widely between different regions, with values of up to 200 cases per 100,000 populations (Orduña *et al.*, 2000). Human brucellosis as geometrical survey show is known to exist in 37 of 49 (75.5%) African countries (Thimm, 1982).

Particularly in developing countries, prevalence rates in man are often high, because of close contact to animals and traditional food consumption customs (Nicoletti, 1984). However, there is a lack of good data on the occurrence and impact of zoonotic infections including brucellosis in many developing countries (Mc Dermott and Arimi, 2002). There are few health centers in most parts of rural sub-Saharan Africa and even fewer that do any testing for brucellosis. Some of the constraints in laboratory diagnosis, particularly in the pastoral arid and semi-arid, include: (1) long distances of health centers from both pastoralist areas and towns, (2) difficulty in obtaining reagents and materials used for testing (3) low number of qualified technicians, (4) little awareness of the importance of brucellosis, even in areas where its risk is high. Recent reports highlight the advantages of laboratory diagnosis and the problems of both clinical and laboratory diagnosis in relation to other flu-like ailments. In a study conducted in Maasailand, Narok, Kenya reported diagnosis of 55% of flu-like cases as malaria and 21.1% as brucellosis (Muriuki *et al.*, 1997). In a similar study in the same area patients revealing with flu-like symptoms were tested for brucellosis (RBPT), malaria (blood smear test), typhoid (Widal test), streptococcal infections (anti-streptolysin O test) and the rheumatic fever (Rheumatoid factor test) (Maichomo, 1997; Maichomo *et al.*, 1998; Maichomo *et al.*, 2000). Results showed that brucellosis (12%) and typhoid (40%) were more common than expected by health workers. Tests on patients having general malaise or joint pains and/or constant head aches in Kampala, Uganda showed malaria to be the most common cause (73%) but also highlighted that brucellosis (13.5%) was an important contributing disease in such patients (Mutanda, 1998). Table 3 shows the sero-prevalence of brucellosis in man in Africa.

Table 3: Human sero-reactors to *Brucella* antibody in Africa

Country	Number tested	Prevalence%	Serology	Authors and years
Nigeria	13,999	7.6 – 29.8	SAT	Chukwu, 1985
Nigeria	738	5.55	SAT	Chukwu, 1985
Tanzania	540	22.6	SAT	Chukwu, 1985
Uganda	3,164	6.4	SAT	Chukwu, 1985
Djibouti	108	6.5	CFT	Chantel <i>et al.</i> , 1994
Eritrea	130	7.1	CFT	Omer <i>et al.</i> , 2000
Eritrea	21	4.6	CFT	Omer <i>et al.</i> , 2000
Eritrea	105	3	CFT	Omer <i>et al.</i> , 2000

The occurrence of human brucellosis in Ethiopia was documented as early as 1972-1974 after the isolation of *Brucella* organisms from 3-blood culture (Efrem, 1981). The same author had reported serological and clinical brucellosis in a 12-year-old girl from the highland area of Bale who suffered from fever, chills, sweat, headache, backache, epistaxis and weakness. The patient had been drinking raw milk; her sister had similar illness, and died afterwards. Upon physical examination the spleen and liver were enlarged, fortunately the patient recovered after 3 weeks of treatment (Efrem, 1981).

In Abernossa ranch of in Oromia region Zewdu (1989) reported a seroprevalence rate of 8% in farm attendances. Sero-epidemiological study of brucellosis in occupationally exposed persons in Addis Ababa abattoirs enterprise and different dairy farms showed that an over all

seroprevalence rates of 4.8%. This is an indicative for presence of the disease in Ethiopia. The extent of the prevalence of the disease in human brucellosis expected to be higher, given that there is a high human–animal contact (Kassahun *et al.*, 2006).

The possibility of infection occurring by drinking of infected milk necessitates pasteurization of milk by officially approved methods .Pasteurization render naturally *Brucella* contaminated raw milk safe for human consumption. However, most cases in humans are occupational and occur in farmers, veterinarians, and butchers. The organism can be isolated from many organs other than the udder and uterus, and the handling of carcass of an infected animal may represent sever exposure. The importance of the disease in humans is an important justification for its eradication (Radostits *et al.*, 2000).

Table 4: Human *Brucella* antibody sero-reactors in Ethiopia

Authors and years	Total tested	Number positive	Prevalence%	Serology
Taye, 1991	8	1	12.5%	RBPT,CFT
Gebreyesus, 2001	49	12	24.5	RBPT
Kassahun, 2004	38	2	5.8%	RBPT, CFT
Tolossa, 2004	126	3	3.4%	RBPT, CFT
(Kassahun <i>et al.</i> , 2006	336	16	4.8	RBPT/ 2MET

Brucellosis is most common in rural areas and among those involved in animal husbandry it also occurs in urban settings when animals are kept in compounds around houses and among meat-packers, dairy workers and veterinarians (Smits *et al.*, 1999).

Each human case originates from animals (Mustofa and Nicoletti, 1993). Human infection is frequently the first indication of brucellosis occurrence in the animals (Weidmann, 1991).

The disease in humans

B. abortus, *B. melitensis* and *B. suis* are highly pathogenic for humans, causing acute febrile illness-undulant fever, which may progress to a more chronic form and can produce serious complication affecting the musculo-skeletal, cardiovascular and central nervous system (OIE, 2004). Each human case originates from animals (Mustofa and Nicoletti, 1993). Human infection is frequently the first indication of brucellosis occurrence in the animals (Weidmann, 1991). Brucellosis is a true zoonosis and the incidence of *B. abortus* in humans is directly influenced by the incidence in animal species (Nicolletti, 1980).

In general, the incubation period is one to three weeks, but may some times be several months. The case fatality rate is low and usually less than one percent. The disease is septicemic with sudden or insidious onset and is accompanied by continued, intermittent or irregular fever. The symptomatology of acute brucellosis like that of many other febrile diseases includes chills and profuse sweating. Weakness is an almost constant symptom and any exercise produces pronounced fatigue. The common symptoms are insomnia, sexual impotence, constipation, anorexia, headache, arthralgia (joint pain) and general malaise. The disease has a marked effect on the nervous system evidenced by irritation nervousness and depression (Acha and Szyfres, 2001). Symptoms and signs are nonspecific, and may simulate several other febrile illnesses such as glandular fever, influenza, malaria, and enteric infections (Smits *et al.*, 1999).

Source of infection and transmission to human

Brucellosis in humans is probably always acquired from infected domestic animals through direct contact or indirectly by ingestion of animal products and by inhalation of air borne agents. The disease may be transmitted from man to man, but is rare if ever it occurs. (WHO,

1997; Acha and Szyfres, 2001). Transmission of infection to humans occurs through skin abrasions following direct contact with tissues, blood, urine, vaginal discharges, aborted fetuses or placentas. Food born infection occurs following ingestion of raw milk and other dairy products, but rarely from eating raw meat from infected animals. Occupational airborne infections in laboratories and abattoirs have also been documented. Accidental inoculation with live vaccines (such as *B. abortus* stain 19) can also occur, resulting in human infections. There are also case reports of congenital infection in humans (FAO, 2003).

Due to its heterogeneous and poorly specific clinical symptoms, the diagnosis of brucellosis always requires laboratory confirmation, either by isolation of the pathogen or by demonstration of specific antibodies (Morata *et al.*, 2003). On the other hand, blood culture sensitivity is often low, ranging from 50 to 90% depending on disease stage, *Brucella* species, culture medium, quantity of circulating bacteria, and the blood culture technique employed (Yagupsky, 1999). At present, there is a battery of serological tests, which can be used for diagnosis of human brucellosis, although each has its own important limitations. Their sensitivity is poor in the early stage of the disease, during which the levels of antibodies can still be low, and their specificity is reduced in areas where the disease is endemic, in exposed professionals, and in the frequent relapses of the disease (Morata *et al.*, 2003).

Many serological tests have been used for the diagnosis of human brucellosis. The most commonly used tests are the serum agglutination test (SAT), the Coombs anti-*Brucella* test, the Rose Bengal test, and complement fixation (Orduña *et al.*, 2000). The detection of *Brucella* specific immunoglobulin M (IgM) antibodies allows the diagnosis of patients with brucellosis at an early stage or acute disease and may help to discriminate between patients in the early phase of brucellosis and those with chronic brucellosis. In countries where the disease is highly endemic, a large proportion of the population may have persistent *Brucella*-specific IgG antibodies. Under such conditions, the detection of specific IgM antibodies is important to make the laboratory diagnosis of brucellosis in the early phase of the disease. Specific IgM antibodies can be detected by SAT performed in the presence of either 2-mercaptoethanol (2-ME test) or dithiothreitol (SAT-DTT) and by ELISA (Smits *et al.*, 1999). The ELISA IgM and IgG tests achieved a specificity and sensitivity of 100% and 96% respectively, while the positive and negative predictive values were 100% and 94%

respectively. The *Brucella* ELISA is a reliable and sensitive test in the diagnosis of brucellosis. The test is rapid, easy to perform and can be automated (Osoba *et al.*, 2001).

In situations where appropriate diagnostic facilities are lacking, a colorimetric test so called dipstick assay with two horizontal bands an antigen band consisting of reactive *Brucella* antigen (lower band) and an internal control (upper band) could be employed. It is an easy-to-perform method for the quick sero-diagnosis of acute human brucellosis. Due to its robustness and simplicity, the assay is highly suitable for application under field conditions. Ideally, application of two dipsticks, one for the detection of specific IgM antibodies and another for the detection of specific IgG antibodies, would be needed to cover the possibility of both acute or recent and chronic brucellosis (Smits *et al.*, 1999). The PCR-based assay has several advantages over the current microbiological methods for the diagnosis of brucellosis, including speed, safety, high sensitivity (100%) and specificity (88%), therefore, it should be considered (Al-Attas *et al.*, 2000).

Modern therapy has considerably reduced the diseases duration as well as the incidence of relapses (Walker, 1999; Acha and Szyfres, 2001). Without adequate and prompt antibiotic treatment some patients develop chronic brucellosis syndrome with many features of chronic fatigue syndrome (WHO, 1997).

The best drug recommended by WHO is the combination rifampicin at a dosage of 600-900 mg daily with doxycycline at 200mg daily. Both drugs are given in the morning as a single dose and relapse is unusual after a course of treatment continued for at least 6 weeks (FAO/WHO, 1986).

2.10. Control and prevention

The probability of success in any zoonotic disease control/eradication program considers the following pre-requisites; effective method for stopping or reducing agent transmission, high socio-economic importance, epidemiological features that allow good case detection and good surveillance for measuring progress and providing information (Robinson, 2003). The control and prevention measures to be adopted on brucellosis should be realistically based on thorough understanding of local and regional variations in animal husbandry practices, social

customs, infrastructure and epidemiological patterns of the disease (Mustofa and Nicoletti, 1993).

2.10.1. Chemoprophylaxis

Brucella is sensitive to treatment however; treatment is unsuccessful, because of the intracellular sequestration of the organisms in lymph nodes, the mammary gland and reproductive organs. *B. abortus* are facultative intracellular bacteria, which can survive and multiply within the cells of macrophage system. Treatment failures are considered not to be due to the development of antimicrobial resistance, but rather to the inability of the drug to penetrate the cell membrane barrier (Radostits *et al.*, 2000). Treatment of infected cattle with antibiotics is not recommended because of the high treatment failure rate, cost, potential risk of maintaining infected animals, and antibiotic residues in cheese production (Walker, 1999). However, tetracycline and dihydrostreptomycin have been used to treat *Brucella* (Walker, 1999).

2.10.2. Vaccination

Vaccination of exposed herds with inactivated or live attenuated vaccine may be temporarily accepted that carrier animals remain in the herd or in the neighborhood. Under these conditions, the socio-economic situation of the animal holder and the situation of the veterinary infrastructure do not yet allow an elimination of carrier animals (Seifert, 1996)..

Live attenuated vaccine

B. abortus strain vaccine. The most widely used vaccine for the prevention of brucellosis in cattle is the *B. abortus* strain 19 vaccine, which remains the reference vaccine to which any other vaccines are compared. It is used as live vaccine and is normally given to female calves aged 3 and 6 months as single subcutaneous dose of $5-8 \times 10^{10}$ viable organisms. A reduced dose of 3×10^8 to 3×10^9 organisms can be administered subcutaneously to adult cattle, but some animals will develop persistent antibody titers and may abort and excrete the vaccine strain in the milk

Alternatively, it can be administered to cattle of any age as two doses of $5-10 \times 10^9$ viable organisms, given by the conjunctival route; this produces protection without a persistent antibody response and reduces the risks of abortion and excretion in milk (OIE, 2004).

B. abortus strain RB51 vaccine. This has been recently developed and has replaced *B. abortus* strain 19 in a number of countries as the approved calf hood vaccine because it does not interfere with serological evaluation (Walker, 1999). *B. abortus* strain RB51 is a live stable rough mutant of *B. abortus* strain 2308, which lack much of the lipopolysaccharide O-side chain and has been investigated as an alternative to strain 19 vaccines (Radostite *et al.*, 2000).

Killed vaccine

Killed *B. abortus* 45/20 vaccines. This vaccine is prepared from killed rough *B. abortus* strain 45/20 in oil adjuvant; it lacks the smooth LPS antigen that interferes with serological diagnosis. It can be used to animals of all age and to pregnant cows. The duration of immunity by 45/20, vaccine has not yet been established (FAO/WHO, 1986). In Australia, the vaccine is applied for the anamnestic test in order to identify carrier animals in which case simple test methods do not detect incomplete antibodies. After application of 45/20, such hidden reactors produce agglutinating antibodies (FAO/WHO, 1986; Seifert, 1996).

Two doses administered 6 weeks apart in animals over 6 months of age are required with *B. abortus* 45/20. Adult cow vaccination is some times performed as a regulatory effort to control infection in a herd (Walker, 1999). An indication for the application of inactivated vaccines exists, where a systematic brucellosis control scheme is planned for infected herds. Under those conditions, one would start to vaccinate the weaned calves regularly with strain 19 vaccine and protect the adult animals with inactivated vaccine until young immune animals can replace these (Seifert, 1996).

2.10.3. Test and slaughter

When proceeding from a control to an eradication program, test and slaughter program is necessary (Aiello, 1998). All breeding cattle in the herd are tested and those, which are positive, are culled and sent to slaughter. This removes infected cows from the herd and reduces exposure and transmission within the herd. Of particular importance is the detection and removal of infected cows prior to parturition (Radostits *et al.*, 2000). This is made possible when the sero-prevalence is reduced to less than 2% (Walker, 1999; Weidmann, 1991). Animals, which are positive to both RBPT and CFT, are slaughtered.

2.10.4. Hygienic prophylaxis

These include safe disposal of aborted fetus, disinfection of contaminated areas and education of the public not to drink raw milk or eat products made from unpasteurized or otherwise untreated milk (Weidmann, 1991; Mustofa and Necolitte 1993; Bayleyegn, 2004)

Experience shows that vaccination alone cannot bring about the eradication of the disease. From the epidemiology point of the disease, important steps were derived at an early stage, as hygienic prophylactic measures. These include: 1) The isolation of calving animals in separate calving pens, which are subsequently disinfected with 2-5% formalin. 2) Wet and well-grassed calving camps should be avoided and vehicles used for transporting infected animals should be disinfected after use. Aborted fetuses, placentas and uterine discharges must be disposed of, preferably by incineration. 3) All cattle, horses, and pigs brought to the farm should be tested, isolated, for 30 days and retested. 4) Cows, which are in advanced pregnancy, should be kept in isolation until after parturition, since occasional infected cows may not show a positive serum reaction until after calving or abortion. 5) Replacement stock should be purchased from herd free of brucellosis. 6) Chlorhexidine gluconate is an effective antiseptic against *B. abortus* and is recommended for washing the arms and hands of animal attendants and veterinarians who are exposed to contaminated tissues and materials (Weidmann, 1991; Bishop *et al.*, 1994; Radostits *et al.*, 2000).

2.10.5. Prevention and control in humans

The most rational approach for preventing human brucellosis is the control and elimination of infection in animal reservoirs, as has been demonstrated in various countries in Europe and Americas. The direct human health benefits of the American bovine brucellosis eradication program during its early years may be a good example for this. In 1947, 6321 cases of human brucellosis were reported for the country (America). This number had been reduced by more than 95% to 252 in 1966 (Schwabe, 1984; Acha and Szyfres, 2001). Some human population may be protected by mandatory milk pasteurization. Prevention of infection in occupational groups (cattle men, abattoir workers, veterinarians and others who come in to contact with animals or their carcasses) is more difficult and should be based on health education, the use of protective clothing, wherever possible, and medical supervision (Acha and Szyfres, 2001). Ranchers, farmers, or animal managers should clean and disinfect calving areas and other places likely to become contaminated with infective material. All individual should wear sturdy (strong) rubber or plastic gloves when assisting calving or aborting animals, and scrub well with soap and water after ward. Precautions against drinking raw milk and eating of unpasteurized milk by products is also important. Ultimately, the best prevention is to eliminate brucellosis from all animals in the area (USDA-APHIS, 2003). Great care should be exercised, when working with infected tissues and cultures in the laboratory. All *Brucella* cultures should be handled following bio safety level 3 practices because of the potential for laboratory infection. All laboratory procedures should be performed in a manner that prevents aerosol formation (Baron *et al.*, 1994; Quinn *et al.*, 1994; Walker, 1999). Protecting refrigerator plant and slaughterhouse workers against brucellosis is particularly important because they constitute the occupational group at highest risk. Protection is achieved by separating the slaughter area from other sections and controlling air circulation. Employees should be instructed in personal hygiene and provided with disinfectants and protective clothing (Acha and Szyfres, 2001).

The immunization of high-risk occupational groups is practiced in the former Soviet Union and China. In the former Soviet Union, good results have apparently been obtained with the use of a vaccine prepared from strain 19 of *B. abortus*, applied by skin scarification. Annual revaccination is carried out for those individuals not reacting to serologic tests. In china, an attenuated live vaccine made from *B. abortus* strain 104M is applied per cutaneously. These

vaccines are not used in other countries because of possible side effects. Promising trials have also been conducted in France with antigenic fractions of Brucella (WHO, 1997; Acha and Szyfres, 2001). Routine screening testes should be introduced in health institutions, since contact between human and animal products in Ethiopia is high. Owners with infected animals should be advised to pasteurize milk and milk products derived from infected animals before consumption and carcass from clinically and serologically positive animals must be boiled, stewed, roasted before consumption. Health education and publicity campaigns should be a part of control programs (Eshetu *et al.*, 2005).

3. MATERIALES AND METHODS

3.1. Study area

A sero-prevalence study on brucellosis was conducted in two districts (Dibate and Wembera) that are located in Metekele administrative Zone, Benishangul Gumuz Regional state in Northwestern part of Ethiopia .Metekele. Administrative zone is bounded by Kamash Administrative Zone of Benishangul gumuz in the South and south west, in the East and North East by Amhara Regional State and To the West by Republic of Sudan. The Zone has seven administrative districts.

Topographically the Zone is composed of lowland, plains, high land (Wembera district), a few mountains and gorges. The altitude of Metekele Administrative zone ranges from 580-2731 masl, lowlands fall from 1000masl in Pawe to about 600masl near the Ethio-Sudan border.

The rainfall is unimodal and obtains high rainfall from May to October and the annual rainfall of Metekel Zone is 1000 mm in average. The mean minimum and maximum annual temperature is 17⁰C and 32⁰C, respectively.

The study area is covered with a wide range of tree species, specially bamboo thickets, and broad-leaved deciduous woodlands and acacia woodlands. The vast proportion of the river Nile (river Abay) stretches to the south-west part of the study area and river Belese is one of the largest tributary of river Abay is found in this area. The main economic activity in Metekele administrative Zone is mixed crop-livestock system. Cattle population of Metekle Administrative Zone (seven Districts) is estimated to be 193,914, of which 100,589 (52%) were found in Dibate and Wembera districts (CSA, 2003).The two districts were selected for study due to the presence of high cattle population relative to other districts. The dominant animal species in Metekele Zone is cattle, followed by goats. Cattle are used to generate income; they are used for ploughing and are sources of meat and milk. The Map of study area is depicted in the Figure 2.

Figure 1: Map of Ethiopia

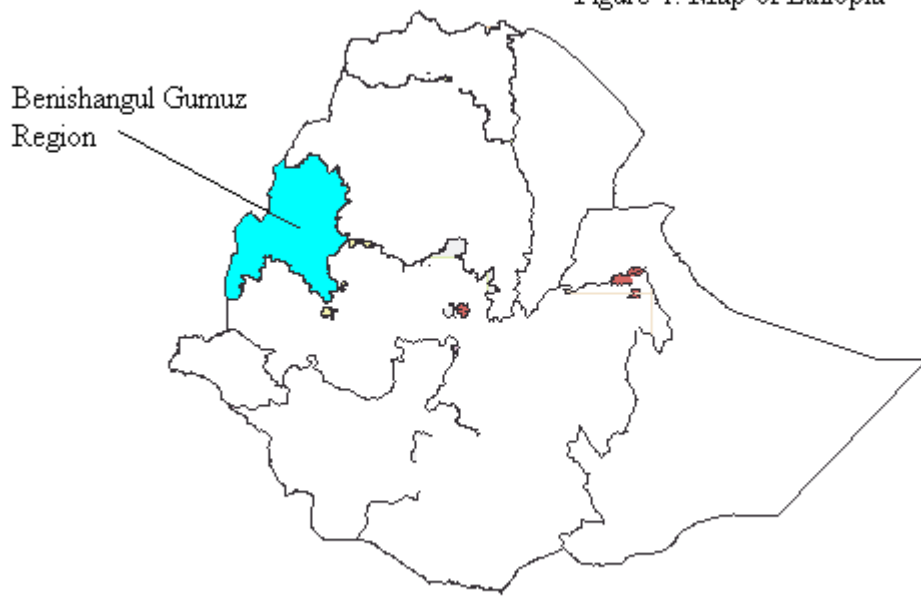


Figure 1: Map of Ethiopia

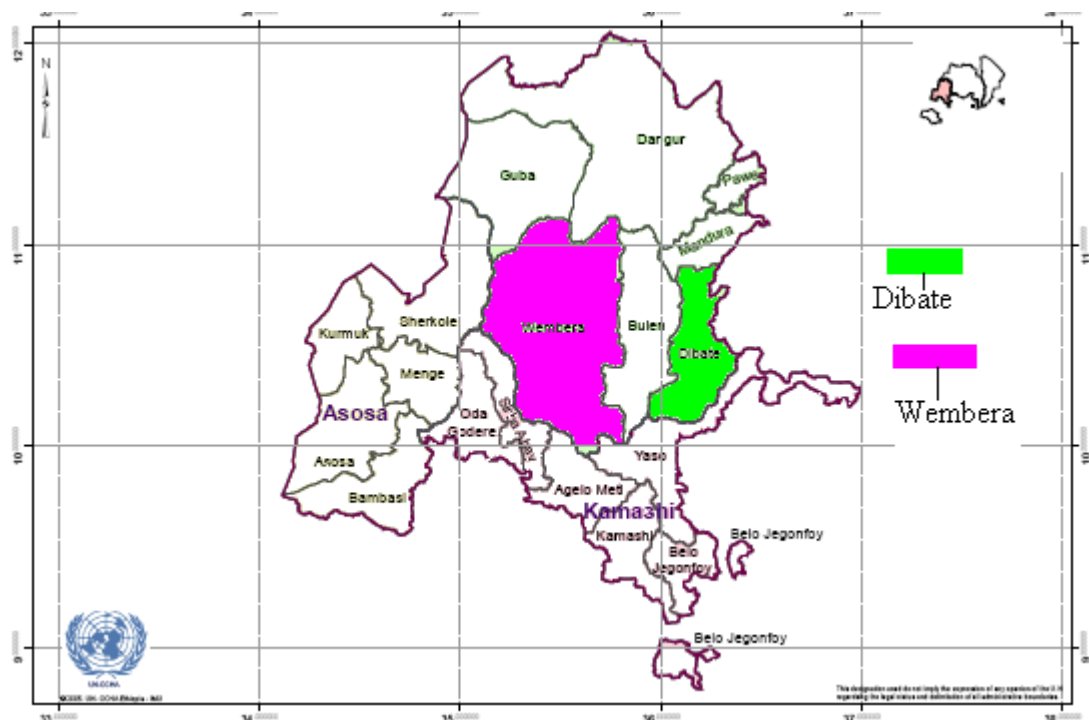


Figure 2: Map of Benishangul Gumuz

3.2. Study animals

Our study animals were cattle found in the districts of Dibate and Wembera in the Benishangul Gumuz Region.

The total cattle population of Metekele Administrative Zone was estimated at 193,914 and Dibate and Wembera districts harbor 100,589 cattle, (CSA; 2003) which form our target population. The 12 Peasant associations (PAs) involved in our study had 36000 cattle head, which formed our study population.

3.3. Study design

3.3.1. Prevalence study

Cross-sectional study was conducted to determine the overall prevalence, herd level prevalence and within herd prevalence. Herd is the total number of cattle population kept in a household.

3.3.2. Sample size and Sampling method

The sample size was determined using cluster sampling formula as described (Thrusfield, 2005).

$$Ts = \frac{g \times 1.96^2 \times p_{exp} \times (1-p_{exp})}{g \times d^2 - 1.96^2 \times Vc}$$

Where,

Ts = Total sample size

p_{exp} = prevalence

g= cluster (12 PAs)

d= desired absolute precision

Vc= between cluster variance

Accordingly, the estimated sample size was 1152 cattle based on the expected bovine brucellosis prevalence of 8.2% (Gebreyesus, 2001), with 95% confidence level, 2% desired absolute precision (d^2) and 12 peasant associations which were predetermined to be selected as primary sampling units(g). The between cluster variance (V_c) for each cluster was not available and thus, determined by guessing the standard deviation (i.e. the average difference expected between an individual cluster prevalence and the overall mean cluster prevalence). Squaring the standard deviation helps to estimate the variance component between clusters (Thrusfield, 2005). Therefore, the standard deviation was 0.021577 and squaring it gave the variance 0.0004656.

To determine sampling unit, the sampling method employed was two-stage cluster sampling. Peasant Associations (PAs) and individual cattle owners were used as the primary and secondary sampling units, respectively. Accordingly, the sampling frame consisted of a list of 34 PAs (primary units) in the two districts. Twelve PAs were randomly selected by a lottery system. The average number of cattle kept in the study area (extensive production system) was assumed to be ($n= 7$) cattle. A total of 164 cattle owners (secondary units) from 12 PAs were selected. Thirteen cattle owners from each PAs were randomly represented, from which all cattle in the household greater than six months of age were sampled. This gave as a total sample of 1152 cattle.

The sample sizes for human risk groups was calculated using simple random formula (Thrusfield, 2005). The expected prevalence was assumed to be 10% and 95%-confidence level and 5% absolute precision were used sample size calculation.

The sampling technique employed for human risk groups was purposive sampling technique, which included farmers, animal health assistants and Butchers. The animal health assistants and butchers sampling was undertaken at their respective working sites while for farmers the serum samples were collected at the respective districts of health centers that were located in the study area.

Blood sample was collected from 138 high-risk groups (106 Farmers, 15 animal health assistants and 17 butchers) in collaboration with *District* health offices. Relevant risk factors (removal of retained fetal membrane, drinking raw milk and eating raw meat) and clinical manifestations (recurrent fever, chills with night sweats, fatigue, muscle and joint pain,

headache, weakness, and anorexia) pertaining to brucellosis were also gathered during serum collection.

$$n = \frac{1.96^2 \times P_{\text{exp}} (1-P_{\text{exp}})}{d^2}$$

Where, P_{exp} =expected prevalence

n=required sample size

d = desired absolute precision

Thus, the calculated sample size was 138 human risk groups

3.3.3. Questionnaire survey

Sixty randomly selected livestock owners from the extensive production system were interviewed using pre-tested structured questionnaire to determine husbandry and management risk factors, which are known or thought to influence the spread and maintenance of brucellosis. These included source of stock replacement, movement of cattle in search of feed and water, dry season watering point, occurrence of abortion, handling of calving and abortion. With regard to the public health significance of the disease, the presence of symptoms suggestive to brucellosis in humans (fever, sweat, anorexia, malaise, weight loss, depression, headache and joint pain), the habit of consumption of raw (unpasteurized) milk, contact with aborted animals and aborted materials were also incorporated in the questionnaire. This result was then compared with serological results.

3.3.4. Serological tests

About 9ml of blood sample was collected from jugular vein of each cattle greater than 6 months of age from the selected cattle using plain vacutainer tubes and needles. The blood samples were allowed to clot at room temperature overnight. Next morning sera were removed (separated) from clotted blood by siphoning them in to sterile cryovials. Finally, sera samples were kept at -20°C until tested by Rose Bengal Plate Test (RBPT) and Complement fixation Test (CFT).

Rose Bengal plate Test

All serum samples collected were screened using RBPT. The antigen consisted of a suspension of *Brucella abortus* obtained from (Institut Pourquier 326, Rue de la Galera 34097 Montpellier Cedex 5, France) inactivated by heat and 0.5% phenol, adjusted to pH 3.65 and colored with Rose Bengal. The procedure described by Alton *et al.* (1975) was used. Briefly, test sera and antigen were left at room temperature for half an hour before the test. Then 25µl of each test serum was taken and placed on a clean glass slide, after which 25 µl of RBPT antigen was added to the side of each test serum using a dropper. Then, the antigen and test serum mixed thoroughly by an applicator. The slide was rocked by hand for 4 minutes and, the results were read by examining the degree of agglutination in good light source. Interpretation: After four minutes rocking, any visible agglutination was considered as positive (OIE, 2004)).

Complement Fixation test

Complement fixation test was undertaken at the National Veterinary Institute Department of Immunology, and preparation of the reagents was performed according to the protocols recommended (OIE, 2004). The CFT is a widely used and accepted confirmatory test although it is complex to perform, requiring good laboratory facilities, and adequately trained staff to accurately titrate and maintain the reagents. Numerous variations of the test occur, but each may be most conveniently carried out using micro titration plates (OIE, 2004). All sera, which tested positive by the RBPT, were further tested using CFT for confirmation. Standard *B. abortus* antigen S 99 for CFT (CVL, New Haw Weybridge, and Surry KT15 3NB, UK) was used to detect the presence of anti-*brucella* antibodies in the sera. The control sera and complement were obtained from the Federal Institute for Health Protection of Consumers and Veterinary Medicine Berlin, Germany and 2% RBC from National Veterinary Institute, Debre Zeit, Ethiopia.

Sera with strong reaction of approximately 100% fixation of the complement (4+), at a dilution of 1:5; Sera with about 75% fixation of the complement (3+), at a dilution of 1:10; Sera with about 50% fixation of the complement (2+) at a dilution of 1:20; Sera with about 25% fixation at a dilution of 1:40 (1+) were classified as positive (OIE, 2004).

4. DATA ANALYSIS

The data obtained from both serological tests and questionnaire were entered in to a computer on Microsoft excel spreadsheet. Statistical analysis was performed using statistical package for social sciences (SPSS), version 11.5 for windows. Cattle tested positive to both RBPT and CFT serially were said to be sero-positive. Clusters (herds) having at least one sero-positive cattle were considered to be positive. The individual animal level sero-prevalence was calculated based on RBPT and CFT positive results divided by total number of animals sampled. Similarly, herd sero-prevalence was computed as the number of herds with at least one positive animal divided by the total number of herds tested. The within herd sero-prevalence was calculated by dividing the number of positive reactors in the herd by total number of animals tested in that herd (Thrusfield, 2005).

Questionnaire data was expected to provide some information about the existence of brucellosis. Accordingly, the data included risk factors associated with source of cattle replacement, movement of cattle during dry season in search of feed and water, method of disposal of fetal membrane and dry season watering point were administered. The results obtained from questionnaire were compared with that of serological results.

The association between different risk factors (categorical variables) and the sero-prevalence was assessed using chi-square (χ^2) test. Those risk factors, which showed an association with the prevalence of bovine brucellosis, were further analyzed by logistic regression (univariable and multivariable logistic regression). To calculate the OR with its 95% confidence interval statistical analysis of continuous variables (age of cattle, herd size, and parity number) were performed by univariable logistic regression. Those factors, which were significantly associated with sero-positivity at univariable analysis, were further tested in multivariable analysis. Odds ratio (OR) was calculated to see the degree of association of the risk factors with the occurrence of brucellosis. The 95% confidence interval and $p < 0.05$ were used to assess the significance of the risk factors in relation to seropositivity.

5 .RESULTS

5.1. Sero-prevalence of bovine brucellosis

Individual animal level prevalence of bovine brucellosis in the two study districts was 1.22% (14/1152) On the basis of RBPT while it was 1% using CFT (11/1152). Out of the 14 sera samples tested positive for RBPT, 11 (79%) were confirmed to be positive with CFT and only 3 (21%) were found to be negative (false positive sero-reactors to RBPT). The agreement number between the two tests was 87.9%.

On the basis of CFT, the overall individual cattle level sero-prevalence in the study area was 1% (11/1152). Out of 164 herds, examined 4.9% had one or more sero-reactore cattle (Table 5). Within herd sero-prevalence ranged between 0% and 15.4%. While considering only positive herds (n=8), it varied from 5.3%- 15.4%.

Table 5: Individual (n=1152) and Herd (n=164) sero-Prevalence of bovine brucellosis in Debate and Wembera districts of Metekele Zone, Benishangul Gumuz Region on the basis of CFT result.

District	Individual cattle sero-prevalence				Herd sero-prevalence			
	Number of Cattle examined	CFT positive cattle: number and %	χ^2	P-value	Examined herds	CFT positive herd: number and %	χ^2	P-value
Dibate	596	8 (1.3)	0.960	0.227*	80	6 (7.5)	2.31	0.160*
Wembera	556	3 (0.5)			84	2 (2.4)		
Total	1152	11 (1%)			164	8 (4.9)		

Fishers Exact test^{*}

5.2. Risk factors

Administrative Districts

Comparison of the sero-reactor to *Brucella* antibody in the two administrative districts was conducted to ascertain its status in the two districts. The overall individual cattle sero-prevalence in Dibate and Wembera districts were in the order of 1.3% (n=8) and 0.5% (n=3) respectively. The proportion of herds which had at least one positive reactors was 7.5% (n=6) in Dibate and 2.4 % (n=2) in Wembera respectively. However, there was no statistically Significant difference between the two districts with regards to individual cattle level sero-prevalence (P=0.227) and herd prevalence (Table 5).

Sex

Sex has some implication in the epidemiology of brucellosis; accordingly, comparison was made on the sero-prevalence of female and male. The sero-prevalence of females was 1.4%, and that of males was 0%. It was found out that those sero-reactors were only females. The Chi square analysis result showed that prevalence of bovine brucellosis was significantly associated with sex (p=0.022) (Table 6). However, univariable logistic regression analysis showed that sex was not significantly associated with sero-prevalence of bovine brucellosis.

Reproductive status

The reproductive status of females was categorized in to four groups: pregnant, lactating, dry and heifer. No significant difference in sero-positivity among the four groups were observed (p=0.090).

Abortion (P=0.012) and retained fetal membrane (P= 0.021) were significantly associated to sero-reactor cows (Table 6).

Table 6: χ^2 analysis for possible association of risk factors with prevalence of bovine brucellosis (n=1152 cattle) detected by CFT

Risk factors	number of cattle	Prevalence: number and %	χ^2	P-value
Sex				
Female	794	11(1.4)	5.008	0.022*
Male	358	0 (0)		
Abortion				
Absent	757	8 (1.1)	12.84	0.012*
Present	37	3 (8.1)		
Retained fetal membrane				
Absent	777	9 (1.2)	13.7	0.021*
Present	17	2 (11.8)		
Reproductive status				
Heifer	251	0 (0)	6.5	0.090
Pregnancy	205	5 (2.4)		
Lactation	302	6 (2)		
Dry	36	0 (0)		

*Fisher's Exact Test

Herd size

Herd size is one of the risk factors that affect the occurrence of brucellosis. In this study highly significant difference (p=0.000, OR=1.34, 95% CI =1.204-1.520) was observed among different herd sizes. Per One cattle, increase in the herd size the chance of acquiring brucellosis infection increases by 1.34 times (Table 7).

Univariable logistic regression analysis of the effect of different risk factors on the prevalence is presented in table 7. Accordingly, age of the animal, herd size, abortion and retained fetal membrane were significantly associated with sero-positive cows. The other studied factors (Reproductive status, Parity number, Sex and the two Districts) did not have significant association with sero-positivity.

Table 7: Univariable logistic regression analysis of the association between risk factors and sero-prevalence of bovine brucellosis detected by CFT

Risk factor	Odds Ratio	95% CI for Odds Ratio	P-value
District			
Dibate	2.508	0.662-9.501	0.176
Wembera	Reference	Reference	
Age			
Age	1.242	1.031-1.495	0.022
Herd size			
Herd size	1.353	1.204-1.520	0.000
Sex			
Male	Reference	Reference	
Female	2.3E+07		0.994
Abortion			
Absent	Reference	Reference	
Present	8.261	2.098-32.532	0.003
Retained fetal membrane			
Absent	Reference	Reference	
Present	11.378	2.26 - 57.21	0.003
Parity number			
Parity number	1.084	0.806-1.457	0.595
Reproductive status			
Heifer	Reference	Reference	
pregnancy	4.0E+07		0.994
Lactation	3.3E+07		0.995
Dry	1.000		1

These factors (age, herd size, abortion and retained fetal membrane) (Table 8) were further analyzed using multivariable logistic regression. The result showed that only herd size and abortion remained to be significantly associated with sero-positivity. The odds of sero-positivity to brucellosis will increase by 1.4 (95% CI OR=1.2-1.5) times by one cattle increase in the herd. Similarly the odds of brucellosis sero-positivity increases by 6.6 (95% CI OR=1.5-29.6) times if abortion is present in the herd than herds no history of abortion.

Table 8: Multivariable logistic regression analysis of the association between risk factors and sero-prevalence of bovine brucellosis detected by CFT

Risk factor	Odds Ratio	95% CI for Odds Ratio	P-value
Age	1.136	0.933-1.383	0.205
Herd size	1.338	1.186-1.510	0.000
Abortion			
Absent			
Present	6.619	1.479-29.636	0.013
Retained fetal membrane			
Absent			
Present	2.161	0.129-36.193	0.592

Questionnaire survey

Sixty randomly selected livestock owners were interviewed with the view to see the association of various management and husbandry risk factors and this was then compared with serological analysis result. The finding revealed that 5% (n=3) of the respondents had sero-positive herds (Table 9). The variation between the respondent of sero-positive herds in the two administrative districts was not significant (p=0.55).

All of the respondents (n=60) kept their cattle on communal grazing land and have used bull service for breeding. The management and husbandry risk factors considered (Source of stock

replacement, way of disposal of fetal membranes, types of housing, Presence of abortion) were not significantly associated to sero-prevalence of *Brucella* antibody.

Table 9: Comparison between serological and questionnaire survey of the two Administrative Districts (Debate and Wembera)

District	Total number of respondents	Sero-positive: herd number and %	X ²	P-value
Dibate (Lowland)	32	2 (6.3)	0.23	0.55*
Wembera(highland)	28	1 (3.6)		
Total	60	3 (5)		

Fisher's Exact Test*=0.55

Study findings in human

From 138 human serum samples tested, none of the farmers, butchers and animal health assistant had *Brucella* antibody in their serum.

Table 10: Chi square test result of management and husbandry risk factors obtained from the Questionnaire survey.

Management and husbandry risk factors	Number of respondents	herd Prevalence: (Number and %)	X ²	P-value
Source of stock replacement				
Market	21	2 (9.5)		
Village breeders	11	1(9.1)	2.8	0.251
Own stock and Village breeders	28	0 (0)		
Awareness about brucellosis				
No	60	3 (5)	-	-
yes	0	0 (0)		
Grazing system				
Communal	60	3 (5)	-	-
Individual	0	(0)		
Mating system				
Natural	60	3 (5)	-	-
AI	0	0 (0)		
Presence of abortion				
No	35	0 (0)		
Yes	25	3 (12)	4.4	0.067
Types of housing				
Fencing with shed	20	0 (0)		
Only fencing	40	3 (7.5)	1.6	0.544
Disposal of fetal membrane				
Burning and burring	17	0 (0)		
Through on the field	43	3 (7)	1.25	0.55

6. DISCUSSION

In the present study, the occurrence of *Brucella* sero-reactors in the two administrative districts (Debate and Wembera) has been investigated in both cattle and humans applying serological analysis and questionnaire surveys.

Over all bovine brucellosis sero-prevalence

In the present investigation the overall individual sero-prevalence of *Brucella* sero-reactor cattle in the two administrative districts (Debate and Wembera), were 1%. This finding agrees with the finding of 1.8% by Fekadu (1999), 0.14% by Tadese (2003), 0.61% by Tolossa (2004) and 1.92% by Kassahun (2004) at individual level.

Herd sero-prevalence

The overall herd sero-prevalence of bovine brucellosis was 4.9%. Tolossa (2004) has reported a herd level sero-prevalence of 14.5% in the extensive production system and Similarly Kassahun (2004) reported herd level sero-prevalence of 13.7% in the extensive production systems, which were higher than our finding.

Agro climate

Comparatively higher sero-prevalence rates were recorded in the lowland district (Dibate) with rates of 1.3% at individual and 7.5% at herd level; sero-prevalence of 0.5% at individual and 2.4% at herd level were found in the highland (Wembera districts). The difference in sero-positivity between the two agro-climates was found not significantly associated at both individual and herd level. However, the numbers of positive cattle were higher in the lowland than the highland. The possible explanation that can be given for high level of sero-reaction in the lowland than the highland is the relatively larger number of cattle kept in the lowland. In the North Eastern Amhara Region, Fekadu (1999) found 3% in the lowland and 0.2% in the highland a similar finding at individual level.

Sex

Univariable logistic regression analysis of the association between sex and prevalence of bovine brucellosis detected by CFT in the study area revealed no significant association between the sex of the animal and sero-positivity ($P=0.994$) (Table 7). However, all sero-positive cattle were females. The reason could be that male animals are kept for relatively shorter time duration than females and thus the chance of exposure is lower for males. Yilkal *et al.* (1998) found higher proportion of female (8%) infected than male (0.11%), though the difference was not significant. Tolossa (2004) has also reported higher proportion of female reactors than males in both extensive and intensive production systems, though the difference was again not significant.

Herd size

In this study highly significant association between herd size and sero-positivity ($p=0.000$, $OR=1.34$) was observed. That means one cattle increase in the herd the odds of acquiring brucellosis infection increased by 1.34 times. Our finding is in accordance with the result of Tolossa (2004) who had reported a significantly higher sero-prevalence with >5 cattle, than those herds having 1-5 cattle under extensive production system. Mussie (2005) found significant variation ($p=0.000$) among the three herd categories: <5 cattle, >5 -10 cattle and >10 cattle in the semi-intensive production. Yilkal *et al.* (1998) have also found significant association between *Brucella* infection and herd size. Kassahun (2004) also reported that in both extensive and intensive systems infection increased as herd size increased from small to large. In Rakungiri district of Uganda, the majority of reactors were detected only in large and medium sized herds (Oloffs, 1996). It is generally accepted that an increase in herd size is usually accompanied by an increase in stocking density and increase in risk of exposure to infection especially following abortion (Nicoletti, 1980; Salman and Meyer, 1984). Stocking density is an important determinant of the potential for transmission between susceptible and infected animals (Crawford *et al.*, 1990; Omer *et al.*, 2000).

Age

In the present study, the prevalence of bovine brucellosis was significantly associated with the age of cattle according to univariable logistic regression analysis but with multivariable logistic regression analysis, the age of cattle was no more significantly associated with the sero-prevalence of bovine brucellosis. It has been reported that susceptibility of cattle to *B. abortus* infection is influenced by age of individual animal. Thus, sexually matured animals and pregnant cattle are more susceptible to infection with *brucella* organisms than sexually immature animals of either sex (Radostits *et al.*, 2000). Tariku (1994) had found higher proportion of old cattle being affected, but the difference among age groups was not found significant.

Reproductive status and sero-positivity

Animals found in the four reproductive phases (lactating, pregnant, dry and heifer) did not show significant difference in sero-prevalence (Table 7). Mussie (2005) has reported that there was no significant difference among the four reproductive statuses ($p= 0.97$). Omer *et al.* (2000) reported comparable finding where high sero-prevalence was found in adult cows irrespective of their lactating or pregnancy status although the proportion of heifers was relatively small. However, sexually mature and pregnant cattle are more susceptible to infection with the organism than sexual immature cattle of either sex (Radostitis *et al.*, 2000). This is because of the localization of *Brucella* in the uterus mostly is associated with pregnancy and the production of erythritol, a carbohydrate found in fetal tissue and possibly to steroid hormones (Nicoletti, 1980; Quinn *et al.*, 1999).

There was no significant association between parity number and sero-prevalence result ($P=0.595$). Mussie (2005) had reported that there was not significant association between parity number and sero-prevalence result in the extensive production system and ranch. However, there was significant association between parity number and sero-reaction in semi-intensive production system. Yilkal *et al.* (1998) had also found a significant association between animals having at least one parturition and in those, which have not.

Retained fetal membrane

The present finding disclosed that not significant association ($p=0.592$) between retained fetal membrane and sero-reactor cattle. Brucellosis is frequently associated with retention of fetal membrane and metritis (Chukwu, 1985).

Abortion

In this study, brucellosis sero-positivity was different among cattle, which experienced abortion than those, which did not ($OR= 6.6, P = 0.013$). Abortion is the most obvious manifestations of brucellosis. Tolossa (2004) reported the significant association between sero-positivity to abortion ($p=0.000$) in the intensive production. Mussie (2005) had also reported that, in semi-intensive production system abortion was found to be significantly associated with sero-prevalence. According to Acha and Szyfres (2001), the predominant symptom in pregnant female is abortion or premature or full term birth of dead or weak calves.

Management risk factors

Sixty randomly selected cattle owners in the extensive production system were interviewed with the view to identify relevant management and husbandry factors associated to the occurrence of the disease under investigation.

None of the management risk factors considered (source of stock replacement, way of disposal of fetal membranes, grazing system, mating system, awareness about brucellosis and type of housing) were found significantly associated to sero-prevalence of *Brucella* antibody. Farmers that used market as the source of stock replacement and disposed the fetal membranes to the field were found to have higher proportion of sero-reactors than farmers that bury the fetal membrane and used own stock as a source of stock replacement. Mussie (2005) reported that ways of disposal of aborted material were not found significantly associated to sero prevalence of *Brucella* antibody. Yilkal *et al.* (1998) likewise reported absence of significant association between disposal of fetal membrane and sero-reactor cattle.

Human

Among 138 human serum samples tested, none of them had *Brucella* antibody in their serum. The study population consisted of 17 butchers (12.7%), 106 farmers (76.8%) and 15 animal health assistants (10.9%). The farmers from whom serum sample were taken did not have any awareness about brucellosis and its source of infection. From the 138 humans sampled for sero-prevalence study of brucellosis, 84.1% humans disposed aborted materials unsafely by throwing it on the field. Moreover, among 138 humans, 82.6% of them consumed raw milk; all these underline the risk of transmission of brucellosis to high-risk group of people. Fortunately, the sero- prevalence of bovine brucellosis in the study area was very low; because of this, sero-positive humans to *brucella* antibody in the health center of the study area was not found. The incidence of brucellosis in humans is clearly correlated with the degree of incidence in the domestic animals. (Weidmann, 1991).The incidence of brucellosis in man is supposed to reflect the incidence of animal disease (WHO, 1986).

7. CONCLUSIONS AND RECOMMENDATIONS

The 1% prevalence of brucellosis in cattle in this study was considerably lower than was expected. The following factors could have contributed to the low prevalence of brucellosis in the study area. (1) The extensive grazing conditions reducing pasture contamination under dry climatic conditions (2) Most of the farmers replaced their animals from their own stock i.e. few bovines were purchased from others.

There was no significant variation in sero-prevalence of bovine brucellosis between the two administrative districts (Dibate and Wembera). Bovine brucellosis sero-positivity was significantly associated with herd size of cattle in the study area. The main clinical sign of brucellosis; abortion was also significantly associated with sero-prevalence of bovine brucellosis. Sex did not show significant variation in sero-prevalence even though all sero-positive cattle were females. The increase in bovine brucellosis sero-positivity was not significantly associated with age. Although there were minor variations among cattle at different reproductive statuses (pregnant, lactating, dry cow, heifer), their difference with respect to sero-reaction was not found significant.

All human sera samples screened for *brucella* antibodies were found negative. This could be due to very low sero-prevalence of cattle in the study area. Seventy two (72) % of interviewed cattle owner in the study area disposed aborted materials unsafely by simply throwing in the field. Moreover, among 138 humans from whom blood samples were taken, 82.6% of them consumed raw milk. These under line the `future risk of transmission of brucellosis to the farmers and the spread of brucellosis in cattle of the study area.

Based on the present study and findings, the following recommendations are forwarded:

- Extension service to aware the livestock owners about the problem and disease transmission should be launched to at all levels.
- For the overall reduction of sero reactor cattle and minimization of the associated risk factors, proper hygienic practices need to be adhered (to proper disposal of fetal

membrane and aborted fetus, proper cleansing and disinfection of the house, use of separate calving pen).

- Brucellosis should be sought as a disease of public health importance and hence, collaboration between veterinary and human health personnel is of utmost important.
- The public in general and high-risk groups in particular should be made aware of the zoonotic importance of the disease through all possible medias available.
- At this stage in time, the only way to protect milk borne transmission of brucellosis is through drinking of boiled milk.
- In order to know the status of brucellosis at the Regional level more comprehensive and systematic studies need to be conducted.

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9. ANNEXES

Annex 1: Questionnaire format for individual owners

Date _____

Region _____ Zone _____ Districts _____

PA _____ name of respondent _____

Age _____ sex _____. Educational status of the respondent _____

Family size _____

Herd size _____

Herd composition/ inventory.

Breeding females

- Dry (non pregnant) -----

breeding bulls-----

- Pregnant -----

Non-breeding males -----

- Lactating -----

Castrated males (ox) -----

- Non-breeding females -----

Young males -----

Heifers -----

Calves (male) -----

Calves (female) -----

Animal acquisition (born, Purchased, gift) and disposed (sold, dead, gift)

Animal type/Cattle	Animal centered		Animal left the herd		remark
	This year	Last year	This year	Last year	
Breeding female(lactating pregnant, Non-pregnant)					
Calf male					
Calf female					
Young male					
Young female/Heifer					
Bull					
Castrated male (ox)					

Management information

1. What type of livestock species are kept in your PA? (Cattle, goat, sheep, equine, camels, others).
2. Where do you get your replacement stock? (Village breeders, market, own herd, other).
3. Do you move your animals to other area in search of feed and/or water? (yes/no)
4. What is your grazing system? (communal, individual, both)
5. What is the water source of your animals in dry season? (river, spring, pond, well, borehole, other)
6. What type of mating do you use? (Artificial insemination, natural, both)
7. What type of house do you use for your animals? (fenced with shed, only fenced, with the family house)
8. Do you have separate calving area? (Yes/no).If yes, where? (separate house, open barn)
9. Do you assist delivery? (Yes/no).If yes, how? (Safe handle pulling, unsafe handle pulling).
10. Do other animals/cattle calve in the same area? (Yes/no).
11. Do you clean the calving area? (Yes/no).
12. Do cows leak calves? (Yes/no).

Disease information

1. What were the most important cattle diseases encountered in your herd during the previous year?
Local name 1 -----2 -----3-----4-----
Scientific name 1 -----2-----3-----4-----
Major symptoms -----
2. Do you know any disease that causes abortion in cattle? If yes, local name -----
scientific name -----
3. Do you know a disease characterized by abortion at the last three month of pregnancy?
Yes/no. if the answer is yes, local name ----- scientific name -----
4. Have you observed abortion or stillbirths in your herds? Yes/no

5. Have you observed retained placenta after abortion or stillbirth? Yes/no
6. Have you observed swelling on knee or testicles in your animals? (Yes/no).
7. How do you dispose aborted materials if animals aborted? (Bury the aborted fetus and after birth, through any where on the field, doing noting, other).
8. Do you know the risk of disease transmission if aborted materials are touched with bare hand? Yes/No,
9. Do you drink milk? Yes/No
10. If the above answer is yes, in what form do you drink milk? a) Raw b) boiled c) sometimes raw sometimes boiled
11. Do you know any diseases, which can transmit by drinking raw milk? Yes/No. if yes, local name ----- scientific name -----
12. Do you slaughter animals at home? Yes/No. if yes, local name ----- scientific name -----
13. Do you have the habit of raw meat consumption? Yes/ No.
14. Do you know any diseases, which could be transmitted by eating raw meat? Yes/No. if yes, local name ----- scientific name -----

Annex 2: Questionnaire format for high-risk human groups

Date _____

Region _____ . Zone _____ Wereda _____

PA _____ Name of respondent _____

Sex _____ Age _____ Educational status _____

Information about factors

1. What is your occupation? A) Farmer b) Butcher c) Veterinarian d) dairy person
2. Do you have cattle? Yes/no.
3. Have you observed abortion or stillbirths in your herds? Yes/no
4. Do you assist delivery? Yes/no. if the answer is no, who assist? a) Veterinarian. b) Other farmer who has experience c) no delivery assists
5. How do you dispose the aborted fetus and the fetal membrane if the animal aborts? a) Bury the aborted fetus and placenta. b) Burn the aborted fetus and placenta c) Leaving in the field.
6. Do you know the risk of disease transmission if aborted materials are touched with bare hand? Yes/No.
7. Do you drink milk? Yes/No
8. If the above answer is yes, in what form do drink milk? a) Raw b) boiled c) sometimes raw sometimes boiled
9. Do you know any diseases, which can transmit by drinking raw milk? Yes/No. if yes, local name ----- scientific name -----
10. Do you slaughter animals at home? Yes/No.
11. Do you have the habit of raw meat consumption? Yes/ No.
12. Do you know any diseases, which could be transmitted by eating raw meat? Yes/No. if yes, local name ----- scientific name -----

13. did any member of the family sick with disease that show the following symptoms?.

Symptoms	Yes	No
Fever		
Headache		
Night sweat		
Backache		
Chills		
Fatigue		
Anorexia		
Weakness		
Joint pain		
Insomnia		
Pain of tests		

Annex 4: CFT test procedure

The test proper, multiple sera technique

The sera were prediluted to 1: 2.5 and incubated at 58⁰ c in a water bath for 3 minutes in order to inactivate the native complement.

- 25 µl of diluted test sera were placed in wells of first and second rows of U- bottom plates and 25µl of VBD was added to all except those of the first.

- Serial doubling dilution were then made by transferring 25µl of serum from the Second row onwards continuing for at least four dilutions

- 25µl of antigen diluted to working dilution excluding those of the anti Complementary controls, which received 25µl VBD instead.

- 25 µl 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 75µl total volume in each case before hemolytic system was added.

- The plate were sealed, incubated at 37⁰ C for 30minutes with agitation

- 25µl of sheep red blood cell suspension was added to each well .The plates were sealed and

reincubated at 37⁰ c for 30 minutes with agitation. Before reading the result the plates were left in the refrigerator at +4⁰ c for 1-hour in order to allow none lysed cells to settle.

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

Positive reactions were indicated by sedimentation of sheep red blood cell and absence of hemolysis. Negative reactions were indicated by heamolysis of sheep red blood cell.

10. CURRICULUM VITAE

Name: Kassaye Erkihun Adugna

Address: Benishngul Gumuz Bureau of Agricultural and Rural Development

Assosa

Nationality: Ethiopian

Date of birth: January 12 1969

Place of birth: South Gondar

Marital status: Single

Education: 1978 – 1982, elementary education. Tagel primary school, Wegda Town

1983 – 1988, Junior and High School at Tagel Secondary school, Wegda Town

1989– 1994, Ukraine Agricultural State University, Faculty of Veterinary Medicine -Kiev

Qualification: DVM from Ukraine Agricultural State University in 1994

Work experience: -As Veterinarian in Benishngul Gumuz Regional State, Metekel Zone office of

Agriculture from 1996-2002.

From 2003- 2004 Team leader of animal production development and Animal health service, In Dibate district

As Regional veterinarian in Benishngul Gumuz Regional State Bureau of Agriculture from 2005-2006

Current duties and responsibilities: Animal health service team leader in Benishngul Gumuz Regional State Bureau of Agriculture-Assosa

On work Training

Training on basic Microbiology in Berlin Germany (2001)

Training on Tsetse fly survey and control Methodology in Bedele, Ethiopia

Language skills

Amharic: fluent written and spoken

English: fluent written and spoken

Russian Language: fluent written and spoken

11. SIGNED DECLARATION SHEET

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

Name: _____

Signature: _____

Date of submission. _____

This thesis has been submitted for examination with my approval as academic Advisors

Dr. Girma Zewde. _____.

Dr. Getahun Ejeta _____.