ADDIS ABABA UNIVERSITY
SCHOOL OF VETERINARY MEDICINE

Seroprevalence of Brucellosis in Small Ruminants and Humans in Amigna District Selected Peasant Association, Arsi Zone, Oromia Regional State, Ethiopia

By

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SEROPREVALENCE OF BRUCELLOSIS IN SMALL RUMINANTS AND HUMANS IN AMIGNA DISTRICT SELECTED PEASANT ASSOCIATION, ARSI ZONE, OROMIA REGIONAL STATE, ETHIOPIA

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Contents

LIST OF ABBREVATIONS

BPAT	Buffered Plate Agglutination Test
CDC	Center for Disease Control
CFSPH	Center for Food Security and Public Health
CFT	Complement Fixation Test
CSA	Central Statistical Agency
DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immuno Sorbent Assay
FAO	Food and Agriculture Organizatio
FPA	Fluorescence Polarization Assay
IgG	Immunoglobulin G
IgM	Immunoglobulin M
Masl	Meter above Sea Level
MRT	Milk Ring Test
MZNT	Modified Zeihl Nelsons Test
OIE	Office International de Epizootic
PAHO	Pan American Health Organization
RBPT	Rose Bengal Plate Test
SAT	Serum Agglutination Test
SRBCs	Sheep Red Blood Cells
TSA	Tryptose Soy Agar
WHO	World Health Organization

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ABSTRACT

A cross sectional study was undertaken in selected five peasant associations found in South East Ethiopia. Amigna district, Arssi Zone, from October 2010 to March 2011 to determine the seroprevalence of Brucellosis in small ruminants and human risk groups (farmers). Sera samples were collected from 100 households and 700 small ruminants (350 goats, 350 sheep). The sampling include small ruminants above 6 month of age and human risk group who had close contact with small ruminants, visited the district's Health Center and had suspected on brucellosis. The sera samples were screened using modified Rose Bengal test (MBRPT), and there was no detection of anti brucella antibodies in 700 animal and 100 human blood samples. During the questionnaire survey, it was found that 28% replace the stock with own stock, 22% from village stock, 50% from both stock. Among the total households 73 owners (73%) used unsafe methods to disposal fetal membrane, whereas 27 (27%) applied safe disposal of fetal membrane. Although antibodies were not detected in sera of animals and humans in the area, there is a human practice favorable for the transmission of the disease from animals to humans. In line with these results, there should be strict regulation in preventing introduction of brucellosis to the area; and humans should avoid practicing risky activities so that transmission of the disease to humans will be prevented.

Key words: *Brucelosis, cross sectional, seroprevalence, Rose Bengal test*

1. INTRODUCTION

Small ruminants are important domestic animals in tropical livestock production systems. About 21% of the world small ruminant population is found in Africa. The population of sheep and goats in Africa represents 17% and 30%, respectively, of the total world population. Small ruminates provide a number of advantages to the producer. They are source of food (Milk and meat), fiber (wool and skin) and cash in a form of saving. Their adaptability to a broad range of environment, short generation cycles and high reproductive rates that leads high production efficiency made small ruminant production an attractive enterprise. Under pastoralist and agro pastoralist production systems, both species of small ruminants could be kept as a source investment and as an insurance against disasters (Devendra and Mc Leroy, 1990; Ibrahim, 1998).

According to recent data, about 26.12 million sheep are estimated to be found in the country, out of which about 74 percent are females, and about 26 percent are males. According to the survey result, the number of goats reported in the country is estimated to be about 21.71 million, out of which about 69 percent are females and 31 percent are males (CSA, 2008).

Brucellosis is a febrile disease of humans described by Hippocrates as early as 450 A.D. Besides being one of the most important zoonoses in the tropics, it is also a disease of domestic animals with important economic consequences (Seifert, 1996).

Infection is often due to occupational exposure and essentially acquired by the oral, respiratory or conjunctival routes. But ingestion of dairy products constitutes the main risk to

the general public. There is also occupational risk to veterinarians, abattoir workers and farmers who handle infected animals and aborted fetus and placentas (Seifert, 1996).
Brucellosis is one of the most easily acquired laboratory infection. Laboratory manipulation of live culture or contaminated materials from infected animals is hazardous as in handling large volume of Brucellosis. Brucellosis in sheep and goats is primarily caused by one the three biovars of *B. mellitensis,* sporadic infection caused by *B. abortus* or *B. suis* have been observed in sheep and goats, but such cases are rare. Pathologically and epidemiologically *B. mellitensis* infection in sheep and goats is very similar to *B. abortus* infection in cattle. In most circumstances the primary route of transmission of Brucelloss is the placenta, fetal fluids and vaginal discharges expelled by infected ewes and goats when they abort or have full term parturition. Shedding of *Brucella* is common in udder secretion and semen; *Brucella* can be isolated from various tissues, such as lymph nodes of the head, spleen, uterus, epididimitis, testes and arthritic lesions (Siefert, 1996).

Brucellosis is a group of closely related disease caused by the members of the genus *Brucella* in animals and humans. Especially *B. mellitensis is* an infectious bacterial disease that can affect most domestic animals, but goats and sheep are susceptible. The bacteria cause a severe debilitating disease in people. B. *ovis* causes infertility in sheep but do not spread to people or other animals. *B. abortus* can, although very rarely, infect sheep and goats. It occurs in small ruminants in Latin America, Southern Europe, the Middle East, Central Asia and Africa. Abortion in late pregnancy, retained placentas, birth of weak offspring and mastitis are the most common signs in newly infected flocks. There may be no signs, or sporadic abortion in flock that has been affected for some time. Goats become persistently infected and can shed the bacteria in their milk and their products throughout their lifetime. Healthy and asymptomatic carriers are source of infection. Other signs include death of weak offspring, low weaning weight, decreased milk production, orchitis and epididymitis and reduce fertility. Goats and sheep infect themselves by licking aborted fetus, placentas, new born offspring, vaginal discharge or by consuming contaminated materials. Respiratory acquired infection can occur when animals or wind disturb contaminated dust. Milkers can spread the infection through unsanitary milking practice. The disease spread to humans from

infected animals through raw milk, unpasteurized products processing meat from infected goats and contact with aborted kids or infective reproductive secretions inhaling contaminated dust and aerosols contact with carcasses or handling wool from infected animals and infected people. This disease is a major cause of direct economic losses resulting from clinical disease and emergency of slaughtering of the infected animals for animal industry. It causes a barrier for the international trade of live animals by being used as impediment through animal movement and export (Maria, 2006; Coelbo *et al.*, 2007).

The eradication of brucellosis is an important goal of public health programs in many affected countries. Ruminant brucellosis may be eradicated by means of adequate testing and slaughtering programmes. However, in areas with high prevalence of the disease, extensive grazing systems and low socioeconomic conditions vaccination is the only practical way to manage the disease. It is generally agreed that the living attenuated *Brucella mellitensis* rev.1 is the most effective vaccine against brucellosis in small ruminants (Schurig *et al.*, 2002; David and Fech, 2007).

In Ethiopia, prevalence of human brucellosis has been reported by a number of people with rates of 12.5% by Yirgu (1991), 2.4% by Tolosa (2004), 53% by Asmare (2004) and 3.78 % by Hailemelekot (2005); fragment studies have revealed the occurrence of small ruminant brucellosis (Tekelye and Kasali, 1990; Yibeltal, 2005; Mengistu, 2007; Teshale *et al.*, 2006; Ashenafi *et al.*, 2007). Small ruminants play major economic roles in the lowland pastoral areas of the country, where they serve as source of milk and meat. Milk from sheep and goats is consumed raw; therefore, it could be possible to expect human brucellosis caused by *B. mellitensis.*

Small ruminants are associated with rural poor. They play a big role in supporting the livelihood system of poorest livestock keepers especially in marginalized areas. In spite of its livelihood contribution and contribution to the livestock economy, this sub sector received only very small attention in the poor countries and disease of small ruminants affect the

income of small holder farmers in sub-Saharan Africa by reducing productivity or through loss of the animal (Ibrahim, 1999; Sebastian, 2004).

Therefore, the objectives of this project survey are:

- To determine the status of small ruminants brucellosis in Amigna district through serological tests, and identify potential risk factors
- To find out whether there is association between small ruminates brucellosis and reproductive disorders.
- To investigate the public health importance of the disease.

2. LITERATURE REVIEW

2.1. Etiology

Brucellosis is an infectious bacterial disease caused by members of the genus *Brueclla* .It is a disease of worldwide importance and affects a number of animal species. *Brucellae* are small cocci; coccobacillae or short rods. They are 0.5-0.7 by 0.6-1.5 mm, nonsporulating and unencapsulated and not acid fast bacteria which stain gram negative. They are arranged as single and less frequently in pairs, short chain or small groups. The different species cannot be distinguished from each other morphologically (staining moreno *et al.*, 2002).

For culturing *Brucellae* require complex media; they grow best if special peptones, like tryptose and trypticase soya peptone are added to the medium at a neutral pH.; 3-10% CO2 atmosphere with an incubation temperature of 37°C is required. Delicate translucent colonies of 2-3 mm in diameter grow on blood or glucose agar. *B. ovis* grows always in the m (mucoid form); *B. abortus* and *B. mellitensis* grow at the beginning in the s (smooth) form and later dissociate in to the R (rough) and the m form (Seifert, 1996).

The species can be distinguished because of their sensibility to phages, their metabolism and their biochemical structure as far as it correlates with their antigenic characteristics (Seifert, 1996).

Some of the *Brucella* species are further classified into biotypes (biovars). The number of biovars of different species is shown is Table 1. The biotypes within the species are differentiated on the basis of combinations of characteristics. These include carbon dioxide requirements, hydrogen disulfide production, urease activity, grows in the presence of dyes, serological specificity, phage typing, oxidative metabolic profiles (Baron and Petersons, 1994; Quinin *et al.*, 2002).

Table 1: **Species, biovars and reservoirs of brucellosis** (Cultor and Cutter, 2006).

Species of *Brucella*	No of Biovars	Reservoir hosts
B. mellitensis	3	Sheep, goat, cattle
B. abortus	7	Cattle
B. suis	5	Swine
B. canis	1	Dogs
B. ovis	1	Sheep
B. neotomae	1	Rodents
B. pinipediae	Not determined	Otter, seal
B. cetaceae	Not determined	Dolphins, porpoise

In humans, brucellosis can be caused by *B. abortus, B. mellitensis* and *B. suis* biovars 1-4 and rarely *B. canis.* Live vaccines for *B. abortus* and *B. mellitensis* as well as *B. canis* m strain are also pathogenic for humans. *B. ovis*, *B. neotomae* and *B. suis* biovars 5 have not been linked to human disease (Renukaradhya *et al.*, 2002). The species of *Brucella* affecting human beings and the levels of their pathogenecity are summarized is table 2.

Table 2: Typical host specificity of Brucella species and their degree of pathogenicity to human

Brucella species	Animal Host	Human Pathogenicity
B. suis	Swine	High
B. mellitensis	Sheep and goats	High
B. ovis	Sheep	None
B. abortus	Cattle, bison	Intermediate
B. canis	Dogs	Intermediate
Marine species	Marine mammals	Rare
B. neotomae	Rodents	None

2.2. Epidemiology

Factors influencing the epidemiology of *Brucella* infections in any geographic location can be classified into factors associated with transmission of the disease among herds and factors influencing the maintenance and spread of the infection within the herd (Crow Ford *et al.*, 1990). Further factors include host factors (age, sex, breed), agent factors and extrinsic factors (environment factors) including management and ecology influence its transmission and maintenance among and within herds (Nicoletti, 1980; Mikolon *et al.*, 1998).

Brucella affects organs rich in sugar erythritol (breast uterus, epididimis, etc). The organism localized in these animal organs causes infertility, sterility, mastitis, abortion or resides in carrier states. Humans in close contact with infected animals (slaughter house workers, veterinarians, farmers, diary workers) are at risk of being infected with *Brucella* organisms (Abdul and Murray, 2007).

Large and small ruminants and humans are susceptible to *Brucella* spp. whereby the species may infect their main host but may also mutually infect other animal species as well as game; camels, horses and buffaloes are also susceptible. Apparently *B. ovis* is only pathogenic for sheep while *B. mellitensis* and especially *B. aboruts* can mutually infect their main host. They also mostly occur in wild animals. The infection is usually introduced into a herd through latently or acutely infected animals. The infection occurs mostly by ingestion of material which has been contaminated with the excretion of female which has aborted but may also have had a normal birth, through brucellosis has to be considered an important venereal disease. Contaminated feed carry large distances when it is traded. Insects and game animals may also carry the infection over long distance (Seifert, 1996).

2.2.1. *Distribution*

Brucellosis is present in many parts of the five continents but the distribution is rather sparse in these parts of the world. This is because of the fact that the disease which belongs to OIE

list of disease remain insidious, in which many countries with limited resources give priority to more other specular diseases like foot and mouth, sheep pox, rift valley fever, peste depetit ruminant, caprine contagious pleuropneumonia (Bookirane, 2006).

They create a serious economic problem for the intensive and extensive animal production systems of the tropics. Its occurrence is increasing in developing countries in an even aggravating epizootological situation. This depends on the policy of many developing countries of importing exotic high production breeds without having the required veterinary infrastructure and the appropriate level of development of the socio economic situation of the animal holder. Furthermore, the increasing movements of animals and the trends towards intensification of animal production favour the spread and transmission of the infection. In intensive dairy production systems of the tropics, an incidence of infection of up to 80% can be found. In the extensive animal production systems of the Sahel an average disease incidence of 25-30 has to be calculated (Seifert, 1990). In eastern Sudan (Butana) an infection rate in cattle of almost 22% and 13.6% in cattle and sheep, respectively, was found (Weiset, 1995).

Areas currently listed high risk are the Mediterranean basin (Portugal, Spain, South France, Italy, Greece, Turkey and North Africa), south and central America Eastern Europe, Asia, Africa, the Caribbean , middle east central Asia to India, China, Mongolia and southern areas of the former Soviet Union. The disease also occurs in central and southern America, Mexico, North Europe, North America and Oceania. An unpasteurized cheese, sometimes called "Village cheese," from these areas represents significant risk to tourists (CDC, 2007; Radostits *et al.,* 2000; Quinn *et al*., 2002). Prevalence varies with and among countries, which could be due to different factors such as herd size, management ecology and biology of the disease. The seroprevalence carried out in different African and middle east countries revealed prevalence that ranged from 0.3 % to 21% in goats and 0.6% to 22% in Sheep, in which most prevalence fell less than 10% at individual level (Benkirane, 2006). The prevalence distribution of small ruminant brucellosis due to *B. mellitensis* and *B. ovis* in some African countries is given (Table 3).

Table 3: Prevalence Distribution of *B. mellitensis* and *B. ovis* in some African Countries (FAO, 2002)

Country	*B. mellitensis*	*B. ovis*
Egypt	+	ND
Ethiopia	+	ND
Kenya	+	ND
Sudan	+	+
Somalia	+	ND
Eretria	ND	+
Libya	+	+
Algeria	ND	ND
Tunisia	++	+
Niger	+	ND
Nigeria	+	+
Cotedevour	ND	+
Zimbabwe	+	+
Botswana	ND	+
South Africa	+	+

++ High prevalence; + sporadic low prevalence; ND: No date

2.2.2. *Occurrence of small ruminant Brucellosis in Ethiopia*

In Ethiopia limited seroprevalence studies carried out so far indicated that brucellosis may be one of the important diseases in goat and sheep raised in the country. A serosurveillance carried out in Afar and Somalia region in 2005, clearly demonstrated that the disease exit in Ethiopia. The serosurveillance finding was 14.6% in goats of afar and 1.6% in sheep, 1.7% in goats in Somalia region (Yibeltal, 2005). In another survey in central high lands of Ethiopia, a seroprevalence of 1.5% in sheep and 1.3% in goats were recorded (Tekelye and Kasalic, 1990). The existence of the disease was also confirmed and reported in Southern Nation and

Nationalities and people's regional state, according to an annual report of Sodo Regional Veterinary Laboratory in 2005. The status of small ruminant brucellosis in Eastern Africa is shown in table 4.

Table 4: Prevalence Distribution of Brucellosis in small Ruminants in some East African Countries

Country	Sheep	Goats	Source
Ethiopia: Central High Lands	1.5	1.3%	Tekelye and Kasali, 1996
Afar region	14.6%	1.62	Yibeltal, 2005
Somalia region	1.6%	1.7%	Yibeltal, 2005
Afar Region	3.2%	5.8%	Ashenafi *et al.*, 2007
Borena	1.09% (RBT)	-	Teshale *et al.*, 2006
Konso and AAC	1.6%	3.2%	Mengistu, 2007
Eriterea	1.4%	3.8%	Omer *et al.*, 2000
Kenya	6.01%	-	Waghela, 1976
Somalia	7.2%	5.2%	Falade and Husecinis

AAC = Awassa agricultural college; RBT = Rose Bengal Test

2.2.3. Transmission and source of infection

The Primary route of infection is through ingestion of contaminated feed and water, inhalation during over-crowding, contact through intact skin and conjunctiva. Lambs may be infected while in the uterus or sucking infected milk of their mother. Venereal transmission by infected rams to susceptible ewes appears to be rare. Transmission may occur by artificial insemination (Seifert, 1996). Transmission between animals occurs readily after massive exposure to aborted materials, contaminated placenta and post partum discharge in infected

female. Transmission to human is as result of contact with infected animal carcass, aborted fetus, placenta, consumption of unpasteurized milk and cheese. It is common to observe human case that is in contact with goats in area where active brucellosis out breaks occur. Raw vegetable and water contaminated with the excreta of infected animals can also serve as source of infection.

Brucella organisms can remain viable in milk, water and damp soil for up to four months (Quinn *et al.,* 2002). The main source of infections is milk and milk products, and aborted materials.

B. abortus and *B. mellitensis* are usually transmitted between animals via contacts with placentas fetus, fetal fluid and vaginal discharges from infected animals. Animals are infected after either abortion or full term parturition. Entry in the body occurs by ingestion and through the mucous membranes, broken skin and possibly intact skin. Most *Brucella* species are also found in semen; males can shed these organisms for long periods or lifelong. *Brucella* can be spread by fomenter including feed and water (CFSPH, 2007).

The source of infection is an aborting animal, as in cattle, the surroundings where lambs are born to infected ewes or where abortion takes place can become greatly contaminated. Animals may contact brucellsis by oral and coetaneous routes or at birth. Infection by inhalation is also possible when healthy and aborting animals are kept in over eroded pens with poor sanitary measures. Transmission of *B. mellitensis* from flock to flock usually follows the movement of infected pregnant females. However, it can also occur via infected males. Wild animals and dogs may transmit parts of aborted fetuses to other areas (Corbel, 1997; Rodostitis *et al.,* 2000). *Brucella* can be found in milk, urine, feces, placenta, and vaginal secretion that accompany natural birth or abortion. In the case of normal full term births, kids from infected ewes are often infected and capable of spreading the disease (Maria, 2006).

The infection is usually introduced into herd through latently and acutely infected animals. The infection occurs mostly by the ingestion of materials which has been contaminated with the excretion of a female which has aborted but may also have had a normal birth. Although *Brucellosis* also has to be considered an important venereal disease, contaminated feed (hay) can spread the infection from pastures over large distance when it is traded. Insects and game animals may also carry the infection over long distance (Seifert, 1996).

2.2.3. Risk factors

The epidemiological variability of small ruminant brucellosis is related to host factors such as (age, sex and breeds) agent factor and extrinsic factors (Environmental Factors), including management, ecology, population and biology of the disease. Some risk factors for ovine and caprine brucellosis in the world include lack of control hygiene, breed, uncontrolled animal movements, shared communal pastures and shared watering places (Al-Talafhah *et al.*, 2003).

2.2.3.1. Management practices

Unless the hygienic conditions or measures to prevent the introduction of the disease and the resistance of the animal improved, all together the condition favors rapid spread of the disease and subsequently results in high prevalence of the disease in that particular herd (Nicoleti, 1980; Rodostitis *et al.*, 2000). Management practices directed at eliminating infected animals and minimizing exposure to aborted tissue greatly reduces the incidence of the disease in commercial livestock expiration (Walker, 1999). Management risk factors for high occurrence of infection are high stocking density, poor housing and lambing, lack of sanitary measures (cleaning, disinfection) and large herd sizes (Mikolon *et al.*, 1998; Crawford *et al.*, 1990). Once infected, the time required to become free from brucellosis is increased by large herd sizes, active abortions, and loose housing (Rodostitis *et al.*, 2002) herd sizes and animal densities are directly related to prevalence of the disease and difficulty in controlling infection in a population (Nocoletti, 1980; Walker, 1999).

2.2.3.2. Host factors

Susceptibility to infection depends on sex, age, breed and pregnancy of the animals. Younger animals tend to be more resistant to infection and frequently clear infection, though latent infection does occur. Only 2.6% of animals infected at birth remain infective as adults and sexually mature animals are much more susceptible to infections regardless of sex. Most animals infected as adult remain infected for life. After reaching sexual maturity the state of pregnancy has a greater influence on the degree of susceptibility than age (Nicoletti, 1980).
It is widely accepted that susceptibility increases with sexual development and pregnancy (Nicoletti, 1980; Morgan *et al.*, 1969). All breeds of sheep appear to be comparable in susceptibility to brucellosis (Rodostitis *et al.*, 2000); susceptibility between breeds has not been reported. Rather there are varying degrees of individual resistance to infection, which are dependent upon exposure dose, age, vaccination and known host resistance factors (Nicoletti, 1980). The introduction of animals through purchase or importation from prevalent places showed a risk association. On the other side, the frequent disinfecting practices in intensive farming had a protective effect (Rodostitis, 2000).

2.2.3.3. Environment

Brucella survives freezing and thawing. Under proper environmental conditions they survive for up to 4 months in the milk, urine, water and damp soil. The environmental resistance of the pathogens depends on whether they are protected against the radiation of sunlight and against high temperatures. Neutral soil PH and moist environment which is rich in organic material are favorable elements for survival. In liquid manure, the *Brucella* survive for 220 weeks in humid feces, up to 4 months in aborted fetuses and after birth, 44 days in the dust of street, in tape water for 30 days, 51 days in sterile water, up to 2 months in the desert soil, up to 2 years in frozen soil. Contaminated straw remains infectious for longer than a month (Weidmann, 1991; Seifet, 1996).

Brucella organisms do not multiply in the environment but they persist and their survival is influenced by the environmental factors (Radostitis *et al.*, 2000). *Brucella* organisms can survive for longer periods in the environment but does not multiply in the environment (Walker, 1999; Quinn *et al.*, 2002).

2.3. Economic and public health significance

2.3.1. Economic Significance

Brucellosis creates serious economic problems for the intensive and extensive animal production systems of the tropics. *Brucella* infection cause tremendous economic losses through reproductive failure in animals (Siemens *et al.*, 2006).

This infection causes significant losses from decreased productivity loss of trade in much of the developing world. *Brucella mellitensis* is considered to be an emerging pathogen, since it has been eradicated from some nations. But the cost of surveillance to remain *B. mellitensis* free is significantly large (CFSPH, 2007).

The economic loss due to brucellosis is attributed to abortion particularly the milking breeds which is susceptible. The result in loss of the young crop in sheep and goats consequently impairs the breeding patterns- when infection is introduced in to a flock or herd, a storm of abortion will occur, hence act as a source of infection (Gall *et al.*, 2001). The adverse socioeconomic impacts impending the foreign livestock trade and products has great significance in the economic development of livestock owners (Carless *et al.*, 1988). This consequently reduces the revenue of the country.

Brucella mellitensis causes heavy economic losses in animal production resulting from clinical disease abortion, neonatal losses, reduced fertility, decrease milk production, emergency slaughtering of the infected animals. In addition, the disease is an impediment to free animal movement and global trade (Rodostitis *et al.*, 2000).

Since the biological and economic efficiency of sheep production is influenced by the number of lambs reared per ewe, mortality related production losses is very significant, particularly in view of the contribution of sheep to the household economics of the agricultural population of the tropics. Reproduction wastage is normally considered to cover are losses from mating to the first breeding of the off springs. Reproductive wastage is caused by environmental, genetic, disease and management factors which operate with different severity and in different severity and in different combinations (Jones *et al.*, 1980).

2.3.2. Zoonotic importance

Brucellosis is considered by FAO, WHO and OIE as the widest spread zoonosis in the world (MacMillan, 1985). Human brucellosis caused by *B. mellitentis* is the most pathogenic species in man hence, constitutes a public health priority. In China epidemiological studies revealed that the infection rate of human brucellosis was 7.7% in the region with Brucellosis; 84.5% of 634 strains isolated from the patients with brucellosis were identified as *B. mellitensis* (Dohoo *et al.*, 1985). Although it is notifiable disease in many countries, the disease remains underestimated by the medical authorities at official figure do not reflect the number of human infection. Due to lack of awareness adequate laboratory support to detect the infection it is often considered as cause of fever or unknown origin (Benkirane, 2005). The infection occurs by contact with infected meat, aborted material and consumption of unpasteurized milk and cheese (Agris, 2000).

A study conducted in Mongolia indicated that the infection rate was 19.14% for herd men, 20.01% for leather workers, 11.46% for the veterinarians, 12.74% for slaughter, 4.65% for farmers and 0.42 % for students (Baron *et al.*, 1994). The clinical signs in man start with Malaise Chills and fever, 7 - 21 days after infection drenching with sweats in late afternoon, or evening are common with a temperature range of 39-49 ^{0}C man suffers from body aches headaches and anorexia occasionally infection develops in lung, bone marrow, heart or genital-urinary system and diffuse hepatitis focal necrosis (Radostitis *et al.*, 2000).

Table 5: Reported cases of human Brucellosis in selected Mediterranean countries (Seimenis *et al.*, 2006).

Country	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**
Algeria	1616	1227	1345	1296	3439	2779	2493	3933	3200	3262	2783
Bulgaria	(a)	(a)	(a)	(a)	(a)	1	(a)	(a)	(a)	3	(a)
Cyprus	(a)	(a)	(a)	(a)	(a)	(a)	(a)	2	1	7	5
Egypt	256	309	490	872	1006	(b)	2355	2902	3510	4030	4335
France	127	178	100	67	91	48	49	44	(b)	37	33
Greece	158	174	242	240	360	419	539	548	405	331	240
Italy	1120	1285	1370	1896	1681	1461	1324	1268	923	813	631
Jordan	750	770	770	994	953	684	432	268	275	219	159
Lebanon	(c)	(c)	188	302	326	285	191	224	274	290	193
Libya	131	(b)	(b)	(b)	27	29	71	21	(b)	(b)	(b)
Malta	6	8	225	18	1	4	(a)	(a)	(a)	1	(a)
Morocco	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)
Portugal	1166	1189	915	860	866	794	683	507	381	206	139
Saudi Arabia	6985	4929	4929	4800	5781	7468	5913	5320	4865	4687	4534
Spain	2842	2842	2708	2005	21154	1545	1553	1104	924	893	642
Syria	1391	3457	7701	6868	6991	7402	6958	6487	9971	11764	23297
Tunisia	801	472	182	490	291	206	355	183	321	250	128
Turkey	6795	8383	8506	9480	11812	12330	11482	10742	15510	17553	14572

a) Not reported;

b) b) No information available

c) c) High occurrence

The Mediterranean and Middle East Region is considered as the area where zoonoses are most numerous and widespread. The reasons for and the impact of this concentration are: A)

biodiversity B) human and animal movements and migrations. One of the most important zoonotic diseases in this region is brucellosis and its prevalence distribution in the region is summarized in table 5.

Brucellosis is one of the important Bacterial zoonosis being transmitted to humans mainly from ruminants. Because of its Public health importance; it is a major cause of direct economic losses for animal industry and a barrier for international trade of live animals. Thus, the eradication of Brucellosis is an important goal of public health programs in affected countries (Seimenis *et al.,* 2006).

2.4. Pathogenesis

The susceptibility of animals depends significantly on their natural resistance, age, level of immunity and on environmental stress. If infection is introduced into an infected herd in which all animals are immunologically naïve to brucellosis, so called abortions to rams may occur and almost all pregnant cows will abort (Seifert, 1996). The infection with *Brucella* species takes place through the mucosae or the injured and /or intact skin through the oral ingestion of contaminated feed and the invasion of the pathogen via the upper parts of intestinal tract, but infection through the mucosae of the respiratory system or the eyes occurs also frequently. The transmission during mating is important but perhaps not as much as often supposed (Walker, 1999; Seifert, 1996).

Following entry into the host *Brucella* is either free in the extracellular environment or resides 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 myeloperoxidease H_2O_2 halide system and production of superoxidase dismutase and catalase against oxidative killing. Stress protein protects the organism from hydrolytic enzymes and oxygen radicals (Walker, 1999).

After infection of the regional lymph nodes, bactermia of animals occurs which can last for 1-3 weeks and distributes the organism to the lymphatic system, the large parenchyma and other organs and tissue the facultative intracellular organisms may infect all organs and tissues. In pregnant animals the uterus is a preferred site of infection where it leads to a necrotizing placentitis (Radostitis, 2000). In nonpregnant animals, the first infection often occurs in the udder followed by the infection of the uterus later after the onset of pregnancy, the enhanced virulence of the *Brucella* inside the reproductive system is supposed to be the consequence of the increased level of the sugar erythritol which is maintained in the reproductive system. A characteristic exudative and proliferative process develops in the gravid uterus starting from the epithelium of the villi of the chorion (Wilsion, 1963).

The predilection site for *Brucella* is the reproductive tract of male and females' especially pregnant uterus and reticuloendothelial system. Alanthoic factors, erythritol and steroid hormones which are present in the placenta and male genital tract of cattle, sheep, goats and pigs, but not humans stimulate growth of *Brucella.* Although the exact mechanism of abortion is unknown, the likely possibilities are placentitis interfering fetal circulation direct effect of the organism toxin, and fatal stress due to inflammation response in fetal tissue (Walker, 1999; Quinn *et al.,* 2002). Generally visible lesions after abortion are necrotizing placentitis, intercotyledonary thickening with yellow gelatinous fluid and thick brown exudates. Aborted fetuses become edematous and abomasal contents may be turbid and have lemon yellow color (Seifert, 1996).

2.5. Clinical findings

Brucella mellitensis mainly causes abortion, stillbirths and births of weak off springs. Animals that abort may retain the placenta. Sheep and goats usually abort only once, reinvasion of the uterus and shedding of organisms can occur during subsequent pregnancies. Milk yield is significantly reduced in animals that abort, as well as in animals whose udder becomes infected after a normal birth. However, clinical signs of mastitis are uncommon acute orchitis and epididymitis can occur in males, and may result in infertility. Arthritis is

seen occasionally in both sexes many non pregnant sheep and goats remain asymptomatic. *Brucella ovis* affects sheep and can cause orchitis, epididymitis and impaired fertility in rams (Corbel, 1997; Walker, 1999; FAO, 2002).

Brucellosis in ruminants is characterized by lesions which appear mainly in the male animal (orchitis, epididymitis) as well as inflammation of the joints and bursa. But abortion may also occur in the female presenting the typical yellowish, sticky layers on the placenta. The consequences of brucellosis in small ruminants are infertility, high mortality rate in lambs, mastitis and reduced milk production. The lesions on the placenta are more prominent with the *B. mellitensis* infection than when *B. abortus* is the causal agent (Seifert, 1996).

In small ruminants *B. abortus* infection often may be in apparent both in males and females. After *B. ovis* infection epididymitis and inflammation of the vesicular gland are the most prominent symptoms in the male animals; this has a significant influence on the herd fertility (Grillo *et al.,* 2000). Human clinical findings are observed after an incubation period from 2-4 weeks with fever 38°C-40°C. The clinical sign may appear insidiously or abruptly in the simplest case, the onset of influenza like fever reaching 38°C-40°C. Limb and back pains are usually severe. However sweating and fatigue are marked, headache, malaise back pain myalgia and generalized aches. Particularly at night chills and sweats can occur complication including arthritis, endocarditic mycotic aneurysims, sacrolitis, spondilits, chronic fatigue, epididymitis and orchitis, neurological signs (including personality changes such as meningitis and uvetitis and optic neuritis) anemia, internal abscess, nephritis, endocarditic and dermatitis. Deaths are usually caused by endogamies or meningitis (Quinn *et al.,* 2002; CFSPH, 2007).

Abortion generally occurs during the last 2 months of pregnancy and is followed in some cases by retention of fetal membranes. In the male localization in the testis epididymitis and accessory sex organs is common and bacteria may be shed in the semen. This may result in acute orchitis and epididymitis and later in infertility. Arthritis is also observed occasionally in both sexes. Animals generally abort once also reinvasion of the uterus occurs in

subsequent pregnancies and organisms are shed with the membranes and fluids from non pregnant animals exposed to small numbers of organisms may develop immunizing infection or they may become latent carriers. (LH falldosevia the subcutaneous route in young replacement animals) may induce a long lasting serological response to agglutination test that seriously interfere with serological screening for infected animals (Alton, 1975).

The magnitude and duration of this response can be affected by many factors including virulence of the strain, size of innoculations, age, sex, pregnancy, species and immune status of the hosts (FAO, 1989; Zain *et al.,* 1985). It is clear that an animal infected by brucellosis contains high level of IgM, IgG, IG_2 and IGA type of antibodies, however, it should be noted that IgG_2 and IgA antibody levels are considerably lower than the IgM and IgG_1 antibody levels (Nielsion, 1990).

2.5.1. Immunity

Brucella is a facultative intracellular organism that survives and replicate in both phagocytic cells. The antibody response following infection depends on whether or not the animal is pregnant and on the stage of gestation (Farrel, 1974). Aglutinin and compliment fixation antibodies become positive 4 weeks following experimental infection (Hendry *et al.,* 1985). An important problem in immunity that develops after recovery is from an acute infection the animal will remain with unsterile immunity in females and thus the animals may remain lifelong carrier and can excrete the pathogen. This is especially true of those animals which have developed chronic processes of hygroma, inflammation of joints and bursitis (Seifert, 1996). Spontaneous recovery may occur particularly in goats which become infected which are not pregnant (Radostitis *et al*., 2000).

2.5.2. Humoral immunity

Following infection by natural exposure a serological response can be expected within 2 to 4 weeks but the response is variable and may be absent all together. Invasion of the pregnant

uterus can be expected to produce a large and persistence rise of antibodies but this may be delayed until after abortion or parturition at the normal time.

Invasion of the lactating udder causes a lesser serological response and localization confined to a small number of lymph nodes may fail to stimulate any response at all $^{o}_{2}$ only a minimal one the pattern of the serological response in terms of immunoglobulin production has not been extensively studied in sheep and goats but available information suggests close similarity to that in cattle, LH production of Igm followed within a week or two by a predominance of Igm predominating the serological response is transient and sometimes missing is young sexually immature animals Rev.1 Vaccine strain when applied under standard condition .

2.5.3. Cell mediated immunity

After gaining entrance of infection in to the body, The organism encounter the cellular defense of the host and the fate of invading bacteria is mainly determined by the cellular defense of the host In particular macrophages and T. Lymphocytes. The most wide used correlate of CMI are Lymphocyte stimulation macrophage inhibition delayed type hypersensitivity and interferon induction the response of CMI can be expected as rapidly as a few week but the responded is variable and may not be detected Virulence varies between species and strains. Infection occurs mainly through the mucous membrane of the oropharynx upper respiratory tract, conjunctiva and genital tract. In pregnant animals the uterus is invaded resulting in abortion the udder is an important predilection site for the disease. Humoral and cell mediated immune mechanisms are activated in the infected animals (Walker, 1999).

2.5. Diagnosis

2.5.1. Bacteriological methods

2.5.1.1. Microscopic examination

Gram's stain of fetal stomach contents from aborted fetus placenta, vaginal discharge and semen reveal gram negative cocci, coccobacilli, and small rods. In the modified ziel neelsen (mzn) method smears are made from fetal membrane, fetal stomach contents, vaginal swabs and Lamb semen (for *B.ovis*). The smear is dried and fixed over flame, stained with carbol fuchsin for 10 min., ats, washed in tape water, washed in 0.5% acetic acid for 30 seconds and washed thoroughly with counter stain using 10% methylen blue for 20 second. *Brucella* stains red with a blue background. (Weidmann, 1991; Cobel, 1997) and McDermott, 2002).

2.5.1.2. Isolation and Identification

Appropriate samples are stomach content, lungs, spleen and meconium of aborted fetus, fetal membrane, vaginal swabs, blood, milk, lymph nodes (Alton and Forsh, 1975). For *B. ovis* the valuable samples for culture are tail of epididymitis, seminal vesicles, ampula, bulbo urethral gland and scrotal lymph nodes (West *et al.,* 1993).

Brucella mellitensis does not require supplementation of CO_2 for growth. The optimal temperature for growth is 37^0C, but growth can occur between 20^0C-40^0C. *Brucella* mellitensis grows optimally at a PH of 6.6-7.4. The colonies are pin point, smooth, glistening, transparent raised and convex. The organism is catalase and oxidase positive, reduce nitrate to nitrite, usually hydrolyses urease, does not produce H_2S, does not cause hemolysis on blood agar, does not produce acid on agar containing glucose, and does not ferment lactose. The organism usually grow in the presence of carbol fachsin and thionin at a standard concentration (Corbel and Hendry, 1985; Quinin *et al.,* 2002).

2.5.1.3. Molecular techniques

In order to establish a highly specific test for brucellosis a number of genes and /or species specific PCR assays were designed. Molecular technology like polymerase chain reaction (PCR) is a new approach and applied in many diagnostic works to overcome limitation and difficulties in bacterial culture and serological assays. Polymerase chain reaction shows high sensitivity, specificity and overcome the extraneous intervention of mimicry antibodies from source other than actual infection (Radostitis *et al.,* 2000).

2.5.1.4. Serological methods

An ideal serological test would establish an early diagnosis, identifies chronic infections, and distinguishes between antibodies of vaccination and those of infection. A test also needs to be economical, simple, and possible to repeat on numerous occasions. However no serological test possess of these qualities (Frenster, 1986).

Antibody detection is commonly used for diagnosing brucellosis and in control programs. For routine test, anti *Brucella* antibodies are detected in serum, milk and sometimes in vaginal mucus of semen (Walker, 1999). *Brucella abortus* strain 99 usually is used for preparation of antigen. The antigen primary involved in either case is the surface lipopolysaccharide (OIE, 2004). Although several variegate of serological tests are employed for the diagnosis of bovine brucellosis in different countries it has limited to those methods that were used in this study.

The commonly used serological tests are the Rose Bengal test (RBT), serum agglutination test (SAT), complement fixation test (CFT), Combs test, enzyme linked immunosorbent assay (ELISA), Rivanol test, skin delayed type hyper sensitivity (SDTH) test, fluorescence polarization assay (FPA) and the milk ring test (MRT) FAO). Serological tests are used for diagnosis more often than are cultures, but only a positive culture yields definitive diagnosis (Baron and Peterson, 1994; Klaus, 2002).

Rose Bengal test (RBT) and complement fixation test (CFT) are mostly used for screening and confirmatory tests, respectively. The Rose Bengal plate agglutination, complement fixation and indirect ELISA tests are usually recommended for screening flocks and individual animals. The complement fixation test is the only test prescribed for confirmation and international trade (Biancifiori *et al.,* 1996). Samples to be tested include blood, milk, serum (Siefert, 1996; Walker, 1996; PAHO-WHO, 2001).

2.5.3. Animal inoculation

In animal inoculation guinea pigs are the most satisfactory laboratory animals for detecting the presence of small numbers of *Brucella* organism in animal tissues, secretions and excretions or when they are heavily inoculated. The inoculation is done by intraperitoneal or subcutaneous route. In male pig orchitis is developed. When guinea pig is autopsied, lesions are observed in the spleen, lungs and lymph nodes; after 7 days these organs minced.

2.5.4. Diagnosis in humans

For laboratory diagnosis of Brucellsis samples can be either of clinical or environmental origin. Clinical specimens best suited include blood, sera, infected tissues, abscess materials, bone marrow and tissues from spleen or liver, pleural fluid and urine. Environmental samples can be milk, meat and other animal products.

The classical RBT is often used as screaming test. RBT is based on the agglutination of serum antibodies with a stained whole cell preparation of killed *Brucellae*. For confirmation of the Wright or serum agglutination test (SAT), enzyme Linked immunosorbent assay may be used (West *et al.,*1993; Smites and Kadri, 2005).

2.6. Prevention and control

The strategies for preventing brucellosis have to be adapted to the animal production system. Failures of disease control are mostly due to the application of neither a scheme for which

neither the veterinary infrastructure exists, nor the required reliable serological laboratories and the animal holder does not have the socio economic prerequisites (I.M.A, 2000). Prevention and control of *B. mellitensis* infection is important from the point of views of economic losses to the small ruminants industry as well as the important and wide spread zoonoses in the world. The approaches used to control brucellosis include management, immunizations, and test and removal of infected animal (Godfroid *et al.,* 2000).

2.6.1. Management

Mass Vaccination is crucial for the control and eradication of bovine, ovine and caprine brucellosis but other complementary measures that may need consideration include improved farm hygiene, restriction and control of trade and movement animals and isolation and removal of infected animals. Preventive measures essential to minimize the risk of infection of the human population should include improved food hygiene including the pasteurization of milk and protection from infection of high risk groups such as milkers and other people working in the dairy industry. Health education of risk groups through community participation and health education programs could play an important role to increase the acceptance and use of preventive measures (West *et al.,* 1993; Smits and Kadri, 2005).

An area exposed to infected animals and their discharge should be cleaned and disinfected. Infection in other species are generally presented by controlling *B. mellitensis* in sheep and goats. *Brucella* species are readily killed by most commonly available disinfectants, including hypochlorite solutions, 70% ethanol, isopropanol, Iodophores, phenolic disinfectants, formaldehyde, glutaraldehyde xylen. However, organic matter and low temperature decrease the efficacy of disinfectants. Disinfectants reported to destroy *Brucella* on contaminated surface include 2-5 % sodium hypochlorite, 2-3°C caustic soda, 20% freshly slaked lime suspension, 2% formaldehyde solution (Abela, 1999; Schurig *et al.,* 2002). The prevention of human Brucellosis is best achieved by control or eradication of the disease in animals prevention in child health education and pasteurization of milk (WHO, 1997). Currently methods used to prevent infection are test and slaughter of seropositive animals, vaccination, hygiene measures and management (Banai, 2002).

2.6.2. Immunization

Vaccination of exposed herds should be vaccinated with inactivated or live vaccine. It may be temporarily accepted that carrier animals remain in the herd in the neighborhood. Under this condition the socio economic situation of the animal holder and the situation of the veterinary infrastructure do not allow the elimination of carrier animals. Live vaccine gives the best protection against brucellosis since a permanent immune response of the organism against the intracellular bacteria must be attained. This is best stimulated by live but attenuated bacteria which are thus permanently present. However enhancing this vaccines that is a risk of accidental self vaccination of the person and then of subsequent infection of male animals cannot be vaccinated with live vaccine because it may cause inflammation of the sexual gland (Jimentz Debagues *et al.,* 1991).

There are two types of vaccines used for immunization of small ruminants against Brucellosis. These are Rev.1 Live vaccine and H-38 attenuated vaccine. Rive.1 live vaccine was obtained by attenuation of *B. mellitensis* smooth strain. The strain was originally depending on streptomycin for its growth but lost this property up on further culture in mice and guinea pigs. It gives protection to sheep and goats against *B. mellitensis* and to rams against *B. ovis.* The vaccine induces abortion when given during the last trimester of gestation and for this reason the vaccine was recommended to be given prior to the first gestation at 3 to7 months of age. The use of Rev.1 Vaccine in cattle has been investigated proved better immunity than S19 (Schurig *et al.*, 2002). The H-38 is inactivated vaccine for small ruminants produced from attenuated *Brucella mellitensisis* containing aluminum hydroxide as adjuvant and gives good protection but the induced immunity is not as solid as live Rev.1 Vaccine (Alton, 1996; Blasco, 2005). Vaccination of livestock is crucial to the control of Brucellosis. Effective reduction of disease prevalence in livestock through mass vaccination eventually will also lead to a reduction of brucellosis in the human population. However vaccination alone is not sufficient and should be accompanied by other measures such as restriction of animal movement and trade, culling of infected animals and improved

farm sanitation to reduce the further spread of disease. In addition a surveillance system is essential to control the efficacy of control measure to identify out breaks in early stage. Clearly the control of Brucellosis requires significant efforts both in terms of human and financial resource (Schurig *et al.*, 2002).

As mentioned above we can conclude that Rev.1 strain is presently recognized as the best available vaccine for the prophylaxis of brucellosis of sheep and goat. Vaccine Rev.1 confers a high degree of protection and the effect of vaccination is quantitative resulting in reduced and shortened excretion of *Brucella* in case of infection and hence in a reduced transmission and contamination of the surroundings (WHO, 1997).

3. MATERIALS AND METHODS

3.1. Study area

The study was conducted in selected peasant associations in amigna district from October, 2009 to March, 2010. The district is situated in south east Oromia region, 250 km for away from Addis Ababa. It has an altitude of 1380-2537 masl with bimodal unevenly distributed rainfall pattern. The average annual rainfall is 864.35 mm and consisting of short rainy season (February-April) and long rainy season (July-September). The district has minimum temperature of 12.03 °C with a relative humidity of 760. Clay is the predominant soil type with sand (14.4), silt (28.4) clay (57.2) in the proportion, respectively and has PH of 7.9. The predominant land area is suitable for livestock production. Goats in most of the areas are the most selected animals for breeding purpose next to cattle and sheep. There are 19 peasant associations in the area. About 150,506 cattle, 15,396 goats, 48,717 sheep, 7865 donkeys, 4995 horses and 31,284 poultry are found in the district. The total human population is about 79,780 of which 39,880 males 39,900 females which are contained in 9727 households (7357 male and 2370 female) (Amigna district Agricultural office).

3.2. Study animals

The study animals were goats and sheep above six month of age. In addition, human risk groups (especially farmers) who had close contact with sheep and goats and visited the districts health center with other symptoms of disease of brucellosis were included in the study. The predominant goat breed in the study area is Arsi-Bale breed which is managed under extensive farming system. Traditional animal feeding and housing systems are practiced in the area.

3.3. Study design

Cross sectional study was conducted between October 2009 and May 2010 in selected peasant associations of Amigna district to determine the seroprevlaence of brucellosis in sheep and goat. Purposive sampling was conducted to include occupationally related human sampling units (farmers) in the study from those patients visiting the districts health center with different diseases. The sampling involved sheep and goats above 6 months of age with the history of vaccination. Risk factors for seroprevalence of small ruminant brucellosis such as species, age, sex, herd size and pregnancy status were considered during questionnaire survey and blood sample collection.

3.4. Questionnaire survey

We were asked 100 farmers in the study area. The information on potential risk factors of small ruminant brucellosis was gathered using a pretested questionnaire. Risk factors such as species, age, sex, herd size, pregnancy status and some of replacement stock, grazing and breeding were considered. Management system together with reproduction disorders such as history of abortion, still birth and other potential risk factors were recorded. Relevant risk factors for human Brucellosis such as removal of retained placenta or foeteal membrane, consumption of raw milk and milk products, contact with infected sheep/goat were recorded with questionnaire format during blood sample collection.

In order to determine the desired sample size of animals, there was no past knowledge about the previous year's prevalence of brucellosis in the district. Hence, the average expected prevalence rate was assumed to be 50% for the area within 95 % Confidence Intervals (CI) at 5 % desired accuracy. Subsequently, the formula used to calculate sample size (n) was as follows:

$$n = \frac{1.96^2 \times P_{ex} \times (1-P_{ex})}{d^2}$$ ..... (Thrusfield, 1995)

Where n = sample size, d = desired absolute precision (0.05), P_{ex} = expected prevalence (50%), thus the desired sample size for p = 0.5 will be n = 384. But, the sample size was increased to 700.

Proportional determination was employed to include 9727 households in the selected peasant associations found in Amigna districts to obtain a total sample of 700 small ruminants (350 sheep, 350 goats). The primary sampling unit was a flock defined as household having at least one sheep or goat. Out of 19 peasant associations 5 PAS were included in the study purposively. This purposive approach was based on accessibility of flocks and other logistics, for example availability of transport. One hundred sera were collected from human risk groups, who visited the district health center and with the symptoms resembling brucellosis.

3.5. Collection of blood samples

Approximately 5 ml of blood samples was collected from jaguar vein of sheep and goats aged above six month and from non vaccinated ones against *B. mellitensis* using sterile plain vacutainer tube and needles. Blood samples were properly labeled and left for 24 hours at room temperature to allow clotting and the harvested sera were stored at 20°C until tested for the purpose of *Brucella* antibodies.

Blood samples were collected from risk groups (farmers) in collaboration with districts health officers in Adeleee Health Center, south East Ethiopia. About 5ml of blood was collected from the brachial veins of humans in collaboration with the districts health institutions. During blood sample collection from human patients to proper ethical procedures were observed. The serological tests employed were mRBT for screening test.

3.6. *Modified Rose Bengal Plate test*

All serum samples (700 animal and 100 human) were screened using modified Rose bengal antigen which constitutes a suspension of *Brucella mellitensis* (obtained from the Institute pourquier most pellier, France), inactivated by heat and 0.5% phenol adjusted to PH 3.65 and

coloured with Rose bengal. Briefly, 75 ml of serum was mixed with 25ml of antigen on a glass plate and the test tube moved vigorously in a wide circle five to six times clockwise, followed by five to six times anticlockwise. The plate was examined after four minutes; any agglutination was considered as positive (OIE, 2008).

3.7. Data management and analysis

Data obtained from both serological tests and questionnaire surveys were stored in Microsoft Excel spread sheet. Descriptive statistical analysis for *Brucella* seroprevalence rate was carried out using SPSS 15.0 for windows. The seroprevalence, 95% confidential interval (CI) unvariable and multivariable logistic regression were used to analysis associations of various risk factors, such as species, sex herd size, pregnancy status, prevalence of abortion and stillbirth with the seroprevalence of the disease.

4. RESULTS

4.1. Seroprevalence of *Brucella* antibodies

A cross sectional study was conducted between October 2009 and March 2010 in selected peasant associations of amigna Destrict, Arrsi Zone, Oromia regional state, Ethiopia, to determine the seroprevalence of Brucellosis in Sheep (350) and Goats (350) and purposive sampling from related human sampling units with the help of Amigna Health Center. All serum samples were collected strictly and with a careful handling dispatched to National Veterinary Institute were tested with mRBPT (Rosebengal test) and none of the sera were positive for Brucella antibodies. The animal samples were recollected and the serological test repeated considering the potential of sample damage during collection and transportation. However, the were no antibrucella antibodies collected upon retesting as well.

4.2. Questionnaire survey

In all study households 100 were questioned about awareness of Brucellosis, source of replacement stock, and presence of veterinary serivce, breeding system and grazing, cause of abortion, frequent abortion, conceiving problems, cause for sale, keeping dogs, season, purpose of milk, consumption of milk, purpose of meat, consumption of meat., treatment of discussed animals, delivery. From this study according to their individual response 100% (100) had no awareness about brucellosis. In addition, 28% replace the stock with own stock, 22% from village stock, 50% from both stock. Out of the total households 57% had veterinary service, 39% self administered health drug, 4% traditional healers. Among the total households 73 owners (73%) used unsafe methods to disposal fetal membrane, whereas 27 (27%) applied safe disposal of fetal membrane. From the total households 34 owners (34%) the cause of abortion was from disease, 23 owners (23%) from mechanical trauma, 43 owners from both causes.

Table 6. Frequencies and percentage for the questionnaire survey (n=100) of respondent of small ruminant in the study area

Factors	Category	No of respondents	Frequencies (%)
Flock size	1-5	2	**2**
	5-10	32	**32**
	>10	66	**66**
Purpose of milk	Home	36	**36**
	Selling	29	**29**
	both	35	**35**
Consumption of milk	Cooked	55	**55**
	Raw	1	**1**
	Both	44	**44**
Purpose of meat	Home consumption	34	**34**
	Group share	40	**40**
	Ceremony	6	**6**
	Emergency Slaughter	20	**20**
Consumption of meat	Cooked	49	**49**
	Raw	1	**1**
	Both	50	**50**
Treatment of diseased animals	Traditional Healers	4	**4**
	Self Administered Vet. Drugs	39	**39**
	Vet. Clinics	57	**57**
Delivery	Hand Pulling	39	**39**
	Naturally	39	**39**
	No Assistance	22	**22**
Disposal of fetal mambranes	Burring	27	**27**
	Offering to dogs	7	**7**
	Leaving in the fields	66	**66**
Replacement Stock	Own stock	28	**28**
	Village Stock	22	**22**
	Both	50	**50**
Keeping Dogs	Yes	96	**96**
	No	4	**4**
Veterinary Service	Yes	90	**90**
	No	10	**10**
Disease Awareness	Yes	-	**-**
	No	100	**100**
Dry Season Watering	Rivers	9	**9**
	Ponds	21	**21**

	Artifitial/Traditional Wells	49	**49**
	Springs	21	**21**
Wet Season Watering	Rivers	41	**41**
	Ponds	23	**23**
	Artifitial/Traditional wells	8	**8**
	Springs	28	**28**
Cause of Abortion	Disease	34	**34**
	Mechanical Trauma	23	**23**
	Both	43	**43**
Frequently Abort	Sell	67	**67**
	Slaughter	33	**33**
	Keeping	-	-
	Others	-	-
Concieving Problems	Sell	63	**63**
	Slaughter	37	**37**
	Keeping	-	-
	Others	-	-
Cause for Sell	Disease	43	**43**
	Infertility	**57**	**57**

5. DISCUSSION

Brucellosis is an important infectious disease of sheep and goats and is considered to be the most serious zoonotic disease for humans. The present study was under taken from 350 sheep and 350 goats in amigna districts of Oromia Regional state.

The herd level seroprevalence of the sera tested were negative. This finding is lower than flock seroprevalence of 22.3% reported in Eastern Amhara region (Shimels, 2008) and 12.1% in morocco (Benkiram, 2006), prevalence of 43% is Uganda (Kabagambe *et al.,* 2001) 37% in west Asia and 495 in North Africa (Benkirnae, 2006) and 61% in goats and 30% in sheep in Tunsia (Benkirane, 2006) were higher than the findings in the study.

The study indicated that the overall individual animal level seroprevalence of brucellosis in small ruminant was negative in both species. It means this seroprevalence of small ruminant brucellosis is comparable to the seroprevalence of 0.5% is South Omo and 0.11% in Somalia Region (Melese *et al*., 2007). Furthermore, the overall individual specific seroprevalence was in agreement with prevalence reported in Africa and Middle East of 0.3% to 21% in goats and 0.6 % to 22% in sheep, respectively (Benkirane, 2006). in this study, Both seroprevalence of goats and sheep are the negative but it is not similar to the result from 3.2% in goats and 1.6% is sheep reported in southern Ethiopia (Mangistu, 2007) 3.8% in goats and 1.4 sheep in Eritrea (Omer, 2000) and 4.1% in goats and 1.4 in goats and 1.6% in sheep in east Moroco (Benkirane, 2006). Seroprevalence of 16.7% in goats and 14.2% in sheep in Afar (Yibeltal, 2005) and 5.8% in goats and 3.2% in sheep in Afar (Ashenfi *et al.,* 2007).

The occurrence of brucellosis in both sex had the same result, no anti brucella antibodies. This finding is not comparable with 3.2% and 1.2% is male and female, respectively, reported in Southern Ethiopia (Mengistu, 2007).

Furthermore, anti Brucella antibodies were not detected in all age of small ruminants.

The diagnostic alternatives for the recognition of brucellosis are more varied than with any other infectious disease. This depends on the reaction of the organisms on the chronic infection which partly occurs. The organism is intracellular, which can be detected only incompletely with the usual serological techniques. In order to carry out eradication scheme with in infected herds it is, however essential to identify all chronically or latently infected animals. There are also difficulties in recognizing latently infected animals especially in autochthonous breeds of tropics. Apparently the autochthonous breeds of the tropics react to a certain level only with so called incomplete, non-agglutinating antibodies in the infection a phenomenon which cannot be identified with the classical serological techniques (Seifert, 1996).

During the questionnaire survey, it was found that 28% replace the stock with own stock, 22% from village stock, 50% from both stock. Among the total households 73 owners (73%) used unsafe methods to disposal fetal membrane, whereas 27 (27%) applied safe disposal of fetal membrane. From this it can be noticed that potential risk factors for brucellosis, that is using replacement stock from outside the farm and also unsafe disposal of fetal membranes, are practiced in the study area. Although there was no detection of anti Brucella antibodies in animal and human sera, there is a risk human practice for future in contracting brucellosis from animals.

6. CONCLUSION AND RECOMMENDATIONS

In the present study an attempt was made to determine the seroprevalence of brucellosis in sheep and goat sera, as well as human sera collected from Amigna District using modified Rose Bengal plate test. There was no detection of anti brucella antibodies either in small ruminant or in human sera. Although antibodies were not detected in sera of animals and humans in the area, there is a human practice favourable for the transmission of the disease from animals to humans.

Therefore, in line with the above conclusive statements, the following points of recommendation are forwarded:

- There should be strict regulation in preventing introduction of brucellosis to the area.

- In case brucellosis is introduced to the area, humans should avoid practicing risky activities so that transmission of the disease to humans will be prevented.

- Potential genetic resistance of sheep and goats in the area to the disease may be investigated in the future.

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Annexes

Annex : Modified Rose Bengal texstx forx (M-RBT)

Materials used

- MRBT Brucella antigen
- Postive control system
- Negative control system
- Enamel Plate
- Mictopipette
- Plastic applicator

Test Procedure

All the sera from ruminants and human being were screened using MRBT--- Generally, the test sera and the antigen were left at room temperature for half an hour before the test was conducted.

- 75 ml of the test serum was taken and put on clean enamel plate.
- 25ml MRBT antigen was added to the side of each test serum using a micro pipette.
- Then the test serum and the antigen were mixed using a plastic applicator.
- Finally, the enamel plate was shacked gently by hands for 4 minutes and the result were read by examining the degree of agglutination in a good source of light with or without a magnifying glsass base on the degree of agglutination.
- Interpretation: After 4 minutes of gentle shacking, any visible agglutination was consided as postive.

Curriculum Vita

Name	Getachew Yohannes
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Educationa back ground

Elementary School (6^{th} grade) in Minilik elementary school, 1n 1968, Ethiopia calendar
Secondary school ($7\text{-}8^{th}$) is Minilik Secondary school from 1968-1969 E.C.
High School ($9\text{-}12^{th}$) grades): Minilik Junior school from 1976-1973 E.C.
First Degree in Veterinary Medicine in Czechoslovakia Republic from 1973-1980 E.c

- **Work Experience:- In Arsi region as a field Veterinarian from 1983 up to now.**

Annex 3:- Questionnaire on management and husbandary risk factors

1. General Information

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

Village ----------

Name of responant ---------- Age --------- Sex ------- Date ----------

Flock size ----------(a. Small<,b,Middle5-10,c.Large.10.

Owner Experience ----------(qualifiction)----------family size----------

1. What do you to milk

   a) Home consumption b) Selling for cash income c)both

2. How do you consume Milk?

   a) Cooked b) Raw c) Both d) other treatments

3. Do you slaughter animals at home? (Y/N). If yes for what purpose?

   a) Home Consumtiop b) Goup share c) Ceremony d) Emergency slaughter

4. How do you consume meat?

   a) Cooked b) Raw c) Both d) other treatments

5. How do you treat your disease animals?

   a) Traditional healers b) Self administrered vet drugs c) vet clinics

6. How do you assist delivery?

   a) Hand pulling b) Naturally c) No assistance

7. How do you dispose of fetal membranes?

   a) Burring b) offering to dogs c) Leaving in the field

8. Where do you get your replacment stock?

   a) Own stock b) Villages' stock c)both

9. Do you keep dogs at your home?

   a) yes b) No

10. List at least five endemic diseases in your area ----------

11. Do you have regular veterinary service?

    a) yes b) No

12. Do you have awarness of small ruminants/humans brucellosis or other disease?

    a) yes ---- b)---

13. Watering points in the different seasons

I. Watering points in the different season

a) Rivers b) ponds c) Artifical/Traditional wells c) Springs

14. What do think about the cause of abortion

a) Disease b) Mechanical trauma c) both d) others

15. What do you do to animals those frequently abort?

a) Sell b) Slaughter c) keeping d) others

16. What do you do to animals those do not conceive?

a) Sell b) Slaughter c) keeping d) others

17. Do you sell breeding female/males? (Y/N) if yes reasons for selling

a) Disease b) Infertility c) Shortage of Money

18. House types (floor tyoe)?

a) Concrete b) Soil c) other

19. Feed type?

a) Concentrates b) Only grazing C)Grazing and concentrates d)others

Annex 4 Questionnaire format for human risk groups

Address	Name	Sex	Age	Consumption of raw milk and milk products	Consumption of raw meat and meat products	Signs of illness						
						Orchitis	Joint or back pain	Recurrent fever	Chills and night sweats	Head ache	Genetal fatigue	steril

Signed declaration

This thesis is my Original work has not been presented for a degree in of other University and that all sources of material used for the thesis have been duly acknowlaged.

Name _____

Signature _____

Date of submission

This thessis has been submitted for examination with our apporval as senciesors advisors

Advusirs

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