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STUDY ON SHEEP BRUCELLOSIS IN SELECTED WOREDAS OFTHE EASTERN AMHARA REGIONAL STATE, NORTH EASTERN ETHIOPIA

BY

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TABLE OF CONTENTS

PAGE

LIST OF TABLES

PAGE

LIST OF FIGURES

PAGE

LIST OF ABBRREVIATIONS

GRIS	**Domestic Animals General Resources Information Systems**
APHIS	**Animal and Plant Health Inspection Services**
BoARD	**Bureau of Agriculture and Rural Development**
^{o}C	**Degree Centigrade**
C-ELISA	**Competitive- Enzyme Linked Immuno Sorbent Assay**
CFT	**Complement Fixation Test**
CSA	**Central Statistics Agency**
DBARC	**Debre Berhan Agricultural Research Center**
ELISA	**Enzyme Linked Immuno Sorbent Assay**
EPAAT	**Ethiopian Participatory Applied Assessment Team**
FPSR	**False positive Serological Reaction**
FAO	**Food Agricultural Organization**
H_2S	**Hydrogen Sulfide**
I-ELISA	**Indirect- Enzyme Linked Immuno Sorbent Assa**
ILRI	**International Livestock Research Institute**
IgA	**Immunoglobulin A**
IgG_1	**Immunoglobulin G_1**
IgG_2	**Immunoglobulin G_2**
IgM	**Immunoglobulin M**
LMA	**Livestock Marketing Authority**
MRT	**Milk Ring Test**
MZN	**Modified Zeihl Neelsen**
OIE	**Office International des Epizootics**
OR	**Odds Ratio**
PCR	**Polymerase Chain Reaction**
PFE	**Pastoral Forum Ethiopia**
RBPT	**Rose Bengal Plate Test**
R-LPS	**Rough Lipopolysacchride**
SARC	**Sheno Agricultural Research Center**

SAT	**Serum Agglutination Test**
SDA	**Serum Dextrose Agar**
S-LPS	**Smooth Lipopolysaccharide**
SDHT	**Skin Delayed type Hypersensitivity Test**
SNNPRS	**Southern Nations Nationalities and People Regional States**
TSA	**Tryptose Soya Agar**
USA	**United States of America**
USDA	**United States Department of Agriculture**
U.A.E	**United Arab Emirates**
VBD	**Veronal Buffer Diluent**
WHO	**Worlds Health Organization**

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ABSTRACT

A cross-sectional study was conducted in South Wollo, North Wollo and North Showa Zones of the Amhara Regional state, between octobrr, 10, 2007 to march 1, 2008,with the objectives of determining the seroprevalence distribution and magnitude of brucellosis in sheep and identifying the potential risk factors associated with diseases. Two strata sampling methods were employing, sheep flock in extensive production (n=1838) and sheep flock in intensive production system (n=571) , with an overall serum sample of 2409, collected from sheep above 06 months of age without previous history of vaccination. The overall seroprevalence obtained by Rose Bengal Test was 4.98% (n=120), the 120 positive sera by RBPT was subjected to the Complement fixation (CFT) and 118 (4.89%) sera become positive for antibodies to brucella infection.. The subsequent analysis were based on the 4.89% (n=118) sera that were positive to both the RBPT and CFT (serial interpretation) test results. In the current study, a flock and individual level seroprevalence for brucella antibodies were conducted. A flock was said to be positive if at least one animal reacted positively by the test. The overall individual and flock level seroprevalence for brucella antibodies in sheep were 4.89% (n=118) and 22.26% (n=274) respectively. Analysis of the findings among the two production system indicated significant difference in both individual ($p<0.05$) and flock level ($p< 0.05$) seroprevalence. Brucella seroreactor female and male sheep vary significantly in the extensive production system ($p<0.05$) compared with intensive production system ($p>0.05$%). Analysis of the findings among different age groups indicated the existence of a significant difference ($p<0.05$) in extensive production, whereas in intensive production statistically no significant difference ($p>0.05$). The existence of seroprevalence in the three flock categories shows significant difference ($p<0.05$) in extensive production, while no difference ($p>0.05$) in intensive production system. Concerning breed level seroprevalence for brucella antibody in both production system reveal significant association ($p>0.05$). Moreover, this study was also assessed the association of reproduction and production performance in relation to brucella infection. Generally the present study disclosed the importance of brucella seroreactor sheep in the study areas, hence, care should be taken and warranting more focused investigation, so as to support intervention efforts in the region.

KEY WORDS: Brucella, Sheep, Sero-prevalence, Extensive, Intensive, Production system, North Showa,South Wollo ,North Wollo

1. INTRODUCTION

Until recently, small ruminants were not a priority in research and development programs. Among the various development efforts besides the regular livestock extension program, establishment of sheep breeding and multiplication centers in different parts of the country has been directly associated with sheep production improvement. The first sheep breeding program was started in 1968 with the establishment of a sheep breeding and multiplication center at Debre Berhan and, later, at Amed Guya in1980 (BoARD, 2004). The limited efforts made so far to increase productivity of sheep have not made visible impacts in terms of economic development and poverty alleviation in Ethiopia.

The role and potential contribution of sheep in reducing the poverty and achieving food security at national and household levels is well recognized. The Amhara region holds about 36 % of the national sheep population. But, productivity estimates are relatively low. The existence of considerable diversity among indigenous sheep breeds that are suitable for the different agro-ecological zones provides great opportunity for improvement.

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 in Africa represents 17 % of the total world population while goats represent 30%. Small ruminants provide a number of advantages to the producer. They are sources of food (milk and meat), fiber (wool and skin), and cash in a form of savings. Their adaptability to a broad range of environments, short generation cycles and high reproductive rates that leads high production efficiency made small ruminants production an attractive enterprise. Under pastoralist and agro-pastoralist production systems, both species of small ruminants could be kept as a source of investment and as an insurance against disasters (Devendra and Mc Leroy, 1990, Ibrahim, 1998).

These small ruminants and their products are important export commodity significantly contributing to the national economy. Moreover, they support the livelihood of million of people. Based on Central Statistics Authority (CSA, 2005) Ethiopia hosts over 24 million heads of sheep and 18 million heads of goats. Of these, the high lands support about 75% of sheep and 27% of

goats, while the lowland (mostly pastoral areas) are inhabited by about 25% of sheep and 73% of goats (PFE, 2004). The Amhara region has 6.4 million heads of sheep, which is about 36% of the national sheep population (EPAAT, 2003). In spite of large population of small ruminants in Ethiopia, the comparative huge resource that the country possesses and the economic return gained from this sub-sector do not seem to coincide. The factors that are attributed to this are undernutrition, malnutrition, low productivity, age-old traditional management and disease. Brucellosis is one of the infectious diseases, considered as major constraints for animal productivity (Tamirat, 1985).

Brucellosis is a febrile disease of humans already described by Hippocrates 450 A.D. is one of the most important zoonosis in the tropics. Not only does brucellosis present a serious hazard to human health but it is also a disease in domestic animals with important economic consequences (Seifert, 1996). The causal organism was at first isolated by Bruce in 1870 from the liver of a patient which had died with Undulated fever (Malta fever), on the islands of Malta, he later named *Micrococcus melitensis*. Bruce described later in 1905, that goats were usually infected and that people contracted the disease mainly from infected milk (Seifert, 1996).

Brucellosis is primarily a disease of domestic animals with potential hazard to cause infection in man. It is mainly a disease of ruminants and swine (Freeman, 1979). The principal manifestation of brucellosis is reproductive failure such as abortion in the last trimester of pregnancy or birth of unthrifty newborn and infertility (Radostits *et al*., 2000; OIE, 2004). It is one of the most important zoonoses in the tropics (Seifert, 1996; Acha and Szyfres, 2001). The consequences of brucellosis in small ruminants are infertility: a high mortality rate in lambs and kids, mastitis and reduced milk production (Seifert, 1996; Quinn *et al*., 1999). Sheep are more resistant than goats and abortion is uncommon to occur in ewes (Freeman, 1979; Hirsh and Zee, 1999). It has been shown that brucellosis causes heavy economic loses in livestock industry. Economic losses in small ruminants stem from breeding inefficiency, loss of lambs and kids, reduced wool, meat and milk production as well as impediment to free animal movements and export of animals and their products (Mustafa and Nicoletti, 1993; Renukaradhya *et al*., 2002).

Sheep meat accounts for 12% of the total meat production in the country. Export of mutton and skin from sheep constitute a major source of foreign exchange. At the farm level, sheep contribute a substantial amount of the farm household income, providing food and non-food products such as skin, fleece and manure. Productivity of sheep has frequently been reported to be below. The average growth rate of sheep meat production during 1993-2000 was estimated at 0.3% per annum (FAO, 1989).

According to the strategic plan set by the Bureau of Agriculture and Rural Development (BoARD, 2004) for the Amhara region, small ruminant production is one among the five commodities within the livestock sector. Therefore the objectives of the present study was

- To determine the seroprevalence of sheep brucellosis in two production systems: extensive and intensive and
- To identify risk factor that is likely to influence the occurrence of the disease.

2. LITERATURE REVIEW

2.1. Etiology and Morphology

Brucella species are small gram negative rods by Gram's staining (0.5-0.7 x 0.6-1.5µm) that often appear cocci or cocco-bacillary (Moreno *et al.,* 2002). They are arranged single and less frequently in pairs, short chain or small groups. They are non-motile, non- spore forming, does not posses capsule, flagella, however an external envelope has been demonstrated by electron microscopy (Walker, 1999). Brucella organisms are partially acid fast in that they are not decolorized by 0.5% acetic acid in modified Zeihl – Nelsons stain and thus appear pink in a blue background. The different species cannot be distinguished from each other morphologically. For micro scoping demonstration in or outside of tissues selective staining methods are applied which are able to show the tiny bacteria (Stamp or Hausen staining) (Moreno *et al.,* 2002).

The species of the genus brucella are able to infect a broad range of hosts (Seifert, 1996). The genus is divided into eight species: *B. abortus* (cattle) *B. melitensis* (goats/sheep), *B. suis* (pigs), *B. canis* (dog), *B. ovis* (ram), and *B. neotomae*, (desert wood rat), based on cultural, metabolic, antigenic properties and host specificity (Cloeckaert *et al*., 2001), and more recently types infecting marine mammals are *B. cetaceae and B. maris* (Foster *et al*., 2002). Brucella species isolated from the sea mammals are divided into two groups, based on their carbon dioxide – depending and the host species. All the seal and the other isolates required Co_2 whereas, the cetacean isolates did not (Foster *et al*., 2002). Marine mammals isolates appear to comprise several new species of brucella corresponding to diverse marine mammal hosts, and some species may contain more than one subtype (Bricker, 2002).

Each brucella species has preferred natural host that serves as a reservoir of infection. Secondary hosts play only very small parts if any in the maintenance or spread of a particular brucella species (Quinn *et al.,* 1999). Brucellosis in small ruminants is caused by *B. melitensis* is also a primary causative agent of undulant fever or Malta fever in man and rarely *B. ovis* causes brucellosis in sheep, particularly causing epididimitis in rams (Radostitis *et al.,* 2000). *B. abortus* was also isolated in sheep and goats (Renukaradhya *et al.,* 2002), and also other animals such as

pig, dog and horse may be infected by the same species of brucella. In horses suppurative bursitis, most commonly recognized as “fistulous withers” 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 (Moreno *et al.,* 2002).

There are three biovars of *B. melitensis,* which differ in geographical distribution but no difference in pathogenecity of animal species affected (Benkirane, 2005). To date human infection by brucella organism has been caused by four species: *B. melitensis*, *B. abortus, B. suis*, and *B. canis* in order of pathogencity respectively (Quinn *et al*., 1999).

The species can be differentiated because of their sensitivity to phage (annex 5-6), their metabolism and their biochemical structure. As far as it correlates with their antigenic characteristics the biotypes are differentiated serologically (agglutination) applying specific sera (A, M and R) (Moreno *et al.,* 2002). There is no single test by which a species may be identified with absolute certainty, but a combination of growth characteristics, colonial and cellular morphology, staining properties, agglutinating antisera and biochemical reactions will allow an accurate identification (Bishop *et al*., 1994). The genera *Bordetella, Campylobacter, Moraxella* and *Actinetobacter* are morphologically related to the Genus *Brucella*. *Yersinia enterocolitica* (YE O9) is antigenically closely related to brucella and is agglutinated by brucella antisera. It can be differentiated from brucella because of its motility (Weidmann, 1991).

2.2. Epidemiology

Factors associated with brucellosis include host factor (age, sex and breed), agent factor and extrinsic factors (environmental factors) including management and ecology (Nicoletti, 1980). Large animals and small ruminants and humans are susceptible to brucella species; whereby the species may infect their main host but many also mutually infect other animal species as well as game, camels, horses, buffaloes are also susceptible. Apparently *B.ovis* is only pathogenic for sheep; while *B.melitensis* and *B.abortus* can mutually infect their respective main host. They also mostly occur in wield ruminants (Crawford *et al*., 1990).

The infection is usually introduced into a herd through latently or acutely infected animals. The infection occurs mostly by the ingestion material which has been contaminated with the excretion of a female which has aborted but may also have had a normal birth, though brucellosis also has to be considered an important venereal disease, contaminated feed (hay) can spread the infection from infected pastures over large distances when it is traded. Insects and game animals may also carry the infection over long distances. Since the period of lambing is limited in sheep infection occurs mostly during the lambing season (Nicoletti, 1980).

2.2.1. Occurrence and Global distribution

Brucellosis is present throughout the five continents, but information on its distribution is rather sparse in many parts of the world. This is probably due to the fact that the disease which belongs to OIE, list B disease remain insidious, while many countries with limited resources give priority to more spectacular disease like Foot and mouth disease (FMD), Sheep pox, Rift valley fever (RVF), Pest des petites ruminants (PPR), Contagious caprine pleuropneumonia (CCCP) (Benkirane, 2005). Prevalence rate shows variation from place to place, which could be due to different factors such as herd size, management, and biology of the disease. The seroprevalence carriedout in different African and Middle East countries revealed prevalence rates ranging from 0.3 to 21% in goats and 0.6% to 22.8 in sheep, of which, most prevalence rates fall less than 10% at individual animal level. The outbreaks occurred in Algeria in 1989 showed seropositivity of 2.2% in sheep and 12% in goats, and at the herd level the prevalence has 43.5% and 42%, respectively, while in Tunisia in 1990 in a place called Gafse, a herd prevalence rate of 61% in goats and 30% in sheep are recorded, in Morocco, in 1996 an outbreak occurred showing flock prevalence of 12.1% and 2.4%, respectively, in sheep and goats (Benkirane, 2005).

Brucellosis is of major economic importance in most countries of the world and it affects approximately 50% of the livestock population world wide and continues to increase in distribution (OIE, 2004). Small ruminant brucellosis has been shown to occur worldwide. It is mostly present in Mediterranean countries, Middle East, Africa, India, China, Mexico and parts of Latin America (Kimberling, 1998; Smith and Sherman, 1994). Small ruminant brucellosis was well studied in certain parts of the world. For instance prevalence rates of 1.6% in sheep and 4.1% in goats in Morocco, 4.0% in sheep and 18.0% in goats in Tunisia, 8.2% in sheep in Israel,

2.4% in sheep and 8.2% in goats in Egypt, 3.0% in both species in Iran and 14.2% in sheep and 16.7% in goats in Khartoum region of Sudan were recorded (Benkirane, 2005). In Kenya prevalence rate of 6.01% in sheep and goats (Waghela, 1976), in Somalia prevalence rate of 7.20% in sheep and 5.29% in goats (Falade and Hussein, 1997) and in Eritrea prevalence rates of 3.8 in goats and 1.4% in sheep were reported (Omer *et al*., 2000).

A report of 1.3% made from Sudan, El-Ansary *et al* .(2001), 6.01% in Kenya Waghela (1976), 5.29% in Somalia Falade and Hussein (1179), 3.8% in eastern low land of Eritrea Omer *et al.* (2000), 4% in Eastern Sudan, El-Ansary *et al* .(2001), 4.75% in Nigeria Waghela (1976), showed the significance of caprine brucellosis in some African countries. However, in Ethiopia only few studies were reported concerning caprine brucellosis with prevalence rate of 16.5% in Afar and 1.7% in Somalia region Yibeltal (2005), and 1.3% in central Ethiopia Tekeleye and Kasali (1990).

2.2.2. Occurrence in Ethiopia

In Ethiopia limited sero-serveillances carriedout so far indicated that, brucellosis may be one of the important diseases in goat and sheep raising country. A seroprevalence study carried in small ruminants in Afar and Somali region in 2005, clearly demonstrated that the disease exists in Ethiopia. The seroprevalence findings were 4.6% in sheep, 16.2% in goats in Afar region and 1.6% in sheep, 1.7% in goats in Somalia region (Yibeltal, 2005). In another work done in the central high land of Ethiopia seroprevalence of 1.5% in sheep and 1.3% in goats recorded (Tekeleye and Kasali, 1990). The existence of the disease was also confirmed and reported in Southern Nations Nationalities and Peoples Regional State (SNNPRS) and Borane pastoral areas (Teshale *et al.,* 2006, unpublished data) and according to the annual report of Sodo Regional Laboratory in the year 2005. In Ethiopia the only high seroprevalence findings that obtained by Yibeltal (2005) disclose the reality that the prevalence of small ruminant brucellosis was much higher in Afar region where communal use of grazing land is practical than Somalia region where clan based flock/herd segregation is common.

Table 1: Prevalence of brucellosis in sheep and goats in East Africa

Country	Sheep	Goats	Refferances
Ethiopia (Central high land)	1.5%	1.3%	Tekeleye and Kassali, 1990
Ethiopia (Afar region)	4.6%	16.2%	Yibeltal, 2005
Ethiopia(Somalia region)	1.6%	1.7%	Yibeltal, 2005
Eritrea	1.4%	3.8%	Omer *et a*l, 2000
Kenya	6.01%		Waghela, 1976
Somalia	7.2%	5.21%	Falade and Husein, 1997

Source: (Benkirane, 2005).

Table 2: Seroprevalence of brucellosis in Sheep in West and North African countries

Country	Year	Concerned area husbandry type	Prevalence %			
			Sheep		Goats	
			Individual	Flock	individual	flock
Morocco	1997	East	1.6	12.1	4.1	
Algeria	1998				1.5	
Tunisia	1992	Nation wide	4.0		18.0	
Israel	1993	Intensive	8.2			
	1995					
Egypt	1998		2.4		8.2	
Sudan	Recent	Khartoum and regions	14.2		16.7	
Jordan			22.8		21.0	
Syria	1988		1.8			
Turkey	1992		0.6			
Iran	1998		3.0		3.0	
Kuwait	1993		5.8	37.0		
	1997		2.4	49.0		
Oman	1989				0.3-6.4	
UAE	1990		2.0		3.4	

Source: (Benkirane, 2005)

2.2.3. Risk factors

The epidemiological variables of small ruminant brucellosis are related to host factors (age, sex and breeds), agent factor and extrinsic factors (environmental factors) including management, ecology, population and biology of the disease.

It is widely accepted that susceptibility increases with sexual development and pregnancy (Nicoletti, 1980; Morgan *et al*., 1969). Young sexually immature animals generally do not become infected following exposure or recover quickly (Radostits *et al*., 2000). Numerous reports now confirm that a small but important percentage of young lambs which are infected in early life are negative to serology tests, and abort or have an infected lambing during the first pregnancy. It is estimated that 2.52% of young lambs born to serologically positive dams reacted in early adulthood and constitute a risk to the flock. Little is known about the factors which may affect latent infection such as severity of infection in the dam, number of organism in the milk and antibody content, time of waning and vaccine administration. It is certain that latent infections are more frequent than previously believed and present serious difficulties in elimination of brucellosis in sheep/goats flocks (Nicoletti, 1980).

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

All breeds of sheep 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. 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).

Brucella is facultative intracellular bacteria, hence has protection from the innate host defense and from therapeutics, moreover, 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 out side the host is influenced by the existing environmental conditions (Radostitis *et al*., 2000). Temperature, humidity, presence of other microorganisms, presence of nutrition, autolytic enzymes, and pH of the environment influence the survival of brucella (Radstitis *et al*., 2000). The organism is sensitive to direct sunlight, disinfectant and pasteurization (FAO, 1989; Seifert, 1996).

Brucella survive for up to 4 months in aborted fetuses and placenta, 22 weeks in humid faces, 33 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 (Weidman, 1991). Disinfectants like caustic soda, formalin 2% and Lysol 10% destroy brucella (Seifert, 1996). Exposure to sunlight kills capable the organisms within a few hours (Nicolleti, 1992).

Goats are highly susceptible to *B. melitensis* infection as compared to sheep. The organism is capable of causing disease in cattle and has been isolated from swine (Banai, 2002). In Europe the incidence of the infection in cattle appears to be increasing and in Malta about a third of cattle reacting positively to the brucellosis agglutination test are infected with *B. melitensis* (Radostitis *et al*., 2000). Strictly speaking, the different species of *Brucella* are not highly host specific. Thus their evolutionary adaptability and inter-host transmission continues to change. These days, *B. melitensis* in cattle has emerged as an important problem in some Southern Europe countries, Israel, Kuwait, SaudiArabia and India (Banai, 2002; Renukaradhya *et al*., 2002).

In camel brucellosis, *B. melitensisis* isolated from camel in Iran, Libya, and Saudi Arabia, *B.abortus* is also isolated from camel in Sudan, Egypt and Kuwait. Contact between camels and sero-reactors were incriminated in transmission of *B.melitensis* to the camel (Linklater, 1979). Camel pastoralists invariably keep relatively large flocks of sheep and goats along side the camels and the frequent isolation of *B.melitensis* from camels in North Africa and Arabian suggest active transmission between seroreactors and camel (Cloeckaert *et al.,* 2001). The

infection of *B. melitensis* to other species of animals is not due to change genetic material, for there is no evidence of natural transfer of genetic material in the organism, but for the pathogen is least specialized being easily transmitted to cattle and human. However, *Brucella* species are obligate parasite of each species having preferred natural host that serve as reservoir of infection (Quinn *et al*., 1999).

Incidence rate of *B.melitensis* infection are evident in breeding age at sexual maturity, because the infection persists in latent form in young one. However, if the kids and lambs are weaned early from their mother and from the infected environment they are usually feed from the infection as adults. The greater the number of abortion and parturitions, the greater will be the exposure risk in the herd. After abortion uterine infection persists for up to 5 months and in the udder the bacteria persists for years (Radostitis *et al.,* 2000).

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 contact favor the spread of brucellosis (Weidman, 1991). Management factors for high incidence of infection are high stocking densities, unhygienic housing and lambing, lack of sanitary measures (cleaning and disinfection) and large herd size (Crawford *et al*, 1990; Nicoletti, 1980; Morgan *et al.,* 1969). Frequent farm disinfection is an effective method of control of brucellosis, because of low resistance of brucella (WHO, 1997). Brucellosis seropositivity in goats showed high association with large herds, communal grazing of goats mixed with sheep, the presence of seropositive goats in the ranch or herd and sero-positive dogs in the vicinity and lack of awareness in abortion. 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 (Rafai, 2002).

2.3. Transmission

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 by suckling infected milk of their mother. Venereal transmissions by infected ram to susceptible ewes appear 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. In sheep the degree of infection of milk and in uterine exudates is much lesser than goats. Studies indicate that 70-90% cause of brucellosis infection occur via the skin and mucous membrane by direct contact (Morgan *et a*l., 1969; Nicoletti, 1992). Transmission to man is as a result of contact with infected animal carcasses, aborted fetus, placenta, consumption of un-pasteurized milk and cheese. It is common to observe human cases that are in contact with goats in area where active brucellosis outbreak occurs. 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*., 1999)

2.4. Pathogenesis

Following entry into the host, brucella is either frees in the extra cellular 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 microphages and to lesser extent neutrophiles is enhanced by suppression of the myeloperoxidase H_2O_2 halide system and production of super oxidase 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, bacteriaemia occurs which can last for 1-3 weeks and distributes the organism to the lymphatic system, the large parenchyma and other organs and tissues. 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 (Rafai, 2002). In non pregnant animals, the first infection often occur in the udder followed by the infection of the uterus latter 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 villas of the chorion (Wilson, 1982).

Sero-negative infections prior to sexual maturity and first parturition (latency) perpetuate the disease (Nicoletti, 1992). The predilection site for *Brucella* is the reproductive tract of males and females, especially pregnant uterus and reticuloendothelial system (Quinn *et al*., 1999). Allantoises factor, 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 contribute to the pathogenesis (Weidman, 1991), the period varies considerably and is affected by factors such as gestation, exposure dose, age, vaccination and other unknown host resistance influence (Nicoletti, 1992).

The variable factors of clinical symptoms which are typically for brucellosis are the consequence of the individual level of host defense which is specific for each breed, but also for each individual and which is the result of the sum of influences of genetically determined resistance, level of immunity, age of the animal, productivity, condition, environmental influence as well as virulence of the pathogen (Wilson, 1982). Whenever the disease is enzootic in relatively resistant autochthonous animals as it is mostly the case in the tropical Savannahs, abortion is rear but the infection causes typical signs which lead to a significant reduction in productivity. These may be, late first lambing age, long inter-lambing times flock fertility bellow 60%, comparatively low milk production (Seifert, 1996). After recovery from an apparent or in apparent abortion, female are protected against a renewed infection because of the development of a massive immunity. They may even become fertile again and but if not, it becomes of permanent lesions in the reproductive system, which appear due to metritis as a consequence of a retention secundinarum. The male animal may suffer of orchitis and show symptoms of oligo-and aspermia, this to certain degree leads to a self cleaning of the flocks (Wilson, 1982).

The primary clinical manifestations of brucellosis are related to the reproductive tract. Abortion that occurs in the last trimester (last 2 months) is the most obvious manifestation. Orchitis, epididimitis in male and arthritis are common observations. In acute natural infection a systematic reaction with fever, depression, loss of weight and some times diarrhea, osteoarthritis, synovitis and nervous sign may occur in sheep. In many instance *B. melitensis* reaches a high incidence in a group of animals without of obvious illness (Radostitis *et al*., 2000). Infection may also cause stillbirth or weak lambs, retained fetal membrane and reduced milk yield. Seminal

vesicles, ampullae, testicles and epididimitis may be infected in males, testicular abscesses may occur, and long standing infections may result in arthritic joints, bursa and hygroma in some animals (Nicoletti, 1992; Seifert, 1996).

2.5. Clinical and pathological feature

Brucellosis in small ruminant is characterized by lesions which appear mainly in the male animal (Orchitis, epididymitis) as well as inflammations 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, as high mortality rate in lambs, mastitis and reduced milk production (Seifert, 1996). The lesions on the placenta are more prominent with the *B. melitensis* infections than when *B. abortus* is the causal agent. 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 animal, this also having a significant influence on the herd fertility (Grillo *et al.*, 2000).

2.6. Immunity

Brucella is a facultative intracellular organism that survives and replicate in both phagocytes and non phagocytic cells. The antibody response following infection depends on whether or not the animal is pregnant and on the stage of gestation (Farrel, 1974). Agglutinin and compliment fixation antibodies become positive 4 weeks following experimental infection (Hendry *et al.*, 1985). An important problem in the immunity that develops after recovery is from an acute infection. The animal mostly will remain with un-sterile 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, inflammation of the joints and bursitis (Seifert, 1996). Spontaneous recovery may occur particularly in goats which become infected while not pregnant (Radostitis *et al.,* 2000). Brucella exhibits both humoral and cellular immune responses. Phagocytes play a key role in initiating T-cell response by processing and presenting antigens. T-cells play a major role in the acquired specific resistance to intracellular bacteria determining the resolution of infection (Grillo *et al.,* 2000). The S-LPS is the major surface antigen for humoral immune response. (Nielsen, 2002).

2.6.1. Humoral immunity

Infection with brucella usually results in the induction of both humeral and cell mediated immunity. The magnitude and duration of these responses can be affected by many factors including virulence of the strain, size of inoculation, age, sex, pregnancy, species and immune status of the hosts (FAO, 1989 and Zain *et al.*, 1985). The immunoglobulin isotopes present in the serologically significant concentrations in serum are IgG_1, IgG_2, IgM and IgA, the first isotype produced after an initial heavy infection or immunization is IgM usually detected in the first or second week but is soon followed IgG_1 antibody, which is the most abundant in serum (FAO, 1989).

It is clear that animal infected by brucellosis contains high level of IgM, IgG_1, IgG_2 and IgA isotype of antibodies; however it should be noted that IgG_2 and IgA antibody levels are considerably lower than the IgM and IgG_1 antibody level (Nielsen and Duncan, 1990). Synthesis of IgG_2 is essential component of the antibody response required to eliminate *B. abortus* S19 or field infection and interestingly IgG_2 was noted to be the only antibody isotype to be effective in antibody dependent cell mediated-cytotoxicity (Nielsen and Duncan, 1990). Humoral factors probably have a role in animal resistance to brucellosis by mediating extra cellular killing of bacteria. However, the role of humoral substances in animal resistance to brucellosis ill defined (Hendry *et al.*, 1985), IgM is efficient in normal agglutination Rose Bengal and Complement fixation tests. IgG_1 is active in normal agglutination but efficient in agglutination at pH 3.6 and fixes complement and IgG_2 is active in normal agglutination but not at pH 3.6 and does not fix complement (Nicoletti, 1980)

2.6.2. Cell mediated Immunity

Brucella species are facilitative intracellular pathogens. They readily phagocytised by macrophage and polymorphonuclear leucocytes are capable of surviving within the cell (WHO, 1997). Effective immunity is primary cellular in nature, specifically sensitized T-lymphocytes release cytokines that activate macrophages, which in turn control brucella by reactive oxygen intermediates (Walker, 1999).

2.7. Diagnosis

In many instances diagnosis of *B. melitensis* infection in animals are made only because the infection has been diagnosed in human contacts, provoking the examination of the local animal population (Radostits *et al.,* 2000). Diagnosis of brucellosis is based up on the history of the herd/flock, epidemiological observation, serological examination and demonstration of causal agent considering all those methods together (Stack *et al.,* 1999). The development of the primary binding assays in serology and molecular biology has greatly improved the efficiency of diagnosis of brucellosis (Bricker, 2000).

Diagnosis of brucellosis is made possible by direct demonstration of the causal organism using staining, immunifluoresent antibody, culture, animal inoculation, polymerase chain reaction (PCR) and indirectly by demonstration of antibodies using serological techniques (Alton *et al*, 1988; Weidman, 1991, Corbel, 1979, Quinn *et al*., 1999). Differential diagnosis of brucellosis should be made with diseases causing abortion in small ruminants. These are Chlamydia, Leptospirosis, Camblobacteriosis, Salmonellosis, Toxoplasmosis and Q-fever (Smith and Sherman, 1994).

2.7.1. Bacteriology

Gram staining of fetal stomach contents from aborted fetus, placenta, vaginal discharge and semen reveal Gram-negative cocci, cocco-bacilli and small rods. In the modified Ziehl-Neelsen method direct smears are made from fetal membranes, fetal stomach contents, vagional swabs, semen, and cotyledon, the smear is dried and fixed over a flame, stained with carbol fuchsine for 10 minute washed in tap water, flooded with 0.5% acetic acid for 30 second, washed thoroughly and counter stained using 1% methlene blue for 20 second, the organism appear small red cocco-bacilli in clumps with blue background reflecting the intracellular growth and is a presumptive evidence of brucellosis (Alton *et. al.* 1975).

2.7.2. Bacterial culture and Biochemical methods

Brucella is pathogenic to man and great care should be exercised in handling materials suspected, if possible examination of culture especially of fluid media should be carried out in safety cabinet. Culture of placenta, cotyledon, vaginal discharge, fetal materials, lymph nodes and other

should be made on solid medium since it facilitates recognition and isolation of developing colonies and limit the establishment of non-smooth mutants (Schurig *et al.,* 2002). For the culture of certain fluids notably blood, liquid media permit the culture of larger volume that cannot be dealt with on solid media. Brucella is normally fastidious, slow growing organism and samples are often heavily contaminated and then require enriched selective media. There are many varieties of suitable media. Some of these are serum dextrose agar (SDA), tryptose soy agar (TSA), liver infusion serum agar and special media containing antibiotics. Brucella also grows in blood agar (Putt *et al*., 1988).

Culture examinations are very important in the epidemiology of 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 (Nicoletti, 1980). Materials for laboratory examination should be cooled immediately or frozen and transported to the laboratory as quickly as possible in leak proof containers (Olsen, 2002). Appropriate sample for culture are stomach contents, lung, spleen and meconium of aborted fetus, fetal membrane, vaginal swabs, blood, milk and lymph nodes (supra-mammary, sub-maxillary, internal iliac). Isolation and identification of the brucella is the only diagnostic procedure not subjected to equivocation, however, negative culture result is not sufficient to rule out infection (Weidman, 1991).

Culture media are classified into primary media and selective media. Primary media in turn are divided into solid and liquid media (WHO, 1997). Solid media include nutrient agar, serum dextrose blood agar, and commercial media (tryptose agar, trypticase soya) and liquid media either trypticase soya broth, tryptose broth, or other commercial selective media prepared by addition of antibiotics such as cycloheximide, bactracin, polymixin B and chemical ethyl violet on serum dextrose or serum-potato agar or any of the basal media (Alton *et al.,* 1988; WHO, 1997; Corbel, 1989). 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*., 1999) Bacterial culture represents the gold standard of laboratory diagnosis. Automated systems have reported to detect more than 95% *B. melitensis* positive cultures in seven days of inoculation. In developing countries due to various reasons detection and identification of *B.*

melitensis in clinical specimens by culture may still be a difficult task with significance delay (Smith and Sherman, 1994).

Brucella abortus requires supplementation of 5-10% Co_2 for growth, but *B. melitensis* does not require supplementation of Co_2. Optimum temperature and pH for growth of *Brucella* are 37^{o}c and 6.6- 7.4, respectively, but growth can occur between 20^{o}c – 40^{o}C, after 3-5 days incubation on selective serum agar, colonies are pinpoint, smooth, glistening, bluish, translucent about 3-4 mm in diameter (Moriyon *et al.,* 2004). The organism is catalase and oxidase positive (*B. ovis* and *B. neotomae* are oxidase negative) reduce nitrate to nitrite except *B. ovis*, rapid urease activity, some strains *B. abortus* produces H_2S, brucella does not cause haemolysis on blood agar, does not produces acid on agar containing glucose, and does not ferment lactose, usually grow in the presence of basic fuchsine and thionin at standard concentration (Corbel, 1989; 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 (Quinn *et al*., 1999)

2.7.3. Animal inoculation

Animal inoculation is the most sensitive methods for detection of *Brucella* and is sometimes necessary when very low number of organisms is present. The guinea pig has been found to be the most satisfactory and most sensitive laboratory animals for detecting the presence of small numbers of brucella organisms in animal tissue, secretion, and excretion when samples are heavily contaminated (Godfroid *et al.,* 2000). The inoculation is done by intra-peritoneal or sub-cutaneous routes. Two guinea pigs are inoculated intramuscular 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 bacteriology examination respectively (Moriyon *et al*., 2004). In male guinea pig orchitis will result. When guinea pigs are autopsied lesion observed in spleen, lymph node and in lung, after 7 days and these organs minced and cultured in solid media containing no inhibitory dye and antibiotics. Serum sample subjected to specific test 3 to 6 weeks after inoculation. A positive reaction in the serum agglutination test,

even without a positive cultural result is sufficient to warrant a diagnosis of brucellosis (OIE, 2004).

2.7.4. Serological test

The diagnosis of brucellosis is best established when the causative organism is isolated from blood and other body fluid in suspected cases. However the tedious process of bacteriology, fastidiousness, growth requirement, less viability of organisms in the sample during transportation and prolonged incubation in isolation are limiting factor to examine a large population as in surveys. Hence, the indirect diagnosis through detection of brucella antibodies in the serum and other body fluid is simpler and indicative that the animal has been in contact with the bacteria and may thus be infected (Lord *et al*., 1989).

The antigen used in brucella serological diagnosis is the O-polysaccharide (O-PS) on the cell surface. The whole antigen or smooth lipo-polysaccharide (S-LPS) prepared by chemical extract is employed in serological tests (Haresign, 1985). Because common epitopes are present in *B. abortus, B. melitensis* and *B. suis* S-LPS, virtually all tests in all thus bacteria utilize *B. abortus* antigen as given by OIE. *B. ovis* and *B. canis* are diagnosed by use of their rough lipo-polysaccharide (R-LPS) or protein antigen (Nielsen, 2002). The S-LPS types are designated as A (abortus) and M (melitensis). It exist in different proportions in respective brucella species and can be distinguished by agglutination adsorption reaction from their respective antiserum to get mono specific sera of A and M antibodies. The proportion of A and M antigen *in B.abortus* is 20:1, where in *B. melitensis* the ratio is 1:20 and in *B. suis* the value occur slightly in narrow proportion {Smith and Sherman, 1994).

The antibody response to brucella in animal consists of an early IgM isotype, the timing of which depends on route of exposure, the dose of bacteria and the health status of the animal. The IgM response is followed almost immediately by the production of IgG antibody, later by small amount of IgG_2 and IgA. Most cross reacting antibody, which is antibody resulting from exposure to microorganisms other than brucella species or environmental antigen consists mainly IgM. Serological test that measure IgM are therefore not desirable as false positive results occur, leading to low assay specificity. Since IgG_2 and IgA antibodies accumulate later after exposure

and are usually present in small and inconsistent amounts. Therefore, assays that predominantly measure IgG, are the most useful. Many serological diagnostic tests have been developed for brucellosis with the purpose of screening, confirmation and assays that also distinguish vaccinal antibody (Nielsen, 2002).

No single serological test and antigen combination showed 100% sensitivity and specificity simultaneously (Cloeckaert *et al*., 2001). A battery of serological tests has been developed for the diagnosis of brucella. Combination of tests shows a degree of sensitivity, which appear to be sufficient to detect infected animals. Frequently, highly sensitive but less specific test are used for screening proposes, and this then are followed by more specific test for conformation (Walker, 1999. The commonly used serological tests are the RBPT, SAT, CFT, ELISA (Quinn *et al.*, 1999).

The Rose Bengal plate test is a spot agglutination technique, because the test does not need special laboratory facilities and it is simple and easy to perform, it is used to screen sera for brucella antibodies. The test detect specific antibodies of the IgM and IgG types and is more effective in detecting antibodies of the IgG types than IgM and IgG_2 types (Schurig *et al.,* 2002). Although the low pH (3.6) of the antigen enhance the specificity of the test, the temperature of the antigen and the ambient temperature at which the reaction takes place may influence the sensitivity and specificity of the RBPT (MacMillan and Cockrem, 1985). The Rose Bengal plate test is very sensitive test; however, like all other serological test it could some times give a positive result due to S19 and or Rev-1 vaccination or due to false positive serological reactions (FPSR). Therefore positive reaction should be investigated using suitable confirmatory strategic and epidemiological investigation (Lord *et al*., 1989).

The antigen consists of brucella stained with Rose Bengal and suspended in buffer at P^H $3.65\underline{+}$ 0.05. The test is conducted on ruled enamel strips, on a glass or ceramic tile or WHO hem-agglutination plate. Equal amounts of serum and antigen (0.03ml) are put on white enamel or plastic trays, mixed, and read after 4 minute rocking. The test is usually interpreted as positive if any agglutination is apparent, and the reaction may be graded according to its rapidity and

appearance. The RBPT is economical, simple and rapid, and gives few false negative and false positive results (Moriyon *et al*., 2004; Alton *et al.*, 1975).

Serum agglutination test which is the ancestor of all serological tests is still widely used often in conjunction with the CFT and Ring tests, while not recognized as a prescribed or alternative test, the SAT has been used with success for many years in surveillance and control programs for animal 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 5mM final test dilution to reduce the level of false positive results. Subsequently the pH of 7.2 must be readjusted in the antigen 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 tube are incubated overnight at 37^{o}C, and the degree of agglutination is read by comparing the opacity against standards representing various degrees of agglutination (USDA, 2003). The mixture of antigen and serum dilutions should be incubated for 16-24 hours at 37^{o}C. If the test is carried out in micro-plates, 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 SAT detects antibodies of the classes IgG_2 and IgM (Cloeckaert *et al.*, 2002). It should be stressed that the serum agglutination test (SAT) is generally, regarded as being unsatisfactory for the purpose of international trade (Zain *et al.*, 1985).

The compliment fixation test detects specific antibodies of the IgM and IgG_1 type that fixes compliment. The CFT is highly specific, but it is laborious and requires highly trained personnel as well as suitable laboratory facilities. This makes the CFT less suitable for use in developing countries. Although its specificity is very important for the control and eradication of brucellosis, it may test false negative when antibodies of the IgG_2 type hinder compliment fixation (MacMillan and Cockrem, 1985). The CFT measures more antibodies of the IgG_1 type than

antibodies of the IgM type, as the later are partially destroyed during in activation. Since antibodies of the IgG_1 type usually appear after antibodies of the IgM type, control and surveillance for brucellosis is best done with SAT and CFT (Olsen, 2002).

The Anti-globulin (Coombs) test detects antibodies of the IgG2 type and is used to confirm SAT results. The Coombs test although laborious, is particularly important when the SAT is positive and CFT results are negative or inconclusive. However Coombs test results are indicative for infection only when its titers are at least two times the titers of the SAT. This is the tests main limitation, as not all infected animals show this ratio. The 2-Mercaptoethanol and the Rivanol tests detect specific IgG and are usually used to differentiate between infected and vaccinated animals (Roop II *et al.,* 1987).

The Enzyme Linked Immunosorbent Assay has proven to be specific and as sensitive as the milk ring test (MRT) and SAT in detecting brucella antibodies in milk and serum. ELISA results are usually also in agreement with CFT results. The test can be used for screening and confirmation of brucellosis in both milk and serum. However, depending on the presence of traces of colostrum in the milk or the presence of low concentrations of lacteal Immunoglobulin, the ELISA may give false positive or false negative (Bracewell and corbel, 1980). It seems that the ELISA is less sensitive than the CFT, as some infected animals that test positive with the CFT may test negative with the ELISA. The assay is very costly when only a few samples are tested; therefore, it is unsuitable for testing individual animals but it is the ideal test for screening suspected herds/flock (Gall *et al.,* 2001).

An alternative immunological test is the brucelline skin test, which can be used for screening unvaccinated herds, provided that a purified (free of SLPS) and standardized antigen preparation (e.g. Brucellin INRA) is used (Hendry *et al.,* 1985). The brucellin skin test has a very high specificity, such that serologically negative unvaccinated animals that are positive reactors to the brucellin test should be regarded as infected animals (Pouillot *et al.,* 1997). Also, results of this test may aide the interpretation of serological reactions thought to be FPSR due to infection with cross reacting bacteria especially in brucellosis – free areas. However not all infected animals react, therefore this test alone cannot be recommended as the sole diagnostic test or for the

purposes international trade (Henning, 1956). It is essential to use a standardized defined brucellin preparation that does not contain SLPS antigen, as this may provoke nonspecific inflammatory reactions or interfere with subsequent serological test. One such preparation is brucellin INRA prepared from a rough strain of *B. melitensis* that is commercially available (Saegerman, *et al.,* 1999).

In molecular technology like polymerase chain reaction (PCR) is a new approach and applied in many diagnostic works to overcome limitations and difficulties of bacterial culture and serological assays. In many works carried out PCR show high sensitivity, specificity and overcome the extraneous intervention of mimicry antibodies from sources of other than actual infection. Antigen detection by use of primers, derived from the OMP-31 gene sequence of the *B. melitensis* was developed successfully to diagnose from goat milk samples (Pouillot *et al.,* 1997).

2.8. Economic and public health significance

The economic and public health significance of brucellosis remain particular concern in developing countries of Africa, Asia and some parts of Latin America. This is due to the danger that infected animals constitute in the transmission of this sever zoonosis to human as well as economic losses associated in animals.

2.8.1. Economic aspects

The economic loss due to brucellosis is attributed to abortion, particularly the milking breeds which are susceptible. The result in loss of the young crop in sheep and goats consequently impairs the breeding patterns. When infection is first introduced into a flock or herd, a storm of abortion will occur, hence acts as a source of infection (Gall *et al*., 2001). The adverse socio-economic impact impeding the foreign livestock trade and its products has great significance in the economic development of livestock owners (Carles *et al*., 1988). This consequently reduces the revenue of the country. Surveillance and control of brucellosis cost require considerable amount of many. To mention, in Western Asia and North Africa countries, the plan to eliminate ovine, caprine and bovine brucellosis, required half of the European commission funding for animal disease control measures in 1997 (Benkirane, 2005).

2.8.2. Reproductive wastage

The reproduction performance of sheep in Africa as well as in Ethiopia has been reported in terms of fertility, prolificacy (litter size), fecundity (fertility X prolificacy), lambing and weaning rates (Wilson, 1982; Mukasa- Mugerwa and Tekelye, 1988). Since the biological and economic efficiency of sheep production is influenced by the number of lambs reared per ewe. Mortality related production losses are very significant, particularly in view of the contribution of sheep to the house hold economics of the agricultural populations of the tropics. Reproduction wastage is normally considered to cover all losses from mating to the first breeding of the offspring. Reproductive wastage is caused by environmental, genetic, disease and management factors, which operate with different severity and in different combinations (Jones *et al.,* 1980)..

These factors interfere with ovulation, fertilization or implantation and during gestation and parturition (Haresign, 1985). Early sheep losses have been attributed to infertility and embryonic mortality and neonatal mortality including starvation (Exposure, abortion), stillbirth, infectious disease, accidental death or loss, predators and congenital defects (Ellis, 1983). Among the infectious causes of abortion, *Chlamydia psittaci* (enzootic abortion of ewes), and *Toxoplasma gondii* are of the major importance in Britain and in Africa (Okoh *et al.*, 1981). Leptospiral organisms also cause abortions, stillbirth and death of weak newborn lambs, but the main serotype have not been identified (Ellis, 1983). *Brucella* species have been sited as one of the principal causes of abortion in sheep and goats, in Africa. (Chukwu, 1987). Studies in Angora goats however, eliminated infectious disease and mineral and vitamin deficiencies as causes of abortion, but incriminated nutritional stress and endocrine failure. Despite frequent abortion and related causes of infertility, including stillbirth and early embryonic mortalities, exhaustive investigations of the causative agents have not been undertaken (Okoh and Momoh, 1981).

2.8.3. 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. melitensis* 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. melitensis* (Dohoo *et al.,* 1985).

Although it is notifiable disease in many countries, the disease remains underestimates by the medical authorities, at official figure do not reflect the number of human infection. Due to lack of awareness and adequate laboratory support to detect the infection it is often considered as cause of fever of unknown origin (Benkirane, 2005). The infection occurs by contact with infected meat, aborted material and consumption of unpasteurized milk and cheese (AGRIS, 2000). Human brucellosis occurred in most cases following epidemic of caprine abortion, whenever contact with infected herd is established. Brucellosis is tends to be found in area where there is high concentration of farm animals.

The establishment of *B. melitensis* in cattle has epidemiological importance. It created an alarming situation to give attention in that the disease extending its horizon of transmission to human through milk and other products of cattle (Banai, 2002). Moreover, brucellosis in humans has a strong occupational correlation. A study conducted in Mongolia, indicated that the infection rate was 19.34% for herds man, 20.01% for leather workers, 11.46% for veterinarian, 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, sweats in late afternoon, or evening are common with a temperature range of 39-40^{o}c. The period of nocturnal fever usually continues for week, months or even 1-2 years. Man suffers from body aches, headaches and anorexia. Occasionally infection develops in lung, bone marrow, heart or genitor-urinary system and diffuse hepatitis with focal necrosis (Radostits *et al.,* 2000).

2.9. Treatment

Treatment of brucellosis in domestic animals is not indicated, however are treated with antibiotic (Doxicycline with Rifampicin), relapses are, however, possible.

2.10. 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 socioeconomic prerequisites (LMA, 2001). Prevention and control of *B. melitensis* infection is important from the point of views of economic losses to the small ruminants industry as well as the important and widespread zoonoses in the world. The

approached used to control brucellosis include, management, immunization and test and removal of infected animals (Godfroid *et al.,* 2000).

2.10.1. Management

From the epidemiological point of the disease there are important steps to be implemented at an early stage. These include hygiene at kidding or lambing, separate pens of kidding/lambing of goats /ewes, early weaning of lamb/kids from and their environment (Radostits *et al*., 2000).

2.10.2. Immunization

Live vaccines give the best protection against brucellosis. Since a permanent immune response of the organism against the intra-cellular bacteria must be attained, this is best stimulated by living, but attenuated bacteria which are thus permanently present. However, in handling these vaccine there is a risk of accidental self vaccination of the personnel and thus of subsequent infection-male animals can not be vaccinated with a live vaccine because it may cause inflammation of the sexual glands (Jimenez de Bagues *et al.,* 1991). The disease persists in Mediterranean countries, in the gulf and sporadically all over the world. It is associated with nomadic animal husbandry, large herd size, contamination of pasture facilitating transmission and the difficulty of regular immunization due to movement of animals, which it self is related to developing countries (Carles *et al.,* 1988). For this reason a test and slaughter policy is not realistic in the majority of places where *B. melitensis* is endemic, due to lack of financial resources needed for compensation. Therefore, immunization is a primary control method proposed by international agencies to reduce prevalence significantly, to ensure test and slaughter program in eradication (Banai, 2002).

2.10.3. Vaccines

The *B. melitensis* Rev-1 live vaccine was obtained by attenuation of a *B. melitensis* smooth (S) strain in the 1950's. Originally the strain was streptomycin dependent for its growth but lost this character upon further culture in mice and guinea pig. It gives protection to sheep and goats against infections with *B.melitensis* and to rams against *B.ovis* (Banai, 2002). Rev-1 strain is applied for the vaccination of small ruminants and also for beef cattle. It is not infrequent to isolate *B. melitensis* in cattle in countries with a high prevalence of this infection in small

ruminants (Cloeckaert *et al.*, 2000). There has been some debate on the protective of S19 against *B. melitensis* infection in cattle and it has been hypothesized that Riv-1, should be more effective vaccine in these conditions, however, there is only one report related to this issue that demonstrated that S19 is able to control *B. melitensis* at the field level (Jimenez de Bagues *et al*., 1991).

By contrast no experiments have been conducted showing the efficacy of Rev.1 against *B.melitensis* infection in cattle. Moreover the safety of this vaccine is practically unknown in cattle (Van Drimmelen and Horwell, 1994). The attenuated live vaccine of brucella induces abortion which given during last trimester of gestation, and shade organisms in lactating does. Reversion to virulent strain possibility is unlikely (Olsen, 2002). For it induces abortion in pregnant animals and for the possible excretions in milk of adult vaccinated animals, the vaccination was recommended to be administered prior to first gestation, at 3-7 months of age. Moreover, Rev-1 can be applied in adult animals excluding the pregnant to accelerate the control of disease in regions with high prevalence and few technical and economic resources. The use of Rev-1 in cattle has been investigated and provided better immunity than S19 (Banai, 2002).

The H-38 is the inactivated vaccine for small ruminants produced from attenuated *B. melitensis* containing aluminum hydroxides adjuvant. It gives good protection, through induction of solid and durable immunity is not possessed as live vaccines (Seifert, 1996). A brucella vaccine is one of the important measures for the prevention and control of brucellosis, especially in ruminants. Due to many inconveniences of the older vaccine, development of new vaccine is required by classical technique or genetic engineering.

These days new generation attenuated *Brucella* mutants are developed as vaccine candidate though none have been fully validated and commercially available for use in small ruminants (Jimenez de Bagues *et al*., 1991).

The following two genetic engineering based approaches are being currently used for developing new anti-brucella vaccines.

1. Development of *B. abortus* S-19 and *B. melitensis* Rev-1 mutants deleted in gene coding for antigens of diagnostic significance. The aim is to develop classical vaccines that allow the differentiation of infected from vaccinated animals.

2. Development of rough (R) vaccine candidates through transposon mutagenesis or deletion in S virulent strain of genes involved in O-chain biosynthesis. Accordingly, these mutants would have a reduced virulence and do not interfere with the classical O-chain based serological tests (i.e. the Rose Bengal and Compliment fixation tests) used for the diagnosis of infections induced by S- *Brucella* (Godfroid *et al.,* 2000).

It is usually considered that a brucellosis eradication program by test and slaughter policy justified on economic grounds only when the prevalence of infected animals in an area is 2% or bellow. This strategy has high cost and involves good organization of farmers and veterinary service, and simultaneously the implementation of strict movement control measure so the disease not to be reintroduced.

Eradication with implementation of test and slaughter policy is possible under certain conditions, when sheep and goats flocks are under strict control, the type of husbandry not extensive or nomadic, there is no common grazing or transhumance of the flocks, an efficient identification system of the animals is in place, the financial other resources are available for long period of time and the veterinary service responsible for the program is well organized (Godfroid *et al.*, 2000).

3. MATERIALS AND METHODS

3.1. Study area

The Eastern part of Amehara National Regional State includes five administrative zones; namely, North Showa, Oromia, South Wollo, North Wollo and Waghamra. This wide territorial coverage has different topography and climate with diversified vegetation. A large portion of this area is high plateau ranges from 1500 to 3500 meters above sea level and dissected by gorges and broad valleys. The river Abay, Tekeze and Awash flows between deep gorges and have tributaries. Traditionally, these areas are classified as high land and low land areas apart by chained mountain stretch out from South and north extremities. The low land area is located in the broad rift valley to the east and the high land to the West of the sub region. Although the area is divided into two eco-zones, there are topographical and altitude differences within the classified zones.

This cross-sectional study was conducted in selected weredas of South Wollo, North Wollo and North Showa zone of the Amhara regional state from December 2007 - June 2008. The study woredas consists of, from South Wollo zone (Legambo and Wereilu), which is located, 540 kms, 480kms, 425kms away from Addis Abeba respectively (Annex-1), from North Wollo zone (Delenta and Gubalafto) and from North Showa zone (Lalomeder and Angolela), including Debre Berhan and Amed Guya sheep breeding and multiplication center and Sheno Agricultural research center. Amed Guya sheep breeding and multiplication center is located in the central high lands of Ethiopia 300kms North East of Addis Abeba at 2900 meter altitude. Sheno and Debre Berhan sheep breeding and multiplication center is located at 132 kms North East of Addis Abeba, at 2800 meter altitude (BoARD, 2004), and has similar environmental conditions with that of Amed Guya sheep breeding and multiplication center.

The roles of these two centers have been administered by the head quarters of the Ministry of Agriculture until 1990. Since regionalization both Debre Berhan and Amed Guya fall under the administration of the Amhara National Regional State. At present, both centers are accountable to the technology multiplication unit of the Bureau of Agriculture and Rural Development of the Amhara region. Major activities and achievements of both ranches were sheep breeding program and productivity improvement. The sheep breeding program in these two ranches are, improving

wool and meat production of the local high land sheep through crossbreeding with exotic sheep breeds. For this purpose, few exotic sheep brides including Merino, Romney, Corriedale, Hampshire, Rambouillet sheep breeds were introduced to Ethiopia.

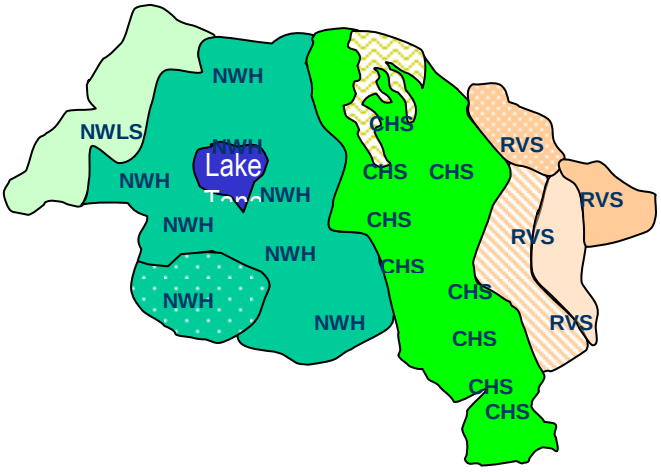


Figure 1: Agro-ecological distributions of the four major sheep types

Table 3: Distribution of sheep population in the Amhara region

Administrative zone	Sheep population		Ratio of sheep to goats	Ratio of sheep to ruminant livestock
	Number	Percent (%)		
South wollo	1724513	26.98	2.21	0.44
North Sheba	1131509	17.71	2.05	0.42
North Wolo	585981	9.17	1.08	0.30
Waghamera	50912	0.80	0.24	0.10
Oromia	24616	0.39	0.17	0.06
South Gonder	645036	10.09	1.41	0.29
East Gojam	953080	14.91	4.66	0.41
West Gojam	532075	8.33	2.06	0.25
North Gonder	416860	6.52	0.50	0.14
Awi	326197	5.10	2.84	0.32
Amhara region	6390779	100.00	1.56	0.32

Source: CSA 2005.

3.2. Study population

3.2.1. Distribution and importance

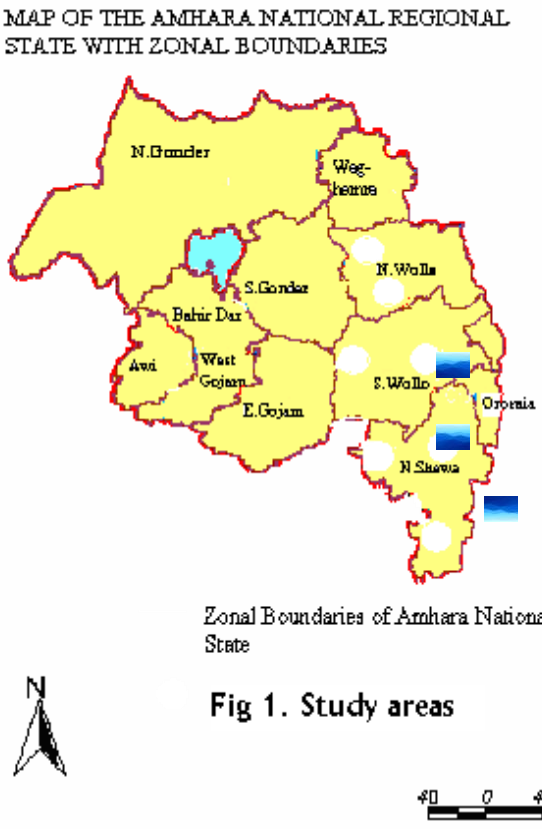
The sheep population of the Amhara region consists a number of local sheep populations named usually after their locality. Based on qualitative and quantitative morphological descriptor variables the sheep types found in the Amhara region were grouped into four major categories (Sisay, 2002). They are central high land sheep (Menz sheep), North-western high land sheep (Agew sheep), North-western low land sheep (Gumez sheep), and Rift valley sheep (Afar sheep).

Figure 2: Study area

Zones: N. Shewa – 2 woradas
S. Wollo – 2 wordas
N. Wollo – 2 Woreda

Sheep Breeding Ranches

- Debre Berhan
- Amed Guya
- Sheno



In the Amhara region as a whole, sheep population outbeats goats in the ratio 1.56:1.00. The majority of the sheep (64%) are found in Eastern Amhara (South Wollo, North Showa, and North Wollo and South Gonder) While about 35% of the sheep are found in Western Amhara zone (East and West Gojam, Awi and North Gonder zones) (Sisay, 2002). Within zones, the sub-moist and moist higher altitude areas (>2500 masl) take a substantial share of the sheep population.

3.3. Cross-sectional study

A cross-sectional survey of brucellosis was conducted from September 2007 to March 2008 to study the seroprevalence of brucellosis and assess the effect of risk factors associated with the distribution.

3.4. Sampling methods and sample size determination

The sample size determination was based on the assumption of the possible seroprevalence rat of the disease recorded in other place 1.5% Yibeltal (2005), expected prevalence rate was considered. The relevant formula to calculate sample size for sheep was the formula used for random sampling method and the study considered 95% levels of significance (Thrusfield, 1995).

$$N = \frac{(tx\sqrt{p(1-p)})2}{L}$$

Where, n = sample size.

tx = student t-value (1.96 at 95%).

P = estimated prevalence

L = desired absolute precision

Then the required sample size were (n=2409

3.5. Sampling strategy

The study population was stratified into two strata based on the management system: sheep flocks in ranches and smallholder farms. The stratification was required because the study population is kept under different management types, which could influence the prevalence of brucellosis antibodies to be estimated. Stratification with a variable sampling fraction will be used and the proportional allocation will be 20% for ranch sheep and 0.25% for smallholder farms.

Stratum1. Sheep flocks on-station: the adult sheep population in ranches was 2706. The sampling frame will be comprised of individual animals from farm records and animal will be sampled by taking 20% random sample. Individual animal (n=571) will be sampled using systematic random sampling. The study is cross-sectional study where the sampling methodology will be stratified

random sampling technique. The study will encompass three zones of the Eastern Amhara national regional state.

Stratum 2. Sheep flocks on smallholder farms: the adult sheep population in selected districts/ woredas will be estimated 510,102 and then, 0.25% of the adult population (n=1838) will be sampled using systematic random sampling. The sample will be proportionally distributed to randomly selected woredas (n=6) and kebeles/localities. Adult animal in each kebele will be sampled using systematic random sampling.

3.5.1. Sample collection

In order to determine the target population, public relation activities will be made with zonal and woreda Agricultural Department personnel. Once the target population determined whole blood will be collected from jugular vein of each sample animal using 10 ml non-heparinized vacutainer tubes. Each serum sample is identified by labeling on the vaccutainer tube. Relevant risk factors: sex, age, breed, herd size management, including reproductive performance (age at first lambing, lambing interval, litter size and annual reproductive rate) were recorded simultaneously with blood collection. Blood samples were allowed to clot at room temperature, and then the serum was separated from clotted blood by centrifugation. The separated sera were stored at -20^{o}C until tested by both, Roth Bengal Plate Test (RBPT) and Complement Fixation Test (CFT).

3.6. Serological tests.

3.6.1. Rose Bengal plate test (RBPT)

The Rose Bengal plate test was used as a screening test of serum samples for the presence of *Brucella* antigens. All blood samples collected were first screened using Rose Bengal Plate Test (RBPT) following the procedure described by Alton *et al*. (1975). The antigen used for RBPT, consider of a suspension of *B.abortus* (Obtained from institute Purquier 326, Rue de la Galera 34097 MANTPELLIER CEDEXS, France), inactivated by heat and phenol, adjusted to pH 3.65 and colored with Rose Bengal. Briefly, test sera and antigen were left at room temperature for half an hour before the test: 30µl of each test serum was taken and placed in a clean glass slide, 120µl of Rose Bengal antigen (1:4) was added to the slide of each test serum using a dropper,

then the antigen and the test serum were mixed thoroughly by an applicator and the slide was shacked by hand for 4 minutes. The result was read by examining the degree of agglutination in good light source and when necessary using magnifying glass deemed. After four minutes rocking and visible agglutination was considered as positive. Material preparations for RBPT are shown in Annex-2.

3.6.2. Complement fixation test

Sera that tested positive to the Rose Bengal Plate Test (RBPT) were further confirmed using Complement Fixation Test (CFT). CFT was conducted at the National Veterinary Institute, at Debre Zeit. Preparation of the reagent was evaluated by titration and performed according to protocols recommended (Annex. 3). Standard *B. abortus* antigen S99 was used as an antigen (CVL, New Haw Weybridge, and Surry KT15 3NB, UK). The antigen, control sera and Complement were obtained from the Bg vv, Berlin, Germany. And 2% sheep red blood cells suspension was prepared before the beginning of the test. In the CFT serum with strong reaction more than 75% fixation of complement (3+) at a dilution of 1:5 and at least with 50% fixation of complement (2+) at a dilution of 1:10 and above were classified as positive (OIE, 2004).

3.7. Questionnaire survey:

A rapid survey was conducted using a semi-structured questionnaire, to determine management risk factors (age, sex, breed, occurrence of retained fetal membrane and abortion) including reproduction performance (age at 1st lambing (days), lambing interval (days), litter size, Annual reproduction rate) that influenced the spread and maintenance of brucellosis (Annex-4). The survey was conducted in purposely selected six villages (two in each zone). A total of 100 farmers were interviewed and responses were recorded. Interviewed farmers identified that Pasteurellosis, Respiratory disease complex, internal parasites (Liver fluke, and Coenurosis), Sheep pox, Diarrhea, Foot rot, Anth*rax* and abortion were the major diseases identified by the majority of farmers in order of importance. When asked about the aforementioned disease susceptibility between the cross breeds (Awassi x local crossbreds) and the local sheep breeds, the majority of interviewed farmers (63%) observed no difference between the two genotypes, 37% of the respondent stated greater susceptibility or lesser chance of recovery of crossbreeds unless they received the necessary medical treatment. They also indicate that, the increased

incidence of abortion problems with retained placenta among crossbreed sheep. Otherwise, no suggestion was given by farmers asserting new disease outbreak or increased disease incidence associated with introduction of crossbred sheep.

3.8. Data analysis:

The data management requirement was handled on the "SPSS" programmed on a microcomputer. The data were compiled by summing up the laboratory findings and field observation into, woredas and zones units accordingly. Epidemiological and statistical analyses were performed as is required. An animal was said to be positive if it tested positive to both RBPT and CFT. A flock having at least one seropositive sheep was considered as positive. Individual sheep level seroprevalence was calculated on the bases of RBPT and CFT positive results divided by total number of sheep tested. Similarly, flock level seroprevalence was computed as the number of flock with at least one positive animal divided by the total number of flock tested. Calculated data were stored in Microsoft EXCEL spread sheet. Descriptive and analytic statistics were computed using intercooled STATA 7.0 soft-wares. Logistic regression and Pearson's Chi-square test (x^2) were employed to see the association of risk factors with that of seropositivity to *Brucella* antibody; the degree of association was computed using Odds ratio (OR) and 95% confidence interval (CI).

4. RESULTS

4.1. Seroprevalence of brucella infection in sheep using RBPT and CFT

Serum samples were collected from selected study woredas and sheep breeding and multiplication centers. A total of 2409 sera (n=1838 serum from smallholder farms and n=571 serum samples from Sheep Breeding and Multiplication Centers) were tested for the presence of serum antibodies against *Brucella* infection in sheep. One hundred and twenty sera were found to be positive to RBPT, upon further testing of the (n = 120) RBPT positive sera with CFT 118 sera become positive, for antibodies to brucellosis. All the seroprevalence estimates presented in the paper are with reference to CFT result.

4.2. Overall seroprevalence of brucellosis

Serum samples were collected from 274 flocks having (n=2409) sheep above six months of age with no history of previous vaccination against brucellosis. The sampling involves the two production systems, intensive farm consists of eight flocks (n= 571), extensive farm 266 flocks (n=1838). The overall individual and flock level, seroprevalence of *Brucella* antibody in sheep were 4.89 (n=118) and 22.26% (n=61), respectively (Table 9). The mean seroprevalence of brucella infection in the smallholder farm were 5.87% and 1.75% in sheep breeding ranches as given in table 4 and table 5 respectively. There was a significant difference in seroprevalence between the two sheep management types ($p<0.001$).

The distribution of *Brucella* seroprevalence was given in (fig.3), among different woredas (Table 4). The seroprevalence of *Brucella* antibodies in wereda level, were higher in Gubalafto 11.51%, followed by 8.54%, 7.8% and 4.27% in Werielu, Delenta, and Leganbo, in their order of importance. The lower seroprevalence of *Brucella* antibody were recorded in Angolela and Lalomider weredas of North Showa zone, 2.26% and 2.33%, respectively (Fig. 3). In sheep breeding ranches the seroprevalence of brucellosis were (2.33%) in Debre Berhan sheep breeding and multiplication center followed by Amed Guya (1.0%) and Sheno Agricultural Research Center (3.57%)(Table 5). This results shows a significant difference between breeding centers for susceptibility to *Brucella* infection ($p<0.001$).

Table 4: Seroprevalence of brucellosis in small holder farm using compliment fixation test

Zone	woreda	Sera		Seroprevalence
		tested	positive	%
North Showa	Lalomider	225	6	2.33
	Angolela	221	5	2.26
South Wollo	Leganbo	515	22	4.27
	Wereilu	386	33	8.54
North Wollo	Delenta	294	23	7.8
	Gubalafto	165	19	11.51
Total		1838	108	5.87

Table 5: Seroprevalence of brucellosis in sheep breeding and multiplication centers

Zones	Sheep breeding Center	Sera		Seroprevalence %
		Tested	Positive	
	Debre Berhan	257	6	2.33
North Showa	Amed Guya	286	3	1.0
	Sheno ARC	28	1	3.57
Total		571	10	1.75

Among pair wise comparison, there is no significant variation among Debre Brehan and Amed Guya sheep breeding center, whereas, a significant difference observed between Sheno and Amed Guya ($p<0.001$) Sheno and Debre Berhan sheep breeding center ($p<0.001$). The seroprevalence distribution in smallholder farm varied among the three zones (Fig 5). The seroprevalence is as higher as 9.15% is North Wollo followed by South Wollo 6.10% and North Showa 2.30% (Table 7). The seroprevalence compared between the three zones has a significant difference ($p<0.001$). Pair wise comparison between North Showa and South Wollo, South Wollo and North Wollo shows a significant difference ($p<0.001$), while, N. Showa and N.Wollo have no significant difference ($p>0.057$). The mean flock seroprevalence of brucellosis with at least one seropositive sheep in small holder farm was 19.92% and 100% in sheep breeding and multiplication center as given in table 9 respectively. In smallholder farm the flock serpositivity varied considerably among the weredas and it was higher in Lalomider (50%) followed by Angolela (48%), Medium in Gubalafto 32% and Legambo 27% and lower in Wereilu 20.6% and Delanta 23.6%, in the order of their importance (Table 6). While all flocks in ranches have at

least one seroreactor sheep, hence the seroprevalence of *Brucella* antibody in Debre Berhan, Amed Guya and Shemo ARC was recorded 100%, 100% and 100% respectively. This shows that there is a significant difference ($p<0.001$) when flock level seroprevalence and management system is considered.

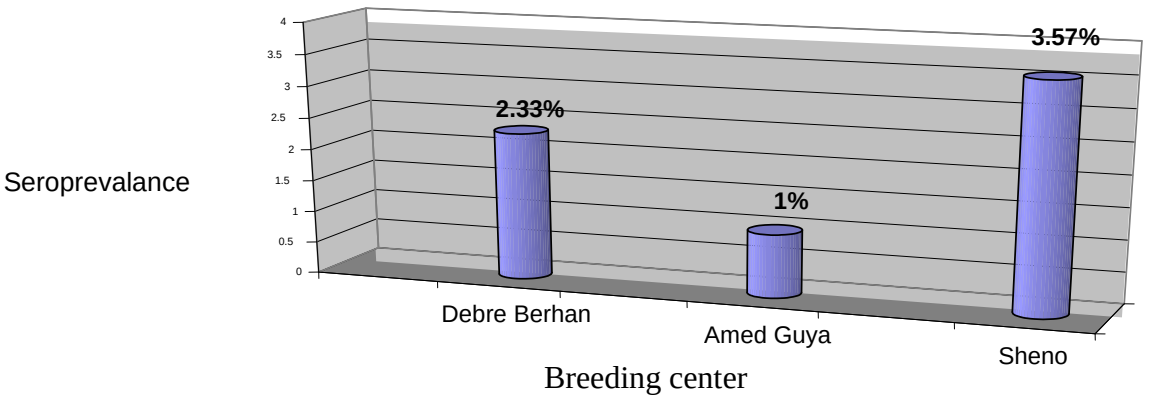


Figure 3: Seroprevalence of brucellosis in sheep breeding and multiplication center

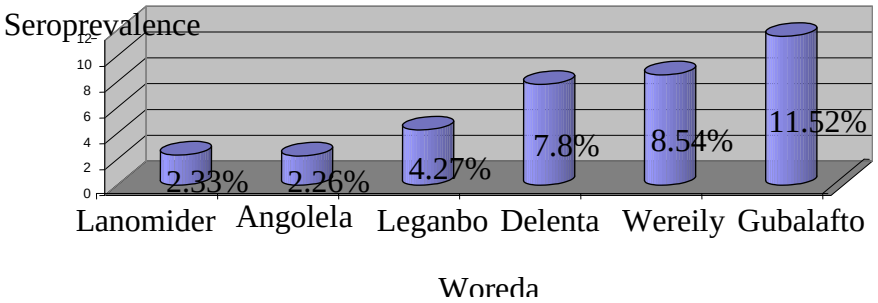


Figure 4: Seroprevalence of brucellosis in smallholder's farms (woreda level) using CFT

Table 6: Flock level seroprevalence of brucellosis in sheep (smallholder and ranches)

Zone	Wereda	Flocks		Flock seroprevalence (%)
		Tested	Positive	
North Showa	Lalomider	74	6	50
	Angolela	65	5	48
South Wollo	Leganbo	37	14	27
	Wereilu	38	11	20.6
North Wollo	Delenta	25	9	23.6
	Gubalafto	27	8	32
Total		266	53	19.9
Sheep breeding center and Sheno ARC	Amed Guya	4	4	100
	Debre Berhan	3	3	100
	Sheno ARC	1	1	100
Total		8	8	100

4.2.1. Zonal seroprevalence

It was necessary to compare the three administrative zones according to the result obtained during the study; this can help to ascertain the status of brucella infection in different localities.
The overall individual sheep seroprevalence among the three Administrative Zones (North Showa, South Wollo and North Wollo) were in the order of, 2.3%, 6.1%, and 9.15% (Table 7) respectively. There was statistically significant difference among the three zones ($p<0.001$) (Table 10). Pair with comparison between zones revealed significant difference between North Showa and SouthWollo ($p<0.001$) and North Showa and North Wollo ($p<0.001$), however N.Showa and N.Wollo zones were not showing significant difference ($p>0.057$). Generally the diseases were distributed with different magnitude through out the entire zones.

4.2.2. Flock level seroprevalence

To ascertain the flock level seroprevalence between the three zones and the two production systems it is necessary to know their serological results. The flock level seroprevalence of the three administrative zones were 7.9 %, 33.3 %, and 32.69% in North Showa, South Wollo and North Wollo respectively (Table 7). The figure shows significance difference between the three

zones (p<0.001), but pair wise sero-computation disclosed a significant association between N. Showa and N.Wollo (p> 0.527). whereas, N. Showa and S. Wollo, S. Wollo and N. Wollo have a significant difference in seroprevalence for *Brucella* antibody (p<0.001) and (p<0.001). The findings of this study ascertain, that the distribution and the magnitude of the disease were vary greatly from place to place and different in different localities.

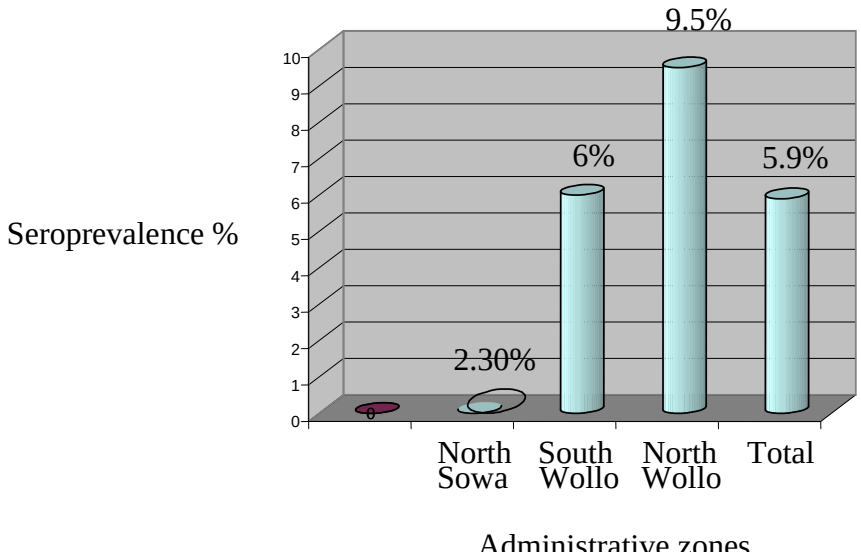


Figure 5: Zonal distribution of brucella seroprevalence in smallholder farms

Table 7: Overall individual and flock level brucella antibody seroprevalence

Zone	No tested	No positive	Prevalence%	No cluster	No positive cluster	Prevalence%
N.Showa	448	11	2.3%	139	11	7.9%
S. Wollo	901	55	6.1%	75	25	33.3%
N. Wollo	459	42	9.15	52	17	32.69%
Total	1838	108	5.87%	266	53	19.92%

4.2.2. Agro-climate

Basically the locations of the study area by itself were found in between the high land and the midland with the high land predominating (2000-2900 masl). The lowland was already excluded from the study, as the lowland areas were out of the study subjects. The majority of animals were

sampled from the highland range land, whereas the minority animals were sampled from the midland range land, this makes the different in localities, otherwise their breed and origins does not makes different between the whole sampled animals. The comparison was made on the seroprevalence of brucella antibody in the highland range land having a seroprevalence of 4.5% and midland range land with a seroprevalence of 9.07% (Table 8). There was a highly significant variation, when the seroprevalence rate and localities is considered (p=0.000) (Table 10).

Table 8: Comparison of brucella antibody sero-reactor in sheep between Midland and highlandin the extensive farm (North Showa, South Wollo and North Wollo zones)

Agro-climate	Number tested	Number positive	Seroprevalence %	Number of cluster	Number positive cluster	Seroprevalence %
Highland "Dega"	1287	58	4.50	201	34	16.9
Midland "Weynadega"	551	50	9.07	65	19	29
Total	1838	108	5.87	266	53	19.9

4.2.3. Production systems

Our focus of attention for the current study was on the two production systems of the area. These include extensive, which consisted local Menz breed of sheep which depend for feed on grazing at the field with minor supplementation at night when they come back home, and intensive mainly composed of exotic, and both cross and local breeds of sheep with the local breeds predominating, which depend highly on feed supplementation at home, occasionally they are allowed to graze around the homestead.

The overall individual level positive reactors to *Brucella* antibody of the two production system were 4.89%, i.e. extensive production has a specific seroprevalence rate of 5.87%, while, intensive production system has a specific seroprevalence of 1.75% (Table 9). The overall individual seroprevalence among the two production systems did vary significantly (p=0.000). Flock level seroprevalence of the intensive farm was 100% computed with extensive production system with flock level seroprevalence of 19.92%. This shows a highly significant difference (p=0.000) among the two production systems in terms of flock level seropositivity. This fact

indicates that, managerial problem can play a vital role in increasing or decreasing disease distribution.

Table 9: Brucella antibody seroreactor sheep in extensive and intensive farming system (North Showa, South Wollo and North Wollo)

Production system	Number tested	Number positive	Seroprevalence %	Number of cluster tested	Number positive cluster	Seroprevalence %
Extensive	1838	108	(5.87%)	266	53	(19.92%)
Intensive	571	10	(1.75%)	8	8	(100%)
Total	2409	118	(4.89%)	274	61	(22.26%)

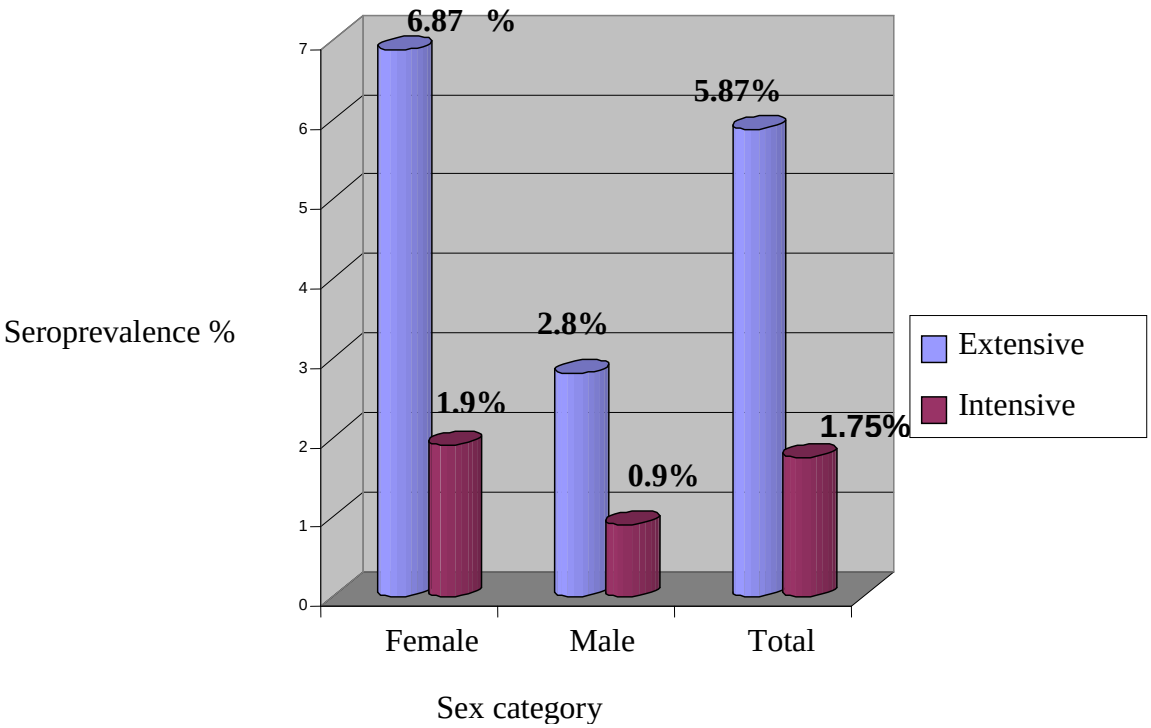


Figure 6: Seroprevalence in terms of sex category in extensive and intensive production system

Table 10: Statistical analysis results of individual and cluster level seroprevalence

Variables	Individual seroprevalence			Flock level seroprevalenc		
	DF	X^2	P-value	DF	X^2	p-value
Zones	2	47.58	0.000	2	65.73	0.000
N.Showa						
S.Wollo						
N.Wollo						
Agro-climate	1	14.692	0.000	1	25.79	0.000
High land						
Mid land						
Production systems	2	5.007	0.086	1	1.95	0.340
Extensive						
Intensive						

DF= Degree of freedom X^2 =Chi-Square P-value = probability value

4.3. Potential risk factors

4.3.1. Sex and seroprevalence

There was a significant sex difference in susceptibility to brucellosis, suggesting sex seems to have an impact on the infection rates of brucellosis. Sex has some implication in the epidemiology of brucellosis accordingly; comparison was made on the seroprevalence of female and male sheep. The seroprevalence of female sheep was, 6.87% and 1.94% for extensive and intensive production system respectively, and of males 2.8% for extensive, 0.9% for intensive production system (Table 11). There is no sex difference in susceptibility to brucella in the intensive production system, while a significant sex difference in susceptibility to brucella was observed in extensive production system. However in both production systems it was found out that seroreactors were at a higher proportion in female than male.

4.3.2. Age and seroprevalence

With regard to age of the whole animals examined there were a big different in seroprevalence of brucella antibody. Similarly the seroprevalence of brucellosis in sheep was found to be higher in

adult animals than younger ones. Age is supposed to have some association with the recovery of antibodies against brucellosis, as sexual maturity is important for the multiplication of brucella organism, therefore, based on its biological relevance age was classified into three categories. In the extensive production seroprevalence of brucella antibody in the three age categories were in the order of 2.56%, 5.9% and 6% with age groups of 0.6-1 year, 2-3 years and >4 years (Table 11), respectively.

In the intensive production system seroprevalence of 0%, 0.8% and 3.1% were found in the three age categories of 06-1 year, 2-3 Years and >4 years respectively (Table 11). Highly statistically significant difference was observed in the three age categories of the intensive production system $p<0.001$. However, the three age categories were not significantly difference in the extensive production system despite the increase in the proportion of seroreactors with age.

4.3.3.. Herd size and seroprevalence

Herd size is one of the risk factors that contribute for the occurrence of brucellosis. Thus, based on the prevailing small ruminants holder of the study area the flock was categorized into three groups. Seroprevalence of 20%, 22.9% and 33% were respectively found from flock categories of 1-10 sheep, >11-20 sheep and > 21 sheep in the extensive production (Table 11). In the intensive production system the current study focus of attention is on >11-20 and >21 flocks which has a sero-prevalence of 100% and 100% respectively. Highly significant association between the two flock categories were the case in intensive production. Whereas, extensive production system revealed a highly significant difference among the three herd categories ($p<0.001$), yet, it was evident that larger sized flocks were found at higher proportion than smaller flocks (Table 11).

4.3.4. Breed and seroprevalence

Based on this fact we have attempted to look into the association of breed to that of seropositivity to brucella antibodies. The comparison was under taken in the intensive production were exotic breed, cross breed and indigenous (Awassi, Awassi x local and local) breeds were kept together. Seroprevalence of 0%, 2.2%, and 2% were found respectively, in the three breeds of sheep, yet there is no significant difference. In the extensive farming system the seroprevalence of brucella antibody in local Menz breeds were 5.97% and the cross (Awassi x local Menz breeds)(very

small in number) were 4.1%, these shows no significant difference among the two breeds of sheep (Table 11).

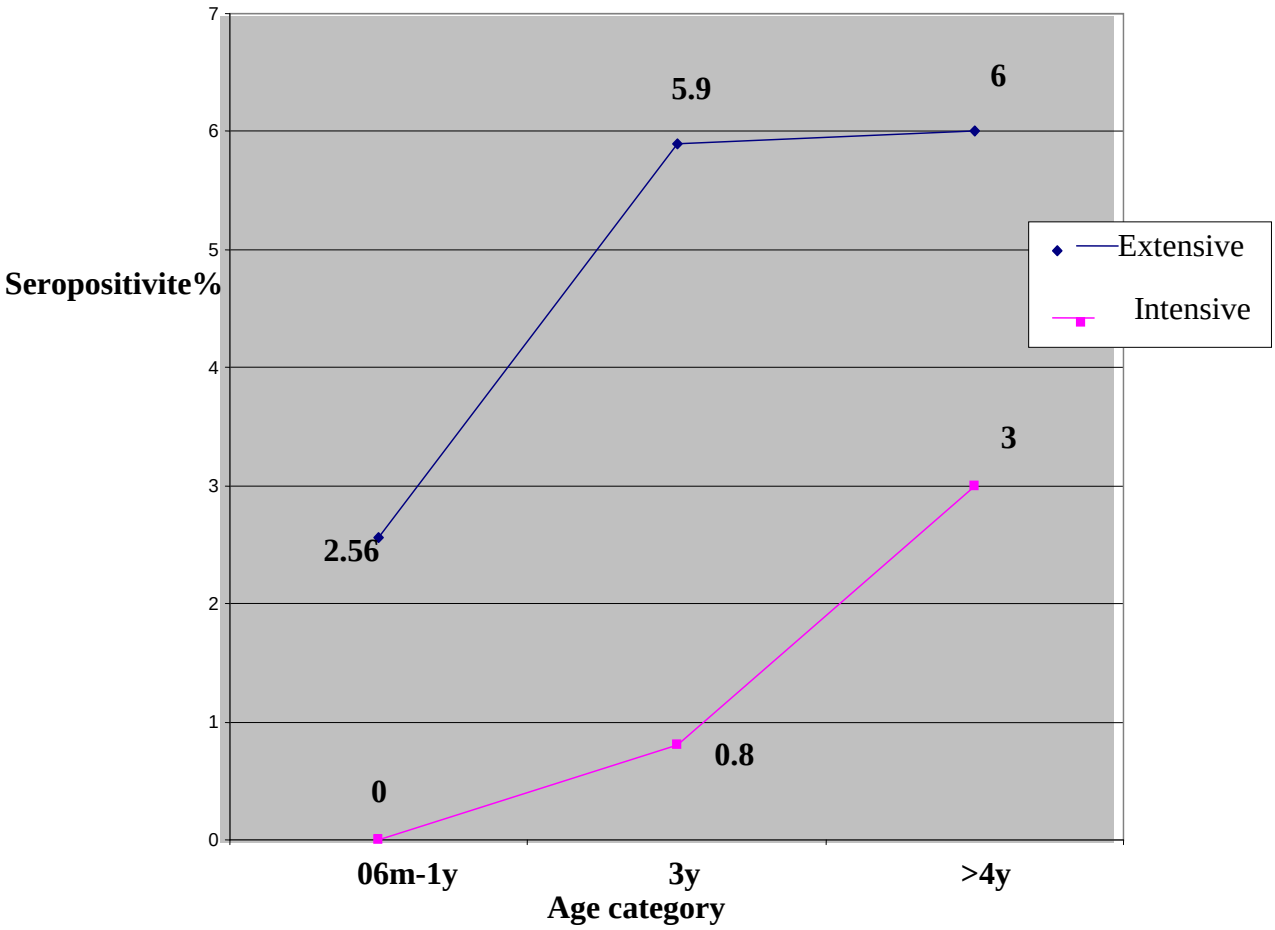


Figure 7: Seroprevalence of brucellosis based on age category of sheep

4.3.5. Reproductive status versus brucella seroprevalence

In the present study the reproductive status of female was categorized in three groups, (Pregnant, lactating ‘‘Nursing’’ and dry), virtually no significant difference in sero-positivity among the three groups was observed, in both production Systems (Table 12). Pregnant sheep again grouped

in to two groups, on the basis of their stages of gestation, where one group consists of sheep below 4 months of pregnancy (1) and the other group having sheep with equal too and above 4 months of pregnancy (2). Incidentally there was significant difference between the two pregnancies

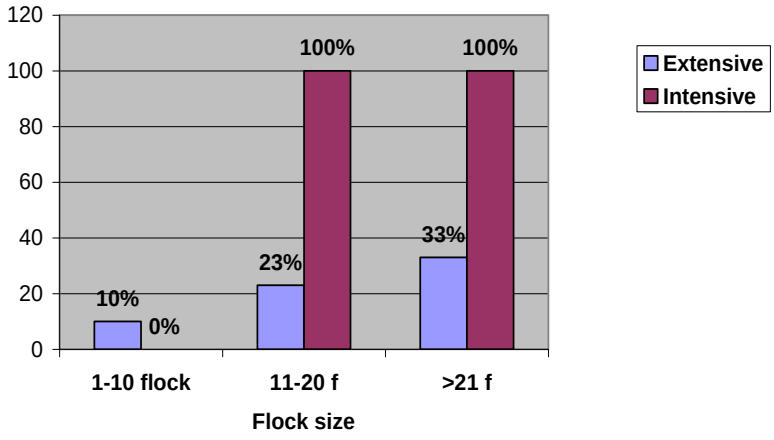


Figure 8: Seroprevalence of brucellosis in relation to flock size

groups in the intensive production ($p<0.001$). But there is no significant difference between the two groups in the traditional management system. To asses the seroprevalence of sheep at different parity number, three categories were created, those with no lambing, those that lambed once and those with two and more lambing accordingly. The extensive production system shows significant variation at the different parity number status ($p< 0.001$). However, no significant difference was observed in the intensive production systems (Table 12).

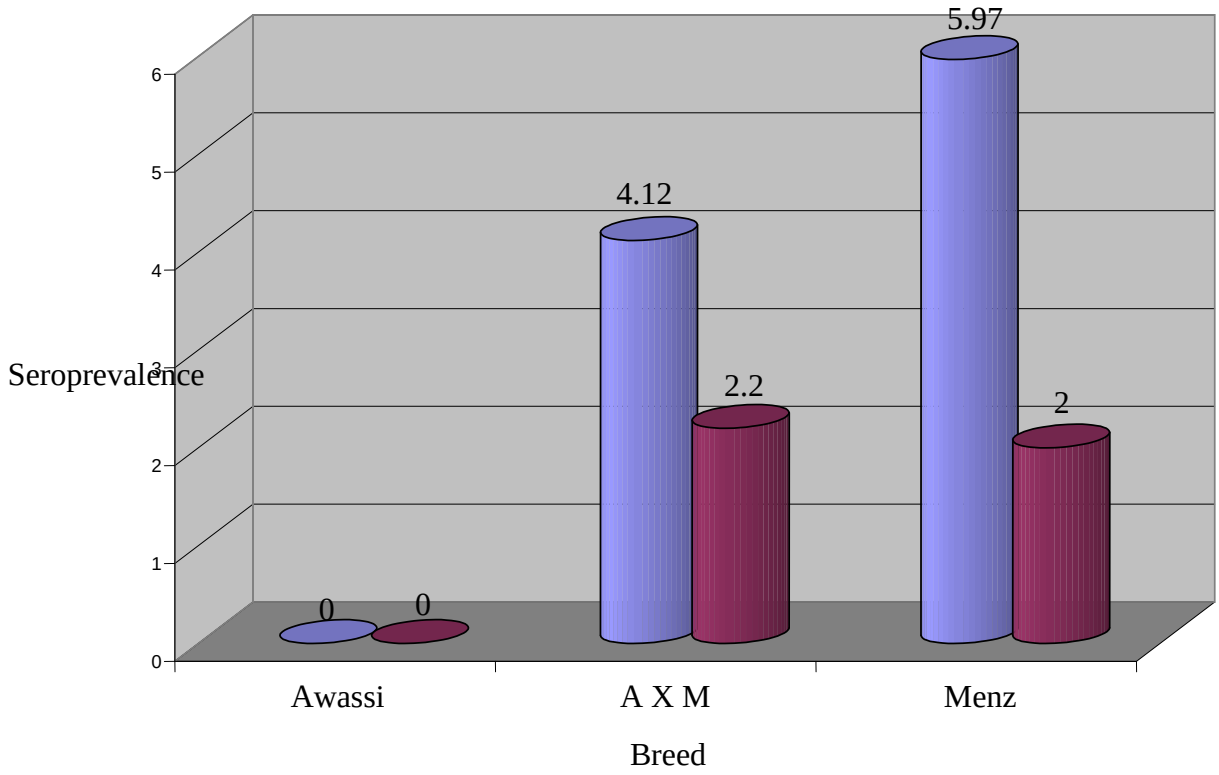


Figure 9: Seroprevalence of brucellosis by breed of sheep in small scale and ranches

4.3.6. Clinical signs and sero-positivity

Based on clinical signs manifestation the animals were categorized as apparently normal and showing overt clinical signs. During the study period a total of 115 cases of (87 cases from extensive, 28 cases from intensive production) reproductive disorders were recorded in sheep. Of these 55, 16, cases had abortion and 32, 12 cases are retained fetal membrane respectively. In this study a highly statistically significant difference was observed in seropositivity between sheep that had previous history of abortion *(X^2 =14.2695, P =0.005),* and retained fetal membrane *(X^2 = 6.213, P =0.001),* compared to those without history of abortion and retained fetal membrane (Table 12). In both productions system abortion was significantly related to brucella antibody seropositivity. (Table 12).

Table 11: Potential risk factors for brucella antibodies seroreactors sheep in extensive and intensive production

Extensive production (Traditional management) system				Intensive production system		
Risk factor	Number tested	Number positive	Seropreval ence (%)	Number tested	Number positive	seroprevalence
Sex						
Female	1381	95	6.87	462	9	1.9
Male	457	13	2.8	109	1	0.9
Total	1838	108	5.87	571	10	1.75
P=0.25, OR.95% CI = [1.27-8.72.46] OR=(0.21)				p= 0.0123, OR= 95%CI [0.31-2.34]		
Age						
06- 1	78	2	2.56	68	0	0
> 2-3	617	37	5.9	250	2	0.8
> 4	1143	69	6	253	8	3
Total	1838	108	5.87	571	10	1.75
P= 1.15, OR, 95% CI=[0.76-2.52]			OR (0.77), P=(0.001)	OR= 95% CI=[0.43- 1.38]		
Herd size						
1-10	110	11	10	0	0	
> 11-20	96	22	22.9	1	1	100
> 21	60	20	33	7	7	100
Total	266	53	19.9	8	8	100
Test statistic	P=(0.27), OR. 95% CI= [0.90-3.21],			OR= (0.69), P=(0.007),		

There is a significant difference in seropositivity between animals having retained fetal membrane at least once in there entire life, and apparently not having retained fetal membrane even once in their entire life (Table 13). To compare the difference in seropositivity with respect to abortion frequency two categories were produced, the first category with a single abortion and the second category have more than one abortion frequencies. Abortion frequency has a significant association with seropositivity in both extensive and intensive production systems (Table. 13).

Table 12: Brucella sero-reactors sheep at different reproductive status in the two production systems

Traditional management system				Intensive management system		
Reproductive status	Number tested	Number positive	Sero-prevalence	Number tested	Number positive	Seroprevalence (%)
Pregnant	308	32	10.38	90	3	3.3
Nursing(Lactation)	425	19	6.16	74	2	2.7
Dry	294	26	8.84	37	2	5.4
Total	1027	77	7.49	201	7	3.48
Duration of pregnancy						
< 4 months	95	9	9.47	51	1	1.96
> 4 months	102	23	22.54	39	2	5
Total	197	32	16.24	90	3	3.3
Parity number						
No parity	150	5	3.3	97	1	1.0
Parity once	267	10	3.7	64	2	3
Parity > once	316	18	5.69	91	4	4.3
Total	733	33	4.5	252	7	2.7

4.3.7. Management and husbandry risk factors

One hundred randomly selected small ruminant owners were interviewed in the extensive production system with the view to see the association of various management and husbandry risk factors, and this then was compared with serological analysis results. The finding revealedthat 18% (n=18) of the respondent had seropositive flocks. From the questionnaire result it was observed that 18% (n = 18) of the respondents had seropositive sheep, with higher proportion in South Wollo zone 22.7% (n=10), to be followed by North Wollo zone 21.87% (n=7). In North Showa zone only one respondent had seropositive sheep as indicated on the table (Table 14). The variations among the three Administrative zones reveal highly significant difference (0,001).

Pair wise comparison between zones revealed significant difference, among North Showa and South wollo and North Showa and North Wollo zone, however South Wollo and North Wollo zones shows a significant association (Table 10).

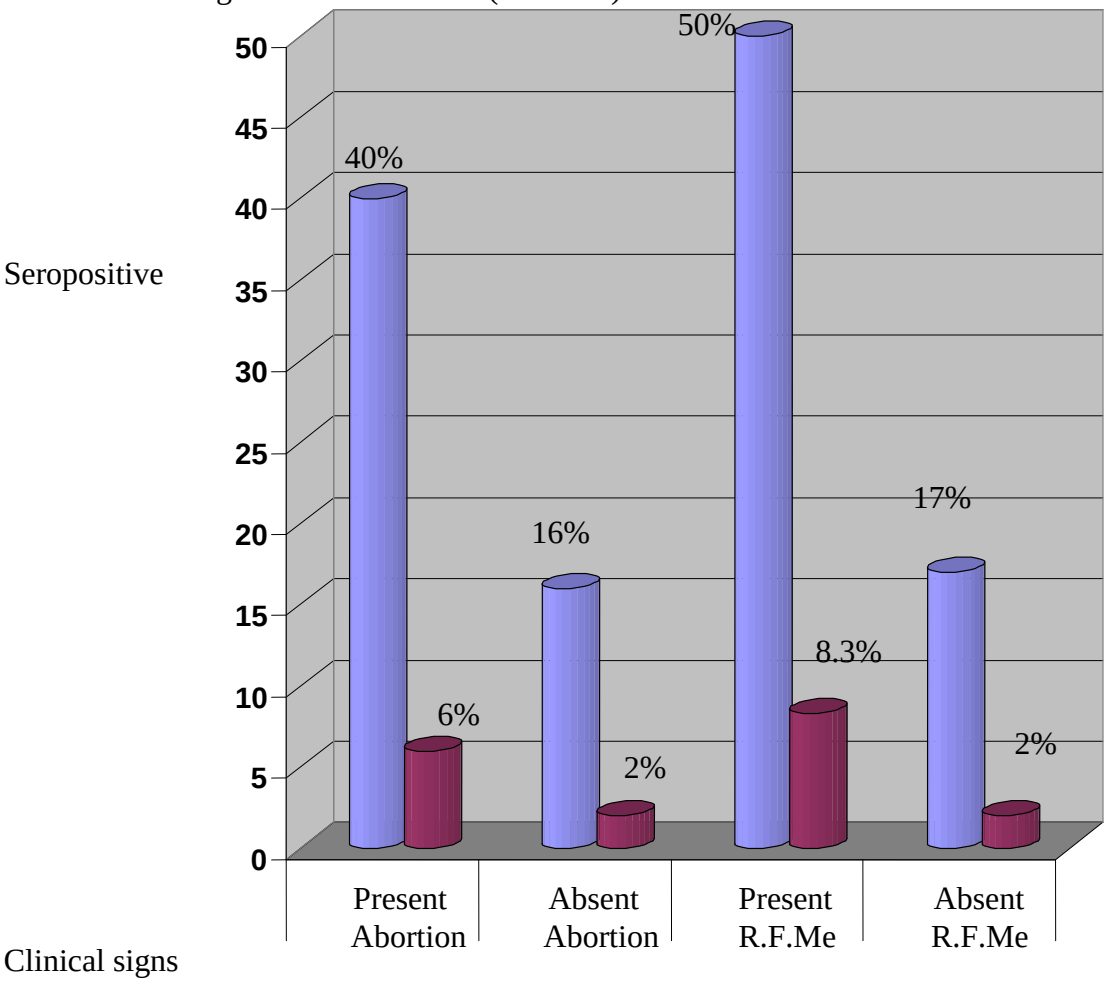


Figure 10: Clinical signs and seropositivity for brucella antibody in both production systems

4.3.8. Reproductive status and seroprevalence

An attempt was made to asses the reproductive parameters of female sheep in the traditional smallholder production system using questionnaire survey. 597 small ruminants' owners were interviewed together and with sample collection, from 6 weredas of South Wollo, North Wollo and North Showa zones. The parameters of interest were, age at first lambing, lambing interval, litter size (prolificacy), number of lamb crops produced per ewe per production life and annual reproduction rates. Accordingly, the mean age at first lambing of the local sheep kept under the traditional smallholder production system was 647 days, while the minimum was 609 and the maximum 620 days and the standard error was 0.04 Similarly, for the indigenous Menz breed of

sheep in the study area, the lambing interval ranged between 268 days and 288 days with an average of 279 days and standard error of 0.02.

Table 13: Seropositivity to brucella antibody and reproductive disorders (Clinical sign) of sheep in the traditional and intensive management systems.

Extensive production system				Intensive production system		
Reproductive disorder	Number of serum tested	Number positive	Prevalence %	serum tested	Number positive	Prevalence %
Abortion						
Present	55	22	40	16	1	6
Absent	218	34	15.59	337	6	1.5
Total	273	56	20.5	353	7	1.7
Test statistics	P=4.22, OR.95% CI=[0.53-32-63]			OR (0.73), p= 0.005, OR.95% CI= [0.28-1.88]		
Retained fetal membrane						
Present	32	16	50	12	1	8.3
Absent	241	42	17	341	6	1.7
Total	273	56	20.5	353	7	1.9
Test statistics	P=3.33, OR. 95% CI= [0.31-22.9]			OR= 1.7, p= 0.001, OR.95%CI=[0.66-4.42]		
Abortion frequency						
Only once	48	19	39.58	13	1	7.69
> once	7	3	42.85	3	0	0
Total	55	22	40	16	1	6.25
Abortion months						
< 4 months	11	5	45	7	0	0
> 4 months	44	17	38.6	9	1	11
Total	55	22	40	16	1	6.25

Table 14: Comparison between serological and questionnaire survey results of the three administrative zones

Administrative zone	Total number of respondent	Number seropositive	Seroprevalence %
North Showa	24	1	4%
South Wollo	44	10	22.7%
North Wollo	32	7	21.87%
Total	100	18	18%

Table 15: Management and husbandry risk factors obtained from the questionnaire survey and serological analysis (N.Showa,S.Wollo and N.Wollo).

Management and husbandry risk factors	Number of respondent	Number of positive	Seroprevalence (%)	P- value	OR. 95% CI=
Present of dog				0.41	0.28-24.19
Yes	32	3	9.37%		
No	68	15	22%		
Disposal of fetal membrane					
Burring	14	0	0		
Throwing to field, river, giving to dog	86	18	20.9%		
Keeping of sheep frequently aborting				0.46	0.60-19-26
Sell and slaughter	64	9	14%		
Keeping	36	9	25%		
Reason for selling breeding female				0.65	0.048-6.74
Infertility	47	11	23%		
Old age and money	53	7	13.20%		

It was also found that the number of lamb crops produced per ewe per production life varied between 4 and 8.5 lambs, with an average of 6.25 lambs and standard error 0.28. Similarly for the indigenous Menz breed in the study area litter size (prolificacy) ranged between 1.06 and 1.29 lambs, with an average of 1.21 lambs with standard error of 0.225.

Table 16: Impact of brucella antibody seropositivity on flock's reproductive performance by comparing serological result with that of questionnaire data.

Reproductive parameter	Mean	Range	Standard	Odds	p-value	OR. 95% CI
	days	Days	error	ratio		
Age at first lambing						
Seropositive	693	630-755	0.10	1.40	0.004	0.38-0.60
Seronegative	601	588-620	0.04			
Total	647	609-688	0.04			
Lambing interval					0.019	0.26-1.18
Sero-positive	290	280-300	0.05			
Sero-negative	268	256-275	0.026			
Total	279	268-288	0.02			
Number of lamb crops /ewes /production life(number)						
Sero-positive	4	3-5	0.26	0.28	0.04	0.07-0.94
Sero-negative	8.5	5-12	0.58			
Total	6.25	4-8.5	0.28			
Litter size(prolificacy)					0.003	
Sero-positive	1.18	1.05-1.24	0.41			
Sero-negative	1.24	1.07-1.35	0.043			
	1.21	1.06-1.29	0.225			
Annual reproduction rate					0.004	
Sero-positive	1.50	1.30-1.68	0.53			
Sero-negative	1.61	1.53-1.65	0.06			
	1.56	1.42-1.67	0.30			

5. DISCUSSION

In the present study the occurrence of brucella seroreactors in sheep in the three Administrative Zone (North Showa, S. Wollo and North Wollo) has been investigated, applying field study, serological analysis and questionnaire surveys. The seroprevalence of small ruminants brucellosis was reported based on the result of both Rose Bengal and Compliment fixation test. A total of 2409 sera (n=1838 serum samples from extensive production and n=571 serum samples from intensive production systems) were tested for the presence and absence of serum antibodies against *Brucella* infection in sheep. One hundred and twenty (120) sera were found to be positive to Rose Bengal Test (RBPT), upon further testing of the 120 RBPT positive sera with Complement fixation test (CFT) 118 sera were found positive. The subsequence analyses were based on the 118 (4.89%) sera that were positive to both the RBPT and CFT (serial interpretation) test results.

The seroprevalence carried out so far in Ethiopia indicate that, brucellosis may be one of the important diseases in sheep raising country. A seroprevalence study carried out in small ruminant in Afar and Somalia region in 2005, clearly demonstrated that, the disease exists in Ethiopia. Other researchers in Ethiopia Tekelye and Kasali (1990), reported seroprevalence rate of 1.5% in sheep and 1.3% in goats in central high land of Ethiopia. Yibeltal (2005) also reported seroprevalence rate of 1.5% in sheep and 16.5% in goats in Afar region, and 1.6% in sheep and 1.7% in goats in Somalia region.

The existence of the disease has been reported in Southern Nations Nationalities and Peoples Regional State (SNNPRS) and Borena pastoral areas (Teshale *et al.*, 2006). Very limited works have been done in Ethiopia with only single information being available on the status of small ruminant brucellosis. The only high seroprevalence findings that obtained by Yibeltal (2005) disclose the reality that the seroprevalence of small ruminant brucellosis was much higher in Afar region where communal use of grazing land is practical, than Somalia region where clan based flock segregation is common.

Comparable seroprevalence were also reported in Africa; a report of 1.6% in sheep and 4.1% in goats made from Morocco, 4.0% in sheep and 18.0% in goats in Tunisia, 8.2% in sheep in Israel, 2.4% in sheep and 8.2% in goats in Egypt, 3.0% in both species in Iran, and 14.2% in sheep and 16.7% in goats in Khartoum region of Sudan (Benkirane, 2005). In Kenya prevalence rates of 6.01% in sheep and goats (Waghela, 1976), in Somalia prevalence rate of 7.2% in sheep and 5.29% in goats (Falade and Hussein, 1997), and in Eritrea prevalence rate of 3.8% in goats and 1.4% in sheep was reported (Omer *et al*., 2000). According to Acha and Szyfres (2001) the rate of infection varies greatly from one country to another and also between regions even within a country.

All the aforementioned study reveals the high sero-prevalence and importance of brucellosis in goats which disclosed that, goats were at a high risk of acquiring brucella infection than sheep. The higher prevalence of brucellosis in goats as compared to sheep might be mainly due to the greater susceptibility of goats to brucella infection than sheep; and partly it could be due to the fact that sheep do not excreta the organism for longer period of time unlike goats. This can reduced the potential of the spread of the disease among sheep population (Radositis *et al*., 2000).

The results of the current study showed that, the overall seroprevalence rate of brucellosis in selected areas of the Eastern Amhara Region were 4.89%. Out of these seropositive sheep 75%, gave very strong reaction (4+ and 3+) to CFT, while 60%, gave 2+ and above reactions. From all above the findings of sero-positive samples during serological test by RBPT 4.97% (n=120), has not difference with that of by CFT 4.89 (n=118). These indicate that, there has been very active *Brucella* infection in the study area with relatively moderate prevalence rate. The 4.89% prevalence of brucellosis in sheep observed in the current study is in fair consent with result of the previous studies in Ethiopia Yibeltal (2005), and other countries like, Karnatake in India (Renukaradhya *et al.,* 2002), in United Arab Emirate (Bankirane, 2005) and in Yemen in imported sheep (Refai, 2002) and in Kenya (Waghela, 1976).

In the present investigation the overall individual and cluster level seroprevalence of *Brucella* in sheep were, 4.89% and 22.26% respectively. The outbreak occurred in Algeria in 1989 showed 2.2% and 43.5% in individual and herd level seropositivity respectively (Benkirane, 2005), which

is relatively support the previous results. Studies in Morocco in 1996, showing flock level seroprevalence of 12.1% in sheep, while in Tunisia in 1990 in a place called Gafse, a flock level seroprevalence rates of 30% are founds in sheep (Benkirane, 2005). Comparable results were also reported from morocco (1996), 12.1%, from Kuwait (1993) 37.0% and 1997 49.0% from Kuwait were also reported (Benkirane, 2005). The present findings shows a significant variation in flock level seroprevalence to brucellosis among the three zones (N. Showa, S. Wollo and N. Wollo), 7.9%, 33.33% and 32.69% respectively.

As the same time flock level seroprevalence in intensive and extensive production system were 100%, and 19.92% respectively. This result shows a significant difference in seropositivity between extensive and intensive production systems ($p<0.05$). This variation might be due to the variation in the management and production system and herd size. The concentration of animals per plot of lands could make the situation favorable for the distribution of disease, yet it was evident that large herd/flock sizes were found at higher risk than smaller herds/flocks (Oloffs, 1996).

Basically, the location of the study area was found in the interface of the midland and highland range lands (2000-2500 masl). The lowland area was already excluded from the study, as the lowland districts were out of the study subjects. During the study the samples were taken from both range lands (midland and highland range lands). However both groups are belongs to the same breed (Menz), and have the same origins, the only difference were their place of grazing lands, watering points and the place where they spend their time on the day time. Our aim is only one that is to know whether these factors can make a difference or not in terms of brucella seropositivity, based on their grazing sites.

Comparatively higher seroprevalence were found in the mid land range than the high land range, both in individual and flock level (Table 8). The difference in sero-positivity between the two agro-climates was found to vary significantly $p<0.05$). The possible explanation that can be given for higher level of sero-reaction in the midland than, the highland is due to shortage of grazing lands in the midland agro-ecology, relatively larger number of sheep were kept in the midland range lands.. Increasing animal's concentration and contact favor the spread of brucellosis. And

the other possibility is the favorability of the environment for survival and multiplication of the organism and its subsequent infection.

The study focuses of attention was on two sheep production systems of the area. This includes extensive which consistes local Menz breeds of sheep, with a low proportion of cross breeds (Awassi X Menz). They depend for feed on grazing at the field with minor supplementation at night when they come back home. Intensive production system which is composed of exotic with low proportion and both cross and local breeds of sheep with the local breeds predominating. They depend highly on feed supplementation at home; occasionally they are allowed to graze around the home stead. In the present study, the variations in seroprevalence of brucella among the two production system investigated were not found significant. However flock level seroprevalence of brucellosis between extensive (19.92%) and intensive (100%) seems to have a significant variation (Table 9).

Generally in the intensive production system low seroprevalence was observed, this is not practically happened in intensively managed farming system. As far as brucellosis was considered as a disease of intensification, more seroprevalence were expected from intensively managed production system. The low individual sheep seroprevalence in the intensive production may be associated with better management practices, through experience which developed recently after being the farm closed due to Maedi-visna virus infection. All sanitary and facility maintenance activities were completed in the center prior to attempting the restocking activities. After facilitating the sanitary measure restocking is takes place. They introduced sheep after being tested By Agar gel immunodiffusion for Maedi visna and Rose Bengal plate test for brucellosis and the routinely cleaning of the house. These may decrease the establishment of infection in the farm and this makes the sheep not being exposed more by brucellosis.

There are reports that indicate the risk of infection with change from extensive to intensive management, whether they have indigenous sheep or introduced (WHO, 1997). Weidman (1991) reported the close correlation between the kind and intensity of husbandry system and rate of infection. Crowford *et al*. (1990) had also described on the influence of methods of management and characteristics of the population on the occurrence of infection in flocks/herds

Generally, the susceptibility of sheep to *B. melitensis* infection is influenced by age, sex, breed, herd size and reproductive status of the individual animal (Radostitis *et al.*, 2000). Bellow an attempt has been made to discus the findings of the current study along the line.

As a whole there was no significant sex difference in susceptibility to brucella infection, suggesting sex seems to have no impact on the infection rates of brucellosis in the intensive production system. Both females and males are equally exposed to the disease. But this is not true in the extensive production system. Despite the higher proportion of females reactor, significant difference in susceptibility to brucella between female (6.87%) and male (1.9%) in the extensive production ($p<0.05$) system. Yibeltal (2005) disclosed that there is no significant sex difference in seropositivity to brucella antibody, sexually mature and pregnant animals are more susceptible to infection with the organism than sexually immature animals of either of sex, which may be due to the fact that sex hormones and erythritol, which stimulate the growth and multiplication of *Brucella* organism tend to increase in concentration with age and sexual maturity (Radostitis *et al*., 2000)

Hirsh and Zee (1999) have described that male animals are less susceptible to brucella infection due to the absence of erythritol which is contradictory to the above statements. Kubuafor *et al.* (2000) had reported as a seroprevalence of 8.5% in females and 1.9% in males in Akwapim-Southern district of Ghana, yet the difference was not significant. Although no control study has been conducted on the relative susceptibility of female and male animals to brucellosis, based on the reactor rates it is possible that rams are more resistant than sexually mature lambs and ewes, however are less resistant than sexually immature lambs (Nicoletti, 1980). It is important to not that serological data may underestimate *B. melitensis* infection in male as infected ram tested were generally non reactors or only had low antibody levels (Crawford *et al.*, 1990).

With regard to age, there was a big difference in prevalence rate of brucellosis. In this study higher proportion of older sheep >4 ages were found more affected than the younger ones. The analysis of *Brucella* sero-reactors among the three age categories revealed a statistically significant difference in intensive production system ($p<0.001$), but not in the extensive production system, the difference among age groups was not found significant in extensive

management system. However, in the extensive production system the three age's categories were found significantly difference, despite the increment in seropositivity with age. Similar results were obtained by Abraham (2005, DVM thesis), which revealed statistically significant difference between different age categories. The increase in infection rate with age of the animal was also reported by Tekelaye and Kasali. (1990). Further more Kubuafor *et al*. (2000) from Ghana reported the significant increase in seropositivity with respect to age. Chukwu (1987) pointed out that sexually mature animals were very susceptible to brucellosis. Walker (1999) had described that younger animals tend to be more resistant to brucella infection.

According to Yibeltal (2005), higher prevalence was found in adult than in younger ones, which support our findings. It has been reported that brucellosis is essentially a disease of sexually mature animals (Quinn *et al*., 1999). Sexually mature and pregnant animals are more prone to brucella infection than sexually immature animals of either sex (Radostitis *et al.,* 2000). On the other hand younger animals tend to be more resistant to infection and frequently clear the established infections, although latent infection could occur (Walker, 1999). This may be due to the fact that sex hormone and erythritol (a polyhydric alcohol which acts as growth factors for brucellosis) which stimulate the growth and multiplication of brucella organism tends to increase in concentration with age and sexual maturity (Radostitis *et al*., 2000).

The findings of significant variation in seroprevalence to brucella among the three herd category were observed with the trend of infection increased together and with herd size. A highly significant variation among the three flock categories 1-10, 11-20 and >21 sheep in the extensive production were observed ($p<0.05$). However the difference was not statistically significant. Though comparatively higher proportion was found in the flock category having >11-20 and >21 sheep than those flocks with <1-10 sheep. As the same time no significant variation among the two herd categories (>11-20 and >21 herd) in the intensive production were observed. In Rakungiri district of Uganda the majorities of reactors were detected only in large and medium sized flock (Oloffs, 1994); it is generally accepted that an increase in stocking density and increase risk of exposure to infection especially following abortion (Nicoletti, 1980); stocking density is an important determinant of the potential between the susceptible and infected animals (Crawford *et al*., 1990).

There is no significant breed difference in susceptibility to brucellosis infection, suggesting different breeds are equally infected and exposed by the disease. An attempt was made to compare the susceptibility to brucellosis between the local Menz breed and cross breed of Menz X Awassi and pure Awassi breed of sheep raised in intensive management systems. But the Awassi pure breed raised in the intensive production was not found affected by the disease, since its number is insignificant, hence, the comparison is going on between the two breeds of sheep raised in the same management systems. However, the seroprevalence estimates between the two breeds not having a significant difference. Apparently in the extensive production system a seroprevalence of 5.97% of local Menz and 4.12% the cross breed sheep (Awassi X Menz) were found, the figure shows significant association in susceptibility to brucellosis between the local and cross breed sheep in the extensive production system, despite a significant variation in their proportion.

This finding was in agreement with the result of the previous study found in Ghana by Kubuafor *et al.* (2000), where no significant difference were observed among the different breed of animals in seropositivity to brucella infection. Radostitis *et al.* (2000) clearly stated that all breeds of sheep appear to be comparable in susceptibility to brucellosis and apparently no specific breed resistances to brucellosis are known. Morgan *et al*. (1969) had also mentioned that susceptibility between different breeds has not been reported.

High seroprevalence was found in adult sheep irrespective of their lactating (nursing) or pregnancy status, although the proportion of young lambs was relatively small. The three reproductive status i.e. pregnancy, lactating (Nursing), and dry did not show significant difference in sero-prevalence among the two production systems. However, sexually mature and pregnant ewes are more susceptible to brucellosis than sexually immature ewes of either sex (Radostitis *et al*., 2000).

This is because of the localization of brucella in the uterus mostly associated with pregnancy and the production of erythritol, (Erythritol, a polyhydric alcohol which acts as growth factor for brucell*a*) present in fetal tissue in high concentrations in the placenta of female animals, and also found in other organs such as the mammary gland and epididymis, which are targets for brucella;

and possibly to steroid hormones (Nicoletti, 1980; Quinn *et al*., 1999). Attempts were also made to see the difference in seroreaction between pregnant sheep less than 4 months of pregnancy and those with greater than 4 months of pregnancy. The seroprevalence between the two pregnancy groups shows no significant difference, both groups are equally affected by brucellosis.

The seroprevalence between extensive and intensive production systems were 9.47% and 1.96% in pregnant animals <4 months and 22.54% and 5% in pregnant animals >4 months respectively. The present study revealed significant association in susceptibility of animals <4 months of pregnancy between the two production systems, whereas analysis of brucella seroreactors among sheep >4 months of age revealed statistically significant difference in intensive production ($p<0.05$) systems. Nicoletti (1980) described the rapid multiplication of the bacteria during the second and third trimester of pregnancy and excretion of large number of organisms at the time of abortion and parturition.

Three parity levels (no parity, only one parity, and more than one parturition) compared to ascertain the association of parity number with that of seropositivity to brucella antibody. There was a significant association between parity number and seropositivity in the intensive production system, while significant association was not observed between parity levels and seropositivity in the extensive production system ($p=0.05$). Accordingly, Radostitis *et al.* (2000) had shown that significant difference exists between animals with no birth and those with at least one parturition.

Abortion is the most obvious manifestation of brucellosis. This study confirmed the significant association observed between seropositivity to abortion in both production systems. According to Acha and Szyfres (2001) the predominant symptom in pregnant animals is abortion or premature or full term birth of dead or weak lambs; however according to Weidman. (1991) the main clinical symptoms in the tropics are not abortion, but are joint inflammation, bursitis and hygromas. Retained fetal membrane has a significant association with seropositivity. The current study disclosed the association of seropositivity to retained fetal membrane, which shows a highly significant difference in seropositivity between sheep that had previous history of retained

fetal membrane to that of with out history of retained fetal membrane. According to Chukwu. (1985) brucellosis is frequently associated with retention of fetal membrane and metritis.

One hundred randomly selected small ruminants owners in the extensive production system were interviewed with the objectives of identifying relevant management and husbandry risk factors associated to the occurrence of brucellosis. For that matter, none of the management and husbandry risk factors associated with seroprevalence of brucella antibody. However those farmers that kept frequently aborting sheep were found to have relatively higher proportion of seroreactors sheep. Similarly those farmers that dispose the fetal membrane to the field, river and giving to dog were found to have higher proportion of seroreactors than those farmers that burry the fetal membrane. However in this study there is no significant association between seroreactors and disposal of fetal membrane and culling of infected animals. The presence of dog and selling of breeding females due to infertility were not important risk factors.

The attempts were made to assess the impact of brucella infection on the reproductive performance of female sheep in the extensive production system. For that matter 597 small ruminant owners were interviewed together and with sample collection. The parameters of interest were, age at first lambing, lambing interval, litter size and annual reproduction rate. Comparison was made on the impact of seropositivity and seronegativity on the reproductive parameter. Accordingly, age at first lambing and the number of lamb born per ewe per production life were found significantly associated with seropositivity, with values of, ($p>0.004$) and ($p>0.04$).

Reports from (Sheno Agricultural Research Center 2002; Debre Brehan Sheep Breeding and Multiplication Center, 2004) disclosed age at first lambing of 599$\pm$ 213 in the same breed; this report is in fair consent with the results of our findings corresponding to the negative seroreacters. It is also found that seropositive ewes had their first lamb protected by four fold than their seronegative counterparts. The lambing intervals of seropositive ewes were relatively longer than the seronegative counter parts. Although there was no significant association between seropositive and seronegative in terms of lambing interval was noticed.

The lambing intervals of seropositive ewes were relatively longer than the seronegative counter parts. Early lambing however reduced replacement costs, increases animal and economic turn over rates and may lead to more rapid genetic improvement (Wilson, 1982; Mukasa-Mugerwa and Tekelye, 1988).

Mean litter size (prolificacy) of seronegative ewes were 1.24 lambs, whereas the seropositive counter parts have a mean litter size of 1.18 lambs, there is no significant difference in litter size between seropositive and seronegative ewes. Most of the time litter size was smaller in primiparous than in multiparous (Wilson, 1987). Mean annual reproductive rate (calculated as litter size X 365 / lambing interval) of seronegative ewes were 1.61 lambs, whereas the seropositive ewes have mean annual reproductive rates of 1.50 lambs.

The numbers of lambs produced per year between the seropositive and seronegative ewes were significantly not difference. Reports of Sheno Agricultural Research Center, 2003; and Debre Brehan Agricultural Research Center (2004), revealed mean litter size of 1.03 and mean annual reproductive rates of 1.30 lambs which is lower than both seronegative as well as seropositive results. According to Seifert (1996), brucellosis causes significant impact which leads to a significant reduction in productivity, these includes late first lambing, long inter-lambing time, flock fertility below 60% and comparatively low milk production.

6. CONCLUSIONS AND RECOMMENDATION

The result of the present brucellosis sero-survey showed that brucellosis is an important sheep disease and well entrenched infection across the selected zone and districts of the Eastern Amhara region, with an overall relatively moderate seroprevalence rate of 4.89%. The traditional husbandry and animal management practices support the spread of brucellosis in the area. These show that brucellosis is becoming an impediment to the exploitation of the huge small ruminant population of the traditional smallholder farmers of the Amhara region. Based on the present cross-sectional study, the seroreactor sheep were found widely distributed however, the occurrence in the two production systems were not found to be statistically significant despite the prevalent in management and husbandry system. Sex, age and herd size were found important risk factors associated with the occurrence of brucella sero-reactor sheep; however, breed were found less important factor with respect to brucella seropositivity. Brucellosis impairs the export of live sheep as the importing countries strictly require brucella free animals. Brucellosis can hamper the sustainability of the revenue generated from small ruminant production.

Generally for the overall reduction of seroreactors sheep in particular and livestock in general and immunization of the associated risk factors, proper hygienic practices need to be adhered. Such measure include proper disposal of fetal membrane and aborted fetus, proper cleaning and disinfection of the house, and the use of separate lambing pen. However, the findings of this study will have practical use not only to the study area, but also to others having similar climate, management, husbandry practice and geographical settings. Hence, the following recommendations are stipulated in surmounting of the problem.

- The status of brucellosis of sheep in Ethiopia is not well known, they may be due to the lack of attention given to small ruminants production sector, absence of research activity in small ruminant disease, poor veterinary development, lack of awareness to the economical and zoonotic impact of the disease have contributed to the less amount of information observed. Hence, there is a need to identify the species and biotypes of *Brucella* involved in small ruminants under Ethiopian conditions.

- In order to mount more specific intervention efforts, knowing the exact status of brucellosis in the area, and conducting detailed and continuous studies are recommended.

- As brucellosis is a known zoonotic disease an attempt should be made to examine human serum samples for the antibody level, and human brucellosis should be considered in the differential diagnosis of certain febrile disease in the area.

- Public education is necessary to increase the awareness of the livestock holder about economic importance of *Brucella* infections, the risk associated with brucellosis and use of food of animal origin should be practiced. Extensive extension service has to be provided to make the animal holder aware of the disease and the impact of husbandry practices on the spread of brucellosis. Educating animal health and extension personnel's as to the control of the disease is paramount important.

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8. ANNEXES

8.1. Rose Bengal Plate Test (RBPT)

The Rose Bengal Plate Test was used as a screening test of serum samples for the presence of brucella agglutinins.

Reagents:

- RBPT brucella antigen
- Positive control serum
- Negative control serum
- Test sera

Apparatus

- Plate
- Micropipette
- Applicator stick
- Magnifying glass

Procedure

Briefly, test sera and antigen were left at room temperature for half an hour before the test: 30µl of each test serum was taken and placed in a clean glass slide, 120µl of Rose Bengal antigen (1:4) was added to the slide of each test serum using a dropper, then the antigen and the test serum were mixed thoroughly by an applicator and the slide was shacked by hand for 4 minutes. The result was read by examining the degree of agglutination in good light source and when necessary using magnifying glass deemed. After four minutes rocking and visible agglutination was considered as positive. Material preparations for RBPT are shown in Annex-2.

Interpretation

Reaction were identified as 0, +, ++, +++, according to Nielsen and Dunken (1990).

0 = No agglutination

+ = barely visible agglutination, using magnified glass

++ = fine agglutination some clearing

+++ = Coarse clumping definite clearing

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

8. 2: Material preparation, evaluation, and titration of Complement fixation test.

Materials required

I. Reagents

1. Veronal buffer: diluents (prepared by mixing 1-vial of the constituents into 1 liter of distilled water (P^H = 7.25). Commercially available or could be prepared as follows and stored in a refeigerator

- Nacl 85.00g
- 5.5 diethyl barbituric acid 5.75g
- Na 5= 5diethyl barbiturate 2.00g
- $Mgcl_26H_2O$ 1.68g
- $Cacl_2$ 0.28g
- Dissolve the barbituric acid in 500 ml hot deionized or distilled water and then add other ingredients.
- Make up to 2:1 with deionized water.
- Autoclave at 121 oCfor 15 mins
- For use, dilute 1/5 in deionized water
- Adjust pH 7.25

2. Sheep RBC

A. Collect blood from jugular vein of a sheep into Alsever's solution at a ratio of 1:1

- Alsever's solution
  - D-glucose (Dextrose) 0.05g
  - Sodium citrate 0.8g
  - Sodium chloride 0.420g
  - Citric acid 0.005g
  - Distilled water 100ml
    - Autoclave at 121oC for 51 mins. Store +4oC
    - Wash erythrocytes in the blood 3 times in Alsever's solution
    - Centrifuge collected blood in a tube at 5000 rpm for 10 mins
    - Discard the supernatant
    - Centrifuge at 1500rpm for 10 mins

- Discard the supernatant
- Continue the washing once more
- This can be used fresh or can be stored at 4oc for 2 weeks.

B. preparation of 4% sheep red blood cell (RBC)

- Transfer an aliquot of the stock erythrocytes in Alsever's solution to a centrifuge tube
- Add veronal buffer and wash cells by gentle pipetting
- Centrifuge at 3000 rpm for 15 mins
- Remove supernatant fluid and discard and add an equal volume of veronal buffer
- Repeat centrifugation until the supernatant fluid is clear
- Finally remove sufficient packed cells to give a 4% suspension in veronal buffer.

3. Hemolysin (available commercially)

4. Standard antigens

- Standard antigens are prepared from appropriate infected mice, embryonated eggs or cell culture etc.
- Some antigens exhibit non-specific hemolytic activity which can be removed by treatment overnight with chloroform, fluorocarbon or absorption with RBC

If necessary in some cases:

- Add 2% chloroform to the antigen suspension and shake vigorously by hand for 2 mints. And then leave overnight at $+4^{o}C$.
- Some antigens are also anti-complementary. They are treated by heating at $56^{o}C$ for 30 minutes or by adding 5-10% inactivated guinea pig serum and incubating the mixture for one hour at $37^{o}C$

5. Complement: commercially available or collect fresh guinea pig serum which could be stored in sealed glass ampoules at $-70^{o}C$ in small aliquots or can also be preserved by Richardson's methods and stored at $4^{o}C$ for 6 months.

A) Preparation of complement

- Starve guinea pig for 24 hours
- Bleed guinea pigs and collect blood in sterile container
- Hold collected blood at room temperature for 1-2 hours
- Carefully separate the clot from the wall of the tube with an applicator stick.

- Store at 4oc overnight
- Decant the fluid and centrifuge at 3000rpm for 15-30 minutes.
- Remove serum

b) Richardson's method for preserving complement

- Borax ($Na_2\ B_4O_7 10H_2O$) 0.57g
- Sodium azide (NaN_3) 0.81g
- Sorbitol ($C_6H_{14}O_6.\ 12H_2O$) 11.74g

Dissolve in saturated NaCl solution and make up to 100ml.

To preserve complement mix 8 parts of guinea pig serum with one part of solution A and then add one part of solution B. store at 4^oC for up to 6 months.

To use in CFT test dilute one part of the preserved serum with 7 parts of distilled water. The effective dilution is 1/10.

6. Standard antisera

- Standard positive and negative sera collected from the same species are used.
- Incubate all standard sera at 56^oc for 30 mins to inactivate the complement
- Many sera are anti-complementary. Reduction of ant-complementary activity is sometimes achieved by mixing equal parts of serum and 1/10 complement and holding the mixture overnight in a refrigerator.

Standardization of reagents

- One unit of a complement (HD50) is the dilution giving 50% lysis with the optimal sensitizing dose (OSD) of haemolysin
- One unit of a complement (HD100) is the dilution giving 100% lysis with the optimal sensitizing dose (OSD) of haemolysin
- One unit of hemolysin (OSD) is the dilution giving most lysis with highest dilution of complement
- Units are assessed simultaneously in a chessboard titration using dilution of complement down the board and dilutions of haemolysin across the board.

Materials

- Preserved guinea pig complement
- Distilled water
- Veronal buffer (VB)

- 4% sheep RBC in VB
- 15 test tubes and rack
- 4x1 ml pipettes
- Syringe
- Refrigerator
- Incubator
- Agglutination plate

Haemolysin Dilution

Prepare the two fold serial dilution starting with 1/100 in test tube

0.1 haemolysin in to 9.9 ml VB = 1/100

1.0 ml 1/100 " 1.0 ml VB = 1/200

1.0 ml 1/200 " 1.0 ml VB = 1/400

1.0 ml 1/400 " 1.0 ml VB = 1/800

1.0 ml 1/800 " 1.0 ml VB = 1/1600

1.0 ml 1/1600 " 1.0 ml VB = 1/3200

1.0 ml 1/3200 " 1.0 ml VB = 1/6400

Control 1.0 ml VB

- Add 1.0 ml 4% sheep RBC in VB to each test tube including the control tube.
- Hold at room temperature for 30 mins.

Complement dilution

Prepare a dilution 1/50 of the complement

3.0 ml 1/50 complement into 1.0 ml VB = 1/67 --- 1/70

3.0 ml 1/67 " 1.0 ml VB = 1/89 --- 1/90

3.0 ml 1/89 " 1.0 ml VB = 1/119--- 1/120

3.0 ml 1/119 " 1.0 ml VB = 1/159 --- 1/160

3.0 ml 1/159 " 1.0 ml VB = 1/212 --- 1/120

3.0 ml 1/212 " 1.0 ml VB = 1/283 --- 1/280

Position the WHO standard agglutination plate to have 8 columns and 10 rows

- Add 0.2 ml VB into all wells in rows 1-8 and columns 1-7
- Add 0.3 ml of VB into 8 wells of columns 8

- Add 0.1 ml volumes of the complement dilutions. The 8 wells of each column receive the same complement dilution. Start with the highest dilution in column 7, column 6, and column 5 etc.
- Add 0.1 ml of the sensitized erythrocytes. The 8 wells of each row receive the same haemolytic dilution; start with the highest haemolysin dilution in row 7.
- Add 0.1 ml of the RBC control into the 8 wells of row 8.
- Incubate the plate at 37^{o}c for 45 mins shaking at 5, 30 and 45 mins.
- Hold for 2 to 4 hours at 4^{o}c until the cells have settled.

OSD (optimal sensitizing dose) = haemolysin dilution giving most lysis with highest dilution of complement showing activity =1/400 in the example

HD 50 (median haemolytic dose) = one unit of complement is the dilution giving 50% lysis withOSD is 1/1600.

In the CFT test proper 4 units of complement are used i.e. 1/400

Standardization of antisera

Materials	4x1 ml pipettes
Antiserum	4x 1 pasteur pipettes
Distill water	1x5 ml pipettes
Haemolysin	Veronal buffer
14 tests tubes and rack	4% sheep RBC in VB
Preserved guinea pig complement	water bath at 56^{o}C
Refrigerator	plate
Water bath 37^{o}C	incubator

Procedure

Inactivate the antiserum at 56 ^{o}C for 30mins

Prepare a 2 fold dilution series of thestandard anti serum in the test tube blanks containing 1.0 mlVB starting with 1/8.

0.3 ml antiserum into 2.1 ml VB = 1/8

1.0 ml 1/8 " 1.0 ml " = 1/16

1.0 ml 1/16 " 1.0 ml " = 1/32

1.0 ml 1/32 " 1.0 ml " = 1/64

1.0 ml 1/64 " 1.0 ml " = 1/128

1.0 ml 1/128 " 1.0 ml " = 1/256

1.0 ml 1/256 " 1.0 ml " = 1/512

Prepare a 2 – fold dilution series of the standard antigen in test tube blanks containing 1.0 ml VB from ½ to 1/128.

Position the agglutination plate to have 8 columns and10 rows

- Add 0.1ml volumes of the antigen dilutions. The 8 wells of each row receive the same dilution starting with the highest dilution in row 7
- Add 0.1 ml VB to wells 1-7 in column 8 and row 8
- Add 0.3 ml VB into the 8th wells of column 8 or row 8and into 2nd, 4th , and 5th well of row 9.
- Add 0.4 ml VB into the 1st well of row 9.
- Add 0.1 ml complement diluted in VB to contain 4 units to the 1st well in row 9 mixes and transfer 0.3 ml through to well 5 in rows 9. Discard 0.3 ml of the mixture from the 5th wells.

Incubate for 30 min. at 37^{o}C

- Prepare 4% sheep RBC and sensitize by mixing with an equal volume of haemolysin diluted to give the OSD.(add haemolysin dilution to the cells)
- Incubate the mixture in a water bath at 37^{o}C for 10 min.
- Add 0.1 ml sensitized erythrocytes to all wells
- Shake and incubate the plate at 37^{o}C for 10 min.
- Add 0.1 ml sensitized erythrocytes to all wells
- Shake and incubate the plate at 37^{o}C for 40 min. shaking at 50, 30 and 45 minuets.

- Hold at 4^{o}c for 2-4 hours to allows the cell to settle

Antigen detaction by complement fixation

Materials

- Standard positive serum
- Standard negative serum
- Verona buffer
- Standard antigen
- Suspect antigen
- Distill water
- Preserved guinea pig complement
- Haemolysin
- WHO plate and cover
- 2 test tubes and rack

refrigerator
4% sheep RBC
water bath at 56^{o}C
2x1 ml pipettes
incubator

Procedure

- Inactivate the standard positive and negative antisera by heating at 56oc for 30mins
- Dispense 0.1ml VB into the 8 wells of rows 1,2,5, and 6.
- Dispense 0.2 ml VB into the 1^{st} 7 wells of rows 3 and 7.
- Dispense 0.4 ml VB into the first well of row 9 and 0.3 ml VB into wells 2,3,4,5, and 8 and row 9
- Prepare 2-fold dilution of the standard antigen in the well of row 1 up to the 7^{th} well using a transfer volume of 0.1 ml. Discard 0.1ml from the 7^{th} well
- Prepare 2-fold dilution of the standard antigen in the well of row 3 up to the 7^{th} well using a transfer volume of 0.1ml.
- Discard 0.1 ml from 7^{th} well and row.
- Prepare 2-fold dilutions of the suspect antigen in the well of row 7 up to the 7^{th} well using a transfer volume of 0.2 ml
- Add 0.1 ml of the inactivated standard positive antiserum diluted to contain 4 units of the optimal dilution to the entire well in rows 1 and 5 including the 8^{th} well.
- Add 0.1 ml complement diluted in VB to contain 4 units to all wells in rows 1,2,3,4,5,6 and 7 except the 8^{th} wells in row 3 and 7
- Add 0.2 ml complement diluted in VB to contain 4 into the 1^{st} well of row 9. Mix and transfer 0.3 ml through to well 5 in row 9. Discard 0.3 ml of the mixture from the 5^{th} well. Incubate at 37^{o}C for 45mins shaking at 15, 30, and 45 mins. Hold the plate for 2-4 hrs to allow the cells to settle

Interpretation

The highest dilution of antigen giving a hemolytic reading of 3 or 4 is the antigen titer provided all the controls have responded correctly. For example

8. 3: Questionnaire form regarding each sampled animal.

Kebele_____

Village_____

ID No	Sex	Age	Breed	Occurrence of abortion		Occurrence of retained placenta		Reproduction performance			
				Yes	No	Yes	No	Age at 1st lambing (days)	lambing interval	Litter size	Annual reproduction rate

Questionnaire on epidemiological study of sheep brucellosis in the eastern Amhara region

Date…….. Code…

1. Name of the district……..
2. Name of the farms association …….
3. Name of the village…….
4. Name of the owners (house hold head)……..
5. Type of farming……..
6. If the farming land is fenced? Yes/ no?
7. Animal identification
    - 7.1. Age of the animals
    - 7.2. Sex
    - 7.3. Breed, a) indigenous, b) cross breed c) exotic
8. Flock size
9. Flock structure
    - 9.1. Number of adult male (rams) used for service……
    - 9.2. Number of adult male (Castrates)…….

9.3. Number of young males……

9.4. Number of adult females (ewes)……

9.5. Number of productive females (ewes)……

9.6. Number of served lambs…….

9.7. Number of lambs (birth to winning)……

10. What are the objectives of sheep keeping in your farm?

a) To generate cash b) to supplement family food

c) As a sole means of existence d) other

11. What is the parity status and average lambing interval of the sheep in your farms?

Identity of the lambs	Parity status	Lambing interval

12. Management and housing

13. Type of housing

13.1. Pen, 13.2. Open field 13.3. Human housing

14. General hygiene of the house: good, satisfactory, poor

15. Do you separate ewes during parturition? Yes? No?

16. Do you put them in separate lambing pen?

17. What are the commonly encountered diseases affected your cattle in order of importance?

A)……..B)……C)……..D)……..E

18. Do you know any diseases that cause abortion in sheep? Yes? No?

19. What are the local names of the diseases,...

20. Was any event of abortion in your farm yes? No?

21. If yes which animals and at what time (month) does it occurred?

Ewes identification	Time of abortion

22. How many abortions (how many animals) have aborted in your farm during the last three years...

23. What do you do with them after abortion...?

    23.1. How do you dispose the after birth……..

    23.2. How do you dispose the aborted fetus...?

24. What is the reason of culling in your farm (disease, old age, infertility, poor production,)

25. From where do you get your replacement stock...?

26. Have you ever tested your farm for brucellosis up to know? Yes? No?

27. Have you ever vaccinated your animals for brucellosis up to know? Yes? No?

8.4: Differential characteristics of species of the genus brucella

Species	Colony morphology	Serum requirement	Lysis by phages[a]							
			Tb		Wb	Iz_1	R/C			
			RTD[c]	10^4RTD	RTD	RTD	RTD	Oxidase	Urease activity	Preferred host
B.abortus	S	–[d]	+	+	+	+	–	+[e]	+[f]	Cattle and other Bovidae
										Biovar 1: swine
										Biovar2: swine, hare
B. suis	S	-	-	+	+[g]	+[g]	-	+	+[h]	Biovar3:swine
										Biovar4: reinder
										Biovar5:weiled rodents
B. melitensis	S	-	-	-	-[i]	+	-	+	+[j]	Sheep and Goats
B.neotomae	S	-	-[k]	+	+	+	-	-	+[h]	Desert wood rat[l]
B. ovis	R	+	-	-	-	-	+	-	-	Rams
B. canis	R	-	-	-	-	-	+	+	+[h]	Dogs

a. Phages: Tbilisi (Tb),Weybridge (Wb), Izatnagarl (IZ_1) and R/C

b. Normally occurring phase: S; smooth, R: rough

c. RTD: routine test dilution

d. *Brucella abortus* biovar 2 generally requires serum for growth on primary isolation

e. Some African isolates of *B.abortus* biova 3 are negative

f. Intermediate rate, except strain 544 and same field strains that is negative

g. Some isolates of *B. Suis* biovar 2 are not or partially lyzed: by phage Wb or IZ_1

h. Rapid rate

i. Some isolates are lysed by phage Wb

j. Slow rate, except some strains that are rapid

k. Minute plaques

l. Neotomal lipid

8.5: Differential characteristic of the biovars of brucella species

Species	Biovar	Co_2 requiement	H_2O production	Growth on dyes			Agglutination with monospecific sera	
				Thionin	Basic fuchsin	A	M	R
	1	-	-	+	+	-	+	-
B. melitensis	2	-	-	+	+	+	-	-
	3	-	-	+	+	+	+	-
	1	+[b]	+	-	+	+	-	-
	2	+[b]	+	-	-	+	-	-
	3	+[b]	+	+	+	+	-	-
B. abortus	4	+[b]	+	-	+[c]	-	+	-
	5	-	-	+	+	-	+	-
	6	-	-	+	+	+	-	-
	9	+ or -	+	+	+	-	+	-
	1	-	+	+	-[d]	+	-	-
	2	-	-	+	-	+	-	-
B. suis	3	-	-	+	+	+	-	-
	4	-	-	+	-[e]	+	+	-
	5	-	-		-	-	+	-
B.neotomae	-	-	+	-[f]	-	+	-	-
B.ovis	-	+	-	+	-e	-	-	+
B.canis	-	-	-	+	-e	-	-	+

a. Dye concentration in serum dextrose medium: 20 mg/ml

b. Usually positive on primary isolation

c. Some strains are inhibited by dyes

d. Some basic fuchsin resistant strains have been isolated

e. Negative foremost strains
f. Growth at a concentration of 10µg/ml thionin

9. CURRICULUM VITAE

I. Personal data

Name	Shimeles Abegaz
Place of birth	Addis Abeba
Sex	Male
Maritial status	Married
Nationality	Ethiopian

II. Academic background

1. Grade 1-6: W/ro Zerfeshiwal Elementary School (1961-1966)
2. Grade 7-8: Dejach Wendrade Secondary School (1967-1968)
3. Grade 9-12: Kokebe Thibah High School (1969-1973)

University Education

1. From September 1975 - 1980 August: Attending higher education at USSR-Harkove University, Faculty of Veterinary Medicine and graduated with the Degree of Doctor of Veterinary Medicine.
2. From October 1999-2000: post graduate studies on Tropical Veterinary Microbiology (M.V.Sc.TVMB), Addis Ababa University, Faculty of Veterinary Medicine.

III. Research Experience

1. Problem related to Taenia Saginata, economic and public health significance in Ethiopia, DVM Thesis, Harccov University, Faculty of Veterinary Medicine, and Harccove, USSR, 1988.

2. Seminar on Fascioliasis in Extensive and semi-intensive production systems in Cheffa valley 2000, in ARARI (Amhara Region Agricultural Research institute)
3. Seminar on Lung worm as a respiratory disease complex on the high land of South Wollo zone. (Review paper submitted to, EARO) 2002.
4. Seminar on outbreak of unidentified camel disease in Afar regional state, 2006 (organized by the Afar Regional State, at the Regional Laboratory, Kombolcha)
5. Study on sheep brucellosis in selected woredas of the eastern Amhara region, in partial fulfillment of the degree of Master of Science in Tropical Veterinary Microbiology, 2008.

V. Work Experience

- Awraja Veterinarian in Motta Awraja in Gojam province.(1989-1991)
- District veterinarian in Debre Markos western Amhara zone(1992-1997)
- Research officer in Kombolcha Rgional Veterinary Laboratory Parasitology Department (1998-2002)
- Research officer in Kombolcha Regional Laboratory in Microbiology Department (2003-2004)
- Head of Microbiology Department in Kombolch Regional Veterinary Laboratory (2005-2007)
- MVSc research work on Ovine brucellosis in Addis Ababa University Faculty of Veterinary Medicine from October 2007 to July 2008.

VI. Further Training

1. Snail-borne trematode infections at the institute of Pathobiology, Addis Ababa University, form February 8-19, 2001.
2. Refresher-training entitled Advanced Veterinary Pathology at the Faculty of Veterinary Medicine from March 21 to April 8 , 2005.
3. Refresher-training entitled Applied Veterinary Immunology and Molecular Biology at the Faculty of Veterinary Medicine from March 14 to 29 July, 2005.

VII. Language proficiency

No	Language	Reading	Writing	Speaking	Listening
1	Amharic	excellent	excellent	excellent	Excellent
2	English	V.good	V.good	V.good	V.good
3	Russian	good	good	good	good

References

- Prof. L Muniappa (BVSc, MVSc, PhD, Professor) Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit.
- Dr. Kelay Belihu (DVM, MSc, Assistant Professor), Faculity of Veterinary medicine, Addis Ababa University, Debre Zeit

10. SIGNED / STATEMENT OF DECLARATION.

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

Name _____

Signature _____

Date of submission _____

This thesis has been submitted for examination with our approval as university advisors.

Dr. L. Muniyappa (BVSc, MVSc, PhD, Professor) _____

Dr. Kelay Belihu (DVM, MSc, Assistant Professor) _____