DDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

SERO – EPIDEMIOLOGY OF BRUCELLOSIS IN SMALL RUMINANTS IN SOUTHERN ETHIOPIA

BY

MENGISTU MEKURIA W/ TSADIK

JUNE 2007
DEBRE ZEIT ETTHIOPIA

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A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Veterinary Science in Tropical Veterinary Epidemiology

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DEDICATION

This Paper is dedicated to Lord Jesus Christ

"The fear of Lord is the beginning of wisdom: and the knowledge of the holy is understanding" Proverbs 9:10

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LIST OF FIGURE

ABBREVIATIONS

AAC	Awassa Agricultural College
AARC	Adami Tullu Agricultural Research Center
AFSSA	French Food Safety Agency
BPAT	Buffered antigen Plate Agglutination Test
cELISA	Competitive Enzyme Linked ImmunoSorbent Assay
CFT	Complement Fixation Test
CSA	Central Statistics Authority
CT	Card test
FAO	Food and Agricultural Organization of the United Nations
FPA	Fluorescent Plarisation Assay
iELISA	Indirect Enzyme Linked Immunosorbent Assay
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
masl	Meters above sea level
mRBT	Modified Rose Bengal Test
MRT	Milk Ring Test
NGO	Non-Government Organization
NVI	National Veterinary Institute
OIE	Office International des Epizootecs
OMP	Outer Membrane Protein
OPS	O-chain lipopolysaccharide
PA	Peasant association
PCR	Polymerase Chain Reaction

PFE	Pastoralists Forum Ethiopia
RBT	Rose Bengal Test
RIV	Rivanol precipitation
R-LPS	Rough lipopolysaccharide
SAT	Standard Tube Agglutination Test
SDA	Serum Dextrose Agar
S-LPS	Smooth lipopolysaccharide
SNNPRS	Southern Nations Nationalities and People's Regional State
TSA	Tryptose Soy Agar
WHO	World Health Organization

ABSTRACT

A cross sectional study was conducted in Oromia and Regional state of SNNPRS from August 2006 to April 2007 to determine the prevalence of brucellosis in goats and sheep and areas associated risk factors using mRBT and CFT. A total of 2905 goats and 1059 sheep were included in this study.

The modified Rose Bengal Test (mRBT) prevalence rate in goats' over all is 5.5% (n=2905) while in sheep is 4.0% (n=1059) and total in small ruminants 5.1% (n=3964) in extensive farming system. The mRBT positive rates, in relation to production systems are 7.11% and 0.5% in pastoral and sedentary respectively. In the intensive farming system, in Adami Tullu Agricultural Research Center, no sero-positive animals while in Awassa Agricultural College, the prevalence rate is 20% by mRBT.

The serial test positives (mRBT and CFT) prevalence rate in goats is 3.2% (n=2905) while in sheep is 1.6% (n=1059). The small ruminants' prevalence rates are 3.9% and 0.33% in pastoral and sedentary production systems respectively. In the intensive farming system, in Adami Tullu Agricultural Research Center, no sero-positive animals while in Awassa Agricultural College, the prevalence rate is 18.4 % by serial tests.

Risk factors analysis was carried out in relation to production system, species and sex and significant differences were observed to counter categories ($p<0.05$). Age factor showed no significant difference in this analysis, which may be due to low number of observations at higher ages.

Flock prevalence was determined only in pastoral production system, but not in mixed crop livestock production system because of small holdings and difficulty to consider as herd (flock). Herd (flock) prevalence was observed only in pastoral production system. Herd prevalence by serial test in Borerna (Oromya) is 38.8 %(n=73), while in South Omo and Konso (SNNPRS) is 22.7 %(n=75). The cut-off value for flock prevalence was greater or equal to one.

In general brucellosis in small ruminants is prevalent in the low land pastoral production system at individual and herd level. In the sedentary areas at higher altitude the prevalence is 0%, except in some fringed areas bordering the pastoral production system as in konso district. Based on the result of the study concluding remarks and recommendation are forwarded.

Key words: Brucellosis, sero-prevalence, risk factors, serial, mRBT/CFT, goats, sheep

1. INTRODUCTION

Small ruminant production is one activity in Livestock industry through out the world. The economic benefits contributed are meat, milk, skin, fiber and manure. The special performance of adaptation of small ruminants to different ecological factors has enabled the livestock raising community to diversify income source in exploiting the marginal lands that other domestic animals hardly thrive.

Ethiopia is a country with different agro ecological zones where considerable populations of small ruminants are raised. The small ruminants of the country are estimated to be 24 million heads of sheep and 23 million heads of goats. Of these, the highlands have 75% of sheep and 27% of goats, while the low lands mostly pastoral areas have 25% of sheep and 73% of goats (PFE, 2004; CSA, 2004).

Disease in small ruminants is one of the constraints to be tackled to ensure the expected economic benefits. Brucellosis in small ruminants is a serious disease primarily in goats which is caused by *B. melitensis*. It is the first member of the genus *Brucella* to be isolated by Bruce in 1887 in Malta of Mediterranean Sea from a human case. The disease is widely distributed through out the world except North Europe, North America, Australia and New Zealand. Brucellosis is prevalent in breeding age and pregnant animals. Transmissions between animals occur readly after exposure to aborted materials, contaminated placenta and postpartum discharge. Human infection is through contact of contaminated materials and consumption of raw milk and cheeseDiagnosis of brucellosis is carried out by the history of the disease in the herd, laboratory culture and identification of the organism. Serology is most routinely practiced method of diagnosis of infection. Control of brucellosis is carried out by the use of live attenuated and killed vaccines of *B.melitensis*.

The existence of small ruminants brucellosis in the country is reported by sero-epidemiological surveys carried out in few places so far (Tekeleye and Kasali, 1990; Yibeltal, 2005; Ashenafi, 2006). In preliminary observations sero-positive of goats to brucellosis was reported from some areas of SNNPRS, according to the annual report of Sodo Regional Veterinary Laboratory (2005).

In Ethiopia, goat's production, particularly dairy goats are encouraged for production in rural development projects through non-governmental organizations (NGO), with objectives of

income generation and consumption of milk. The goat in the context is considered as "poor man's cow". With such activities the introduction of improved exotic dairy goats is under taken. However the risk of acquiring brucellosis by such susceptible breeds is present, if the disease enzootically exists. Consumption of raw goat milk in rural community and living together in the same house with goats are also important considerations from zoonotic point of view.

Based on this introductory concept, the objective of this study is as follows:

-To study the sero-prevalence of small ruminants brucellosis in Borena and South Omo zones and parts of SNNPRS.

-To study associated risk factors of small ruminant brucellosis in study areas.

-To observe production system of small ruminants in southern Ethiopia

2. LITRATURE REVIEW

2.1 Epidemiology

2.1.1 Etiology

Brucella species are small gram-negative rods (0.5-0.7×0.6-1.5μm) that often appear cocco-bacillary. They are non-motile non-spore forming and partially acid fast in that they are not decolorized by 0.5 % acetic acid in Modified Zeil Neelson stain. The genus is divided in six species: *Brucella*. *abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B canis* and *B. neotomae*, based on cultural, metabolic, antigenic properties and host specificity (Quinn *et al*., 1994).

B. melitensis is the primary causative agent of brucellosis in small ruminants and undulant or Malta fever in human. *B. ovis* causes brucellosis in sheep, particularly causing epididimitis in rams (Radostits *et al*., 2000). *B. abortus* was also isolated in sheep and goats (Renukaradhya *et al*., 2002).

There are three biovars of *B. melitensis* that differ in geographical distribution, but no difference in pathogenecity of animal species affected (Benkirane, 2005). To date, human infection by *Brucella* organisms has been caused by four species: *B. melitensis*, *B. abortus*, *B. suis* and *B. canis* (Quinn *et al*., 1994).

2.1.2 Occurrence

Global distribution

Small ruminants brucellosis is prevalent in Mediterranean and Middle Eastern countries through central Asia to India, in China, Mongolia, and southern area of the former Soviet Union. The disease also occurs in Central and Southern America, Mexico and some areas of Africa. It is not prevalent in North Europe, North America, and Oceania (Radostits *et al*., 2000; Deqiu *et al*., 2002; Benkirane, 2005).

Different prevalence studies showed variation from place to place, which could be due to different factors such as herd size, management and biology of the disease. The sero-surveillances carried out 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 (Tables 1 and 2). The outbreak which occurred in Algeria in 1989 showed a sero-positivity of 2.2% in sheep and 12% in goats and at the herd level the prevalence was 43.5% and 42%, respectively, while in Tunisia in 1991 in a place called Gafsa, a herd prevalence rate of 61% in goats and 30% in sheep are recorded, in Morocco, in 1996 an outbreak occurred with flock prevalence of 12.1% and 2.4%, respectively, in sheep and goats (Benkirane, 2005).

Occurrence in Ethiopia

The status and distribution of brucellosis of small ruminants in Ethiopia is not widely studied. This may be due to the lack of sufficient disease investigation in small ruminant. Few sero-surveillances so far carried out indicate that, brucellosis may be one of the important diseases in small ruminants. A sero-surveillance study carried out in small ruminants in Afar and Somali Regions in 2005 and 2006, clearly demonstrated that the disease exists in Ethiopia. The sero- surveillance findings were 14.6% in sheep, 16.2% in goats in Afar Region and 1.6% in sheep, 1.7% in goats in Somali Region (Yibeltal, 2005). Another study carried out in Afar region showed a prevalence of 5.8% in goats (n= 1005), in sheep 3.2% (n= 563) and in both species sero- positive rate of 4.8% (n= 1568) by using CFT as confirmatory test (Ashenafi, 2006). A preliminary investigation showed the presence of brucellosis in small ruminants in the Borena range land pastoral system with a value of 2% (n= 183) by using RBT (Teshale *et al.*, 2006). In another work done in the central highlands of Ethiopia, a sero - prevalence of 1.5% in sheep and 1.3% in goats is recorded (Tekeleye and Kasali, 1990). The existence of the disease was also confirmed serologically and reported in Southern Nations Nationalities and People's Regional State (SNNPRS), according to the annual report of Sodo Regional Veterinary Laboratory in the year 2005.

Table 1. Prevalences of small ruminants brucellosis in East Africa

Country	Sheep	Goat	Author
Ethiopia (Central high land)	1.5%	1.3%	Tekeleye and Kasali, 1990
Ethiopia (Afar Region)	14.6%	16.2%	Yibeltal, 2005
" (Somali Region)	1.6%	1.7%	"
Ethiopia (Afar Region)	3.2%	5.8%	Ashenafi, 2006
Eritrea	1.4%	3.8%	Omer *et al*., 2000
Somali	7.2%	5.29%	Falade and Husein, 1979

Table 2. Prevalences of small ruminants brucellosis in some West Asia and North African countries

Country	Year	Area & type of Husbandry	Prevalence		
			Sheep		Goats
			Individual	Flock	Individual
Morocco	1997	East	1.6%	12.1%	4.1%
Algeria	1998				1.5%
Tunisia	1992	Nation wide	4.0%		18.0%
Israel	1993 -1995	Intensive	8.2%		
Egypt	1998		2.4%		8.2%
Sudan	Recent	Khartoum	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)

2.1.3 Host

Goats are highly susceptible to *B. melitensis* infection, sheep less so. The organism is capable of causing disease in cattle and has been isolated from swine. In Europe, the incidence of the infection on cattle appears to be increasing and in Malta about a third of cattle reacting positively to the brucellosis agglutination test is 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, in cattle, infection with *B. melitensis* has emerged as an important problem in some southern European countries, Israel, Kuwait, Saudi Arabia and India (Banai, 2002; Renukaradhya *et al*., 2002).

In camel *B. melitensis* is isolated from camels in Iran, Libya and Saudi Arabia and *B. abortus* is also isolated from camel in the Sudan, Egypt and Kuwait. Contact between camels and small ruminants were incriminated in transmission of *B. melitensis* to the camel. Camel Pastoralists invariably keep relatively large flocks of sheep and goats along side camels and the frequent isolation of *B. melitensis* from camels in North Africa and Arabia suggest active transmission between small ruminants and camel (Abba and Agab, 2004).

2.1.4 Transmission

The transmission between animals occurs readily after massive exposure to aborted materials, contaminated placenta and postpartum discharges in infected female. In sheep, the degree of infection of milk and in uterine exudates is much lesser than goats. Infection is by ingestion, inhalation and abraded skin. Studies indicate that 70–90% cases of brucellosis infection occur via the skin and mucous membrane by direct contact. Transmission to man is as a result of contact with infected animal carcasses aborted fetus, placenta, consumption of un-pasteurized milk and cheese (Deqiu *et al*., 2002). It is common to observe human cases that are in contact with goats in areas where active brucellosis outbreak occurs. Raw vegetables 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*., 1994; Wallach *et al*., 1997).

2.1.5 Risk factors

The epidemiological variables of caprine brucellosis are related to management, population and biology of the disease.

Management

Brucellosis sero-positivity in goats showed high association with large herds, communal grazing of goats mixed with sheep, the presence of sero-positive goats in the ranch or herds and sero–positive dogs in the vicinity and lack of awareness in abortions. 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 (Mikolon *et al*., 1998; Reviriego *et al*., 2000; Kabagambe *et al*., 2001).

Host factor

Incidence rate of *B. melitensis* infection are evident in breeding age at sexual maturity, because the infection persists in latent form in young ages. However, if the kids and lambs are weaned early from their mother and from the infected environment they are usually freed 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 (Radostits *et al*., 2000).

Pathogen factor

Brucella virulence is its ability to survive and multiply within macrophages. The inability of leukocyte to kill virulent *Brucella* effectively at the primary site of infection is a key factor in the dissemination to the regional lymph nodes and other sites. The lipopolysaccharide of smooth bacterial colony (S–LPS) is directly associated with virulence and is thought to play a role in enhancing intracellular survival. Proteins or virulence factors are produced by the organism and induce different actions such as prevention of phagosomal fusion in macrophages, resistance of the harsh intracellular environment, multiplication and infection of other cells (Gyles and Theon, 1993).

2.2 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 severe zoonosis to human as well as economic losses associated in animals.

2.2.1. Economic aspects

The economic loss due to brucellosis is attributed to abortion, particularly the milking breeds which are susceptible. This results in loss of the young crop in sheep and goats, consequently impairing the breeding pattern. When infection is first introduced into a flock or herd, a storm of abortion will occur, hence, acting as a source of infection. The adverse socio economic impact impeding the foreign livestock trade and its products has great significance for the economic development of livestock owners. This consequently reduces the revenue of the country. Surveillance and control costs requires considerable amount of money. To mention, in Western Asia and North African countries to eliminate ovine, caprine and bovine brucellosis, required half of the total European Commission Funding for animal disease control measures in 1997 (Benkirane, 2005).

2.2.2. Zoonotic importance

Brucellosis is considered by FAO, WHO and OIE as the most wide spread zoonoses in the world (Sharma and Adlakha, 1996; MacMillan, 1997).

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* (Deqiu *et al*., 2002). Although it is a notifiable disease in many countries, the disease remains underestimated by the medical authorities, as official figure do not reflect the number of human infections. 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 results by contact with infected meat, aborted material and consumption of un-

pasteurized milk and cheese. Human brucellosis occurred in most cases following epidemics of caprine abortion, whenever contact with infected herd is established. Brucellosis tends to be found in areas where there is high concentration of farm animals (Wallach *et al*., 1997). The establishment of *B. melitensis* in cattle has epidemiological importance. It created an alarming situation to give due attention in that the disease is extending its horizon of transmission to human through milk and other products of cattle (Banai, 2002).

In Ethiopia brucellosis in man is reported in Addis Ababa on occupationally exposed persons in abattoirs enterprise and different dairy farms. Seroprevalence rate of 4.8% was determined (Jiksa et al., 2006).

2.3 Immunity

The genus *Brucella* is a facultative intracellular organism that survives and replicate in both phagocytes and non-phagocyte cells. The antibody response following infection depends on whether or not the animal is pregnant and on the last stage of gestation. Agglutinin and complement fixation antibodies become positive 4 weeks following experimental infection (Radostits *et al*., 2000).

An important problem is the immunity that develops after recovery from an acute infection. The animal mostly will remain with un-sterile immunity in females and thus the animals may remain life long carriers and can excrete the pathogen. This is especially true of those animals, which have developed hygroma (Seifert, 1996). Spontaneous recovery may occur particularly in goats, which are become infected while not pregnant (Radostits *et al*, 2000).

The types of immunity to *Brucella* are 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 intra cellular bacteria determining the resolution of infection (Tizard, 1996). S-LPS is the major surface antigen for humoral immune response in smooth strains of *B. melitensis B. abortus* and *B. suis* (Nielsen, 2002).

2.4 Diagnosis

In many instances a diagnosis of *B. melitensis* infection in animals is made only because the infection has been diagnosed in human contacts, provoking the examination of the local animal population (Radostitis *et al*., 2000).

Diagnosis of brucellosis is based upon the disease history of the herd, epidemiological observations, serological examinations and demonstration of causal agent considering all this methods together (Sharma and Adlakha, 1996). The development of the primary binding assays in serology and molecular biology has greatly improved the efficiency of diagnosis of brucellosis.

Differential diagnosis of brucellosis should be made with diseases causing abortion in small ruminants. These are Chlamydia, leptospirosis, camplobacteriosis, salmonellosis toxoplasmosis and Q-fever (Smith and Sherman, 1994).

2.4.1. Bacteriology

Bacteriological specimens need to be collected aseptically, transported to the laboratory in transport media or in cold chains. Specimens collected are whole fetus or stomach content, spleen, lung, liver, placenta, cotyledon, uterine discharges, lymph node, milk and blood.

Under direct smear examination made from specimens of fetal abomasal content and cotyledon stained with Modified Zeihl-Neelsen, the organism appears small, red, cocco-bacilli in clumps reflecting the intra cellular growth and is a presumptive evidence of brucellosis. Care must be taken in the interpretation of result as other infectious agents with similar morphology such as *Coxiella burnetti* and *Chlamidia psittaci* may pose confusion (Quinn *et al.,* 1994).

During the bacterial culture, great care should be exercised in handling materials suspected, and examination especially of fluid media should be carried out in safety cabinet is the organism is pathogenic in man. Culture from placenta, cotyledon, vaginal discharge, fetal materials, lymph nodes and others should be made on solid medium since it facilitates recognition and isolation of developing colonies and limit the establishment mutants. To culture certain fluids notably blood, liquid media permit the culture of larger volumes that

can’t be dealt with solid media.

The organism *Brucella* is normally fastidious slow growing organism and samples are often heavily contaminated and thus 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 also grows in blood agar. Identification of species is carried out by the cultural growth requirements, colony morphology, staining character, agglutination with specific sera and phage typing (Quinn *et al*., 1994; Sharma and Adlakha, 1996).

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 in clinical specimens by culture may still be a difficult task with significant delay.

2.4.2. Serology

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

The antigen used in serological diagnosis is the O-polysaccharide (OPS) on the cell surface. The whole antigen or smooth lipo-polysaccharide (S-LPS) prepared by chemical extraction is employed in serological tests. Because common epitopes in *B. abortus, B. melitensis* and *B. suis* contain S-LPS, virtually all tests in all these 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 exists 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 (Sharma and Adlakha, 1996).

The antibody response to *Brucella* in animals consists of an early IgM isotype response, 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 and later by small amount of IgG2 and IgA. Most cross-reacting antibody, which is antibody resulting from exposure to microorganisms other than *Brucella* species or environmental antigen consists mainly of IgM. Serological tests that measure IgM are therefore not desirable as false positive results occur, leading to low assay specificity. Since IgG2 and IgA antibodies accumulate later after exposure and are usually present in small and inconsistent amounts. Therefore assays that predominantly measure IgG1 are the most useful (Nielsen, 2002).

Many serological diagnostic tests have been developed for brucellosis with the purpose of screening, confirmation and assays that also distinguish vaccinal antibodies. The tube agglutination test (SAT) detects IgM and is less specific and prone to false positive reactions by cross-reacting antibody. Therefore, modifications were made to destroy or inactivate IgM agglutinins. Among these modifications, the acidified antigens (RBT), Buffered antigen Plate Agglutination Test (BPAT), rivanol precipitation (RIV) and the use of 2-mercaptoethanol are in common use in various laboratories (Radostits *et al*., 2000; Nielsen *et al*, 2002).

Rose Bengal test (RBT) by some authors is recognized as a quick cheap and effective test for the diagnosis of brucellosis in small ruminants (MacMillan, 1997) and mostly subjected to other confirmatory test commonly Complement Fixation test (CFT). The standard Rose Bengal (RB) and Complement Fixation (CFT) tests are the main serological tests used to detect antibodies against *B. abortus* and *B. melitensis* infections. Both tests have been used for several decades, for eradication of bovine and small ruminants brucellosis. Nevertheless, there is evidence that both tests are significantly less effective for the diagnosis of brucellosis in sheep and goats than in cattle. In some eradication programs it is reported that a relatively high proportion of sheep and goats from *B. melitensis* infected flock show negative results in the standard RB test but positive in CF test, questioning the sensitivity of the former screening test. The difference may have originated in the differences proportion of antigen epitopes of type A and M in *B. abotus* and *B. melitensis*, which reflects difference in antigen antibody reactions. To improve the sensitivity of the RBT, simple modification, increasing the volume

sera to be tested has been recommended. It is the use of serum of 75µl, mixed with 25µl of antigen and named Modified Rose Bengal Test (Bercovich *et al.,* 1998; OIE, 2004; Ferreira *et al.,* 2003).

Indirect enzyme linked immunosorbent assay (i-ELISA) do not differentiate vaccinal antibody resulting from *B. abortus* (S19) or *B. melitensis* (Rev-1) vaccinations from antibody induced from pathogenic strain. In works done in sheep combination of tests as CFT and RBT, CFT and SAT, indicated brucellosis in 75% - 88% of infected animals where as the iELISA indicated infection in 97-100% of those animals. It is concluded that screening sheep seras for brucellosis with iELISA improves brucellosis diagnosis and in areas of low prevalence it will help to maintain flock of sheep free from brucellosis (Bercovich *et al.,* 1998). The development of Competitive enzyme linked immunosorbent assay (c-ELISA), detects the monoclonal antibody has largely solved these problems and is a prescribed test by OIE for international trade (Nielsen, 2002).

Fluorescence polarization assay (FPA) is regarded as official test for the diagnosis of bovine brucellosis by OIE is currently under evaluation in sheep and goats (Garin-Bastuji *et al*., 2006). FPA is highly specific, easy to perform, and capable to adapt to field and high-throughput laboratory needs, relatively lower cost than the use of Card Test (CT) and CFT. However, more studies are required for its approval as a diagnostic tool for goat brucellosis, by testing large number of serum samples from brucellosis-free areas, and bacteriological positives and vaccinated animals (Ramirez-Pfeiffer *et al*., 2006).

The milk ring test (MRT), detects *Brucella* antibodies in milk but its use in sheep and goats has not been recommended (OIE, 2004).

2.4.3. Animal inoculation

The guinea pig has been found to be the most satisfactory laboratory animal for detecting the presence of small numbers of organisms in animal's tissue, secretions and excretions or when samples are heavily contaminated. The inoculation is done by intra -peritoneal or sub-cutaneous routes. In male guinea pig orchitis will result. When guinea pigs are autopsied, lesions 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 is subjected to a 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).

2.4.4. Molecular technique

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 overcame the extraneous intervention of mimicry antibodies from sources 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 (Gupta, 2006a). The evaluation of single – step PCR, on tissue and blood from infected goats with *B. melitensis* demonstrated that PCR test is reliable, highly sensitive and specific, reproducible, easily standardized and avoids the risk of infection in laboratory workers. It will complement serological testing and be a practical and reliable tool for diagnosis of brucellosis in goats (Gupta *et al*., 2006b).

2.5 Prevention and control

Prevention and control of *B. melitensis* infection is highly required from views of economic losses to the small ruminants industry as well as the important and widespread zoonoses in the world.

The approaches used to control brucellosis include, management, immunization and test and removal of infected animals.

2.5.1 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 of goats (does), early weaning of kids from does /ewes and their environment (Radostits *et al*., 2000).

2.5.2. Immunization

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 difficulty of regular immunization due to movement of animals which in it self is related to developing countries. 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 eradications. Immunization is carried out by the use of live vaccine REV-1 that gives better protection compared to inactivated vaccine H-38 (Banai, 2002; Blasco, 2005).

2.5.3 Test and removal of infected animals

It is usually considered that a brucellosis eradication programme by test and slaughter policy is justified on economic grounds only when the prevalence of infected animals in an area is 2% or below. This strategy costs and involves good organization of farmers and veterinary service, and simultaneously the implementation of strict movement control measures so the disease is not reintroduced (Minas, 2005).

3. MATERIALS AND METHODS

3.1 Description of study areas

The study areas were considered based on the raising of goats and sheep, on management system (extensive and intensive), on production system (Pastoral and mixed crop livestock or sedentary) in Oromya region (Borena, Adami tulu) and in SNNPRS region (South Omo, Sidama, Kambata Hadya and Konso).

The categorization of production system is based on their altitude and nature of production system. The Pastoral production system is low land areas, altitude below 1600 meters above sea level. The sedentary area is mixed crop- livestock production system and is at higher altitude that ranges 1600-2100 meters above sea level and partly. The geographical positions of the survey areas were in latitude range of N $03^0$45′ to N $07^0$09′, and in longitude range of E 36^0 29′ and E 38^0 47′.

3.1.1 Borena

Borena (Yabello) is located 565 Km south of Addis Ababa, capital city of Ethiopia. Generally, Borena area represents a vast low land terrain in southern Ethiopia covering an area of about 15000 Km^2. The area is bordering with Kenya to the South, Somali Region to East, Gujji Zone to the North and Southern Nations Nationalities and People's Regional State (SNNPRS). The Borena plateau gently undulates from high mountain massifs in the North, 1650 masl to the South bordering Kenya, 1000 masl with slight variation due to central mountain ridges and scattered volcanic cones and craters (Coppock, 1994).

The annual rainfall ranges from 400 mm in the south to 1100 mm (with an average of 700 mm). The rain pattern is bimodal type with main rainy season, *ganna* (65%) extending from March to May and small rainy season, *haggaya* from mid September to mid November. Annual mean daily temperature varies from 19^0C to 24^0C with moderate seasonal variation. The other two seasons are the cool dry season, *adolessa* extending from June to August and the warmer dry season, *bonna* extending from December to February. The seasons affect herding strategies due to its effect on forage and water resources availability (Coppock, 1994). The vegetation comprises grasses, shrubs and trees, and is dominated by savannah type

containing mixture of perennial and woody plants. The savannah community varies from open grassland to bush encroachment areas. There is a shift in composition in response to heavy grazing browsing, burning and drought. Grazing shift the community to more stress as browsing and burning favors the grass. Several plant species in the area are recognized as valuable livestock forage. Acacia are dominating bush species in the area. Grass availability in quantity and quality varies seasonally, being very high in the wet season and low in the dry season, which makes nutritive stress very high to livestock. In extreme seasons the pasture will be completely exhausted forcing the herd's men to migrate where grazing and water is available (Coppock, 1994; Desta, 2000).

Table 3. Livestock population in different district of Borena zone

District	Livestock					
	Cattle	Goats	Sheep	Camel	Equine	Poultry
Arero	140000	52000	23000	24986	5311	-
Dire	337370	99649	45319	33833	8629	-
Yabello	219000	83606	22045	11458	2969	339078
Miyo	15000	45000	15000	12000	10500	14800
Teltele	169935	121889	54112	850	5670	-
Moyale	59829	19104	6087	5868	3666	11813
Total	941134	421248	165563	88995	36745	365691

Source: Pastoral development Borena, 2006

Animal husbandry is characterized by extensive pastoral production system and seasonal mobility. Cattle are the dominant animal species followed by goats, camel and sheep. Camel and cattle herd splitting into mobile, *forra* and home based, *warra* is practiced as strategy to mitigate forage and water shortage (Desta, 2000).

3.1.2. Southern Nations Nationalities and People's Regional State (SPNNPRS)

South Omo

South Omo is a pastoral area, considered as an area of interest in the research located in SNNPRS. Jinka is the capital town of the zone located at 750 Kms south of Addis Ababa. Topographically, it is massif and plain low land. Two big rivers namely Omo and Mago flow in the area. The altitude is about 400 masl. Mean monthly temperature ranges from18^0C to

32^0C, mean annual rain fall is about 390mm. In the area, the rainfall is erratic and usually bimodal occurring from September to November and from March to May (CSA, 2004).

The livestock production system is pastoral and transhumance, where elders and children remain on their settlement villages and youngsters keep their livestock. South Omo comprises of more than 43% of the regional goat and sheep population.

Table 4. Livestock population in South Omo

Description	Cattle	Sheep	Goats	Horses	Asses	Mule	Camel	Poultry
Number	1392821	1013331	1157201	7214	48353	3091	1300	13925
Regional %(SNNPRS)	15.77	31.96	43.65	2.46	15.85	4.41	100	4.04

Source: CSA 2004

Sedentary areas of SNNPRS

Sidama, Kambata and Hadya are sedentary zones in SNNPRS with semi intensive farming system and located at about 275 and 350 kms from Addis Ababa, respectively. The areas are humid to sub humid with mixed crop-livestock farming system. The annual average rainfall ranges from 700-960mm and bimodal. There are two rainy seasons in the year. The small rainy season *Belg*, is from Februarys to May and the big rain *Meher*, is from mid June to October. The altitude ranges 1725 to 2100 masl.

3.1.3 Intensive farms

In Sidama Zone the other study site/area is Awassa Agricultural College (AAC) located 275 kms south of Addis Ababa. Research work is being done on crossed goats with Toggen burge for production improvements. Similarly, Adami Tulu Agricultural Research Center (AARC) was another area of the survey in East Shewa (Oromiya) engaged in research on goat productivity and health. It is located 170 kms south of Addis Ababa along Shashemane road.

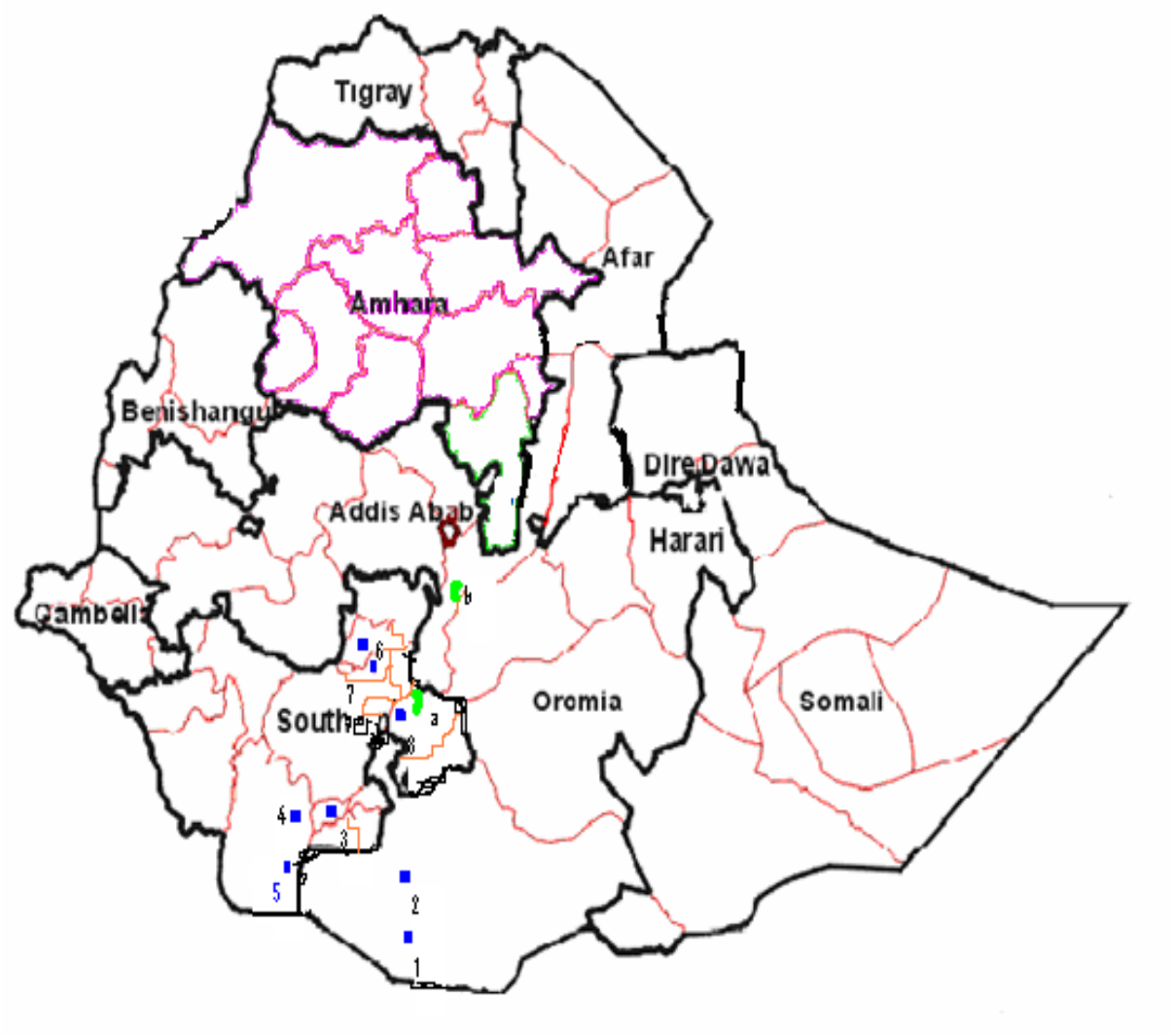

Figure 1. Map of study areas

Keys: Survey districts (zones) - Δ

Farms□

1. Dire (Borena)
2. Yabello (Borena)
3. Konso
4. Bena Themay (S. Omo)
5. Hamer (S. Omo)
6. Lemu (Hadya)
7. Kadida Gamella (Kambata)
8. Awassa zuria (Sidama)

a. AAC
b. AARC

3.2 Study design

3.2.1 Sero-prevalence

The type of study used was cross sectional. The indirect diagnosis through detection of *Brucella* antibodies in the serum is indicative that the animal has been in contact with the bacteria and may thus be infected. Samples were obtained from small ruminant population of two regions (Oromya and SNNPRS). The study site selected from Oromya is Borena with pastoral production system; and from SNNPRS two areas are south Omo with pastoral production system; and with crop livestock production system (Sidama, Kambata, Hadya and Konso). The other selected sites are two intensive production systems AAC and AARC.

3.2.2 Questionnaire study

Questionnaire was administered to farmers during the collection of serum samples. It included history of the disease in the herd and all other relevant questions with the aim of obtaining information on the management, productivity, brucellosis and other prevalent small ruminant diseases (Annex 1).

3.2.3 Risk factors

Determination of potential risk factors associated with sero-positives was carried. Associations between risk factors of production systems, species, sex and age was analyzed. The herd size varies in the different production systems. The pastoral production system is low land (Borena and South Omo) had higher herd size of 70 - 80 and may range 30 to 200 or 300 per house hold. In Konso district a herd size average of 30 is observed in the low land peasant associations; but in the other crop-livestock mixed production system (Sidama, Kambatta, Hadya) are in the higher altitudes greater than 1600 masl and the average flock size of 5-7 sheep and goats per household.

3.3 Sampling methodology

The study areas are selected purposively to include different management and production systems.

Multi-stage sampling method was designed in extensive farming system at different stages such as agro-climatic/farming system, districts, peasant association, village, household (cluster/flock), species (goat and sheep) and individual animal level. For purpose of accessibility and knowledge of the spatial distribution of the disease, the agro-ecological areas district and villages were purposively selected. The peasant associations, households or flock (herd) and individual animals were selected randomly to increase accuracy and make representativeness (Toma *et al*., 1999).

Table 5. Sampling methodology

Ser.N^{o}	Different stages	Sampling methods
1	Agro-climatic/farming	Purposive (to know distribution and disease status)
2	District	Purposive (accessibility)
3	Peasant association	Random sampling (sampling fraction > 10%)
4	Village	Purposive (accessibility)
5	Household (cluster, flock)	Random sampling
6	Species (goats, sheep)	Stratified sampling
7	Individual animal level	Systematic random sampling

3.3.1. Borena and South Omo Zone

Four districts were selected in two zones purposively owing to high small ruminant population and easier accessibility. In each district 5 Peasant Associations were selected randomly, which makes a total of 10. The proportion of PAs is greater than 10% to the total enumerated. An average of seven households was selected in a peasant association and flocks were sampled randomly. For the sample size determination, 50% of prevalence was considered for there was no previous work done in the area.

Sample size was determined using the formula in Thrusfield (2005) and Toma, *et al*. (1999).

$$n = \frac{1.96^2 * p * (1-p)}{d^2}$$

CI = 95%, (p = 0.5, q = 0.5), d = 0.05, n = 384,

CI: Confidence interval n: sample size

P: prevalence d: precission

To increase the precision sample size is multiplied by three times and 1152 samples from pastoral area of Borana and S. Omo each.

3.3.2 Mixed crop livestock (Sedentary) production system of SNNPRS

Sidama, Kambata and Hadya zones and Konso especial district were the sedentary area at high altitude in SNNPRS with semi intensive farming system. These places were taken as one category and the sampling method and sample sizes are planned as mentioned above, 1152 samples.

3.3.3 Intensive farms

In intensive farms of AAC and AARC simple random sampling method was used and the sample size was determined by using the formula in Thrusfield, 2005.

$$n = \frac{1.96^2 * p * (1-p)}{d^2}$$

CI = 95%, p = 0.5, q = 0.5, d = 0.05, n = 384, but the adjustment to the limited population was done using the formula.

$$n_{adj} = N*n / N+n$$

In summary, the following samples were collected from different agro-ecological zones.

Table 6. Sampling strategies of pastoral areas and collected samples

Zones/Region	District	Selected PA'S	Goat	Sheep	Total sampled
Borena (Oromya)	Yabello	5	487	198	685
"	Dire	5	423	231	654
South Omo (SNNPRS)	Bena Tsemay	5	557	141	698
"	Hamer	5	405	275	680
Total		20	1872	845	2717

Table 7. Sampling strategies of sedentary areas and collected samples

Zone	District	Selected PAs	Goat	Sheep	Total. Samples
Sidama	Awassa Zuria	4	325	77	402
	Konso	5	346	65	411
Hadya	Lemu	4	173	-	173
Kambata	Kadida Gamela	4	189	72	261
Total		17	1033	214	1247

Table 8. Sampling of intensive farms and collected samples

Region /Zone	Places	Institution	No of Goat	No of Sheep	Total samples
Oromya/East Shewa	Adami Tulu	AARC	443	_	443
SNNPRS	Awassa	AAC	141	_	141
Total			584	_	584

Hence, a total of 4548 serum samples were collected from all proposed study areas of which 3489 are from goats and the remaining 1059 from sheep.

3.4 Serological examination

Blood sample amounting to 10ml was collected from the jugular vein of goats and sheep using plain vacutainer tubes. The blood samples were allowed to clot at room temperature. Then the serum is separated from clotted blood by centrifugation. Separated sera were stored at -20^0c until tested by mRBT and CFT.

A. Screening test by modified Rose Bengal Test (mRBT)

The samples collected from the field were screened using modified Rose Bengal Test. The standard Rose Bengal (RB) and Complement Fixation (CFT) tests are the main serological tests used to detect antibodies against *B. abortus* and *B. melitensis* infections. Both tests have been used for several decades, proving to be successful for eradication of bovine brucellosis

and small ruminants. Nevertheless, there is evidence that both tests are significantly less effective for the diagnosis of brucellosis in sheep and goats than in cattle. In some eradication program it is reported that a relatively high proportion of sheep and goats from *B. melitensis* infected flock show negative results in the standard RB test but positive in CF test, questioning the sensitivity of the former screening test. The difference may have originated in the differences proportion of antigen epitopes of type A and M in *B. abortus* and *B. melitensis*. The difference is reflected in serology tests of antigen antibody reactions. To improve the sensitivity of the RB increasing the volume of serum to be tested by three fold has been recommended. Hence 75µl of test serum is mixed with 25µl of antigen (OIE, 2004; Ferreira *et al*., 2003).

Materials and equipments needed for mRBT test

1. RBPT antigen
2. Negative and positive control sera
3. Test serum
4. Plastic applicator
5. Enamel plate or glass slide
6. Rocking machine and centrifuge

In the screening test, mRBT was used, that is, 75µl of serum and 25µl of antigen on a white enamel plate and mixed thoroughly with the applicator stick. It was shaked for four minutes and any visible reaction was graded as positive

The positive samples were sent to OIE/FAO Brucellosis reference Laboratory AFSSA (French Food Safety Agency), Paris France under cold chain. The samples there were tested by CFT. Cut value in the CFT was greater and equal to 20 iu/ ml. Some samples unable to be dealt in AFSSA were tested in the National Veterinary Institute (NVI) by the use of CFT positivity at a thresh hold of 1:5. Samples positive by mRBT are confirmed by CFT in the serial test protocol.

B. CFT (Complete Fixation Test)

1. Microwel plates (U-shaped), multi channel and single channel pipettes, pipette tips
2. Flasks and measuring cylinders
3. Water bath, incubator, centrifuge, deep freezer, magnetic stirrer, pH meter and beam (digital) balance

4. Veronal buffer diluent (VBD), Alsever's solution (preservative)
5. Complement, hemolysin (amboceptor's), CFT antigen, control sera and sheep and sheep RBC.

Confirmatory test by Complement Fixation Test

The test procedure has two steps. First a known antigen is incubated with test and control sera to allow the formation of immune complexes. A well-defined amount of complement is added to the reaction mixture. Only in positive reaction, when specific antibodies and antigen meet, immune complexes are formed and the complement will be consumed. In the negative case, there is no formation of immune complexes and hence, no consumption of complement. All these reactions are invisible, and differentiation between positive and negative reaction is not possible up to this point.

In the second reaction step, red blood cells and their specific antibodies are added and form complexes. In the positive case, no complement will be left over to cause hemolysis. In the negative case, the complement added in the first reaction step is still present and will cause visible hemolysis after addition of the hemolytic system.

The detail of Complement Fixation Test (CFT) procedures according to OIE (2004) guide manual is indicated in Annex 2.

The serial positives of the screening mRBT and CFT were taken to compute the sero-prevalence and statistical analysis.

3.5. Data analysis

Data from questionnaires and the laboratory was stored in Microsoft Excel spread sheet program. Descriptive statistical analysis for *Brucella* sero-prevalence rate was carried out using SPSS 11.5 for windows. The serological test prevalence, 95 % confidence intervals (CI) and logistic multi-variate regression test were used for various risk factors analyses such as production system, species, sex and age. The significance differences were observed in odds ratio of the risk factor categories against dependant variable, the disease brucellosis, based on serology.

4. RESULT

4.1 Extensive farming systems

4.1.1 Prevalence at individual animal level by Administrative Zones

In administrative regions, the modified Rose Bengal Test (mRBT) sero-prevalence rate in goats' is 5.5% (n=2905) while in sheep is 4.0% (n=1059) and the overall sero-positivity total in small ruminants was 5.1 % (n=3964) in extensive farming system. In the intensive farming system, in Adami Tullu Agricultural Research Center (AARC), no sero-positive animals were detected while in Awassa Agricultural College (AAC), the prevalence rate is 20% by mRBT.

The serial sero-prevalence rate in goats' was 3.2% (n=2905) while in sheep 1.6 % (n=1059) and overall sero-prevalence in small ruminants was 2.8 % (n=3964) in extensive farming system. In the intensive farming system, in Adami Tullu Agricultural Research Center (AARC), no sero-positive animals were while in Awassa Agricultural College (AAC), the sero-prevalence rate is 18.4% (141).

Table 9. Sero-prevalence of mRBT and serial test by Administrative areas

	goats		sheep		Total		
Zones	mRBT % (n)	Serial % (n)	mRBT % (n)	Serial % (n)	mRBT % (n)	Serial % (n)	95%, CI
Borena	10.2(910)	6.4(910)	8.4(429)	3.7(429)	9.6(1339)	5.5(1339)	4.3 -6.8
S.Omo	4.1(962)	1.3(962)	1.4(416)	0.2(416)	3.3(1378)	1.02(1378)	0.5 - 1.5
Konso	7.8(346)	6.6(346)	0(65)	0(65)	6.6(411)	5.6(411)	3.4 - 7.8
Hadya	0(173)	0(173)			0(173)	0(173)	
Kambata	0(189)	0(189)	0(72)	0(72)	0(261)	0(261)	
Sdama	0(325)	0(325)	0(77)	0(77)	0(402)	0(402)	
Total	5.5(2905)	3.2(2905)	4.0 (1059)	1.6(1059)	5.1 (3964)	2.8(3964)	2.3- 3.3

4.1.2 Prevalence at individual level by production systems

The prevalence rate difference between the two production systems, mRBT is 7.11% (n=2744) in pastoral and 0.5% (n=1220) in sedentary production system and the serial positives (mRBT / CFT) 3.9 % (n=2744) and 0.3 % (n=1220) in the respective production systems (Table 10).

Table 10 Prevalence of mRBT and serial tests at individual level in production systems

Production system	goats		Sheep		Total		
	mRBT,%(n = samples)	Serial %(n= Samples)	mRBT, % (n=Samples)	Serial %(n=Samples)	mRBT, % (n=Samples)	Serial %(n= Samples)	95 % CI
Pastoral	8.1(1898)	4.7(1898)	5(846)	2.0 (846)	7.1(2744)	3.9 (2744)	3.2 - 4.6
Sedentary	0.6(1007)	0.4(1007)	0(213)	0 (213)	0.5(120)	0.3 (1220)	0.0 -0.6

4.1.3 Prevalence at herd (flock) level

Flock level consideration was possible only in pastoral production system in extensive farming system. It was difficult to enumerate flocks in the mixed crop livestock production areas since animals were coming from different households at the time of sampling in field; hence, conducting flock level prevalence analysis approach was not carried out. In the pastoral production system an average of 18 heads of animals per flock or per household was sampled. The cut off value for flock prevalence was greater or equal to one, which is when one or greater than one animal is positive, the herd is considered as positive.

Flock prevalence was calculated in two zones, namely Borena 38.8 % (n=73), South Omo and bordering peasant associations in Konso together 22.7% (n=75).

Table 11. Prevalence rate and positive herds' of mRBT and serial tests in pastoral areas

Areas	No, positive herds	mRBT n (%)	No, positive herds	Serial n (%)
Borena	38	73 (52.1)	28	73 (38.4)
S.Omo,Konso	27	75 (36.0)	17	75 (22.7)

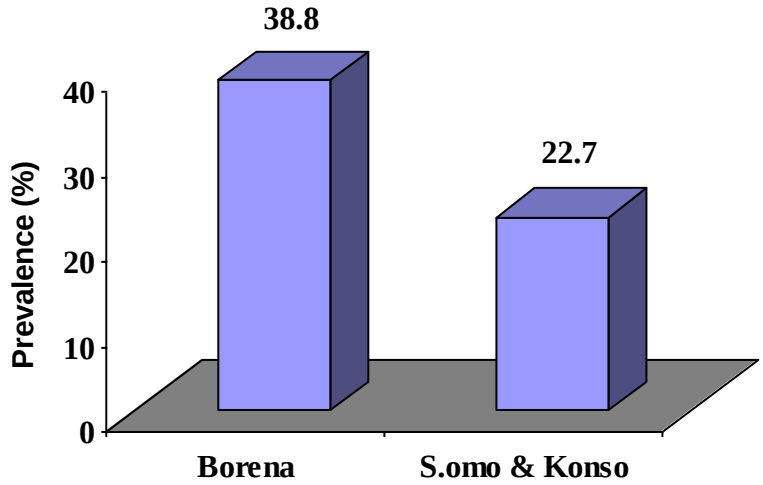

Figure 2. Herd prevalence rates

4.2 Intensive farming system

In the intensive management system two farms were studied, in one farm, Awassa Agricultural College (AAC) a high prevalence was observed, 18.4%, and in the other, Adami tullu Agricultural Research Center, the prevalence was zero.

Table 12. prevalence rate of serial test positives in intensive management system

Regions	Farms	mRBT goats,%(n= Samples)	Serial testgoats,%(n= Samples)	
Oromya,	AARC	0(443)	0(443)	-
SNNPRS	AAC	19.1(141)	18.4 (141)	12 – 24.9
Total		4.45(584)	4.4 (584)	2.4 – 5.5

4.3 Risk factors analysis

4.3.1 Production system

In the sero-surviellance carried out the production system is found to be important and prevalence differences are observed. It was 3.9 % (n= 2744) in pastoral and 0.3 % (n=1220) in mixed crop livestock production system. There is significance difference between pastoral and mixed crop livestock production system with OR value 12.83, (95% CI of OR 4.682 35.164) and (p= 0.000).

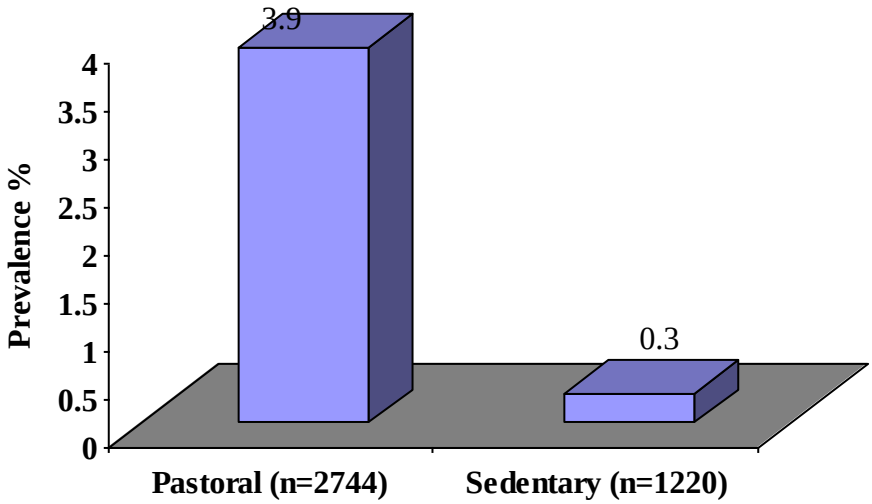

Figure 3. Production system prevalence

4.3.2 Species

In the sero-surviellance of small ruminants goats and sheep were considered. Samples were collected from 2905 goats and 1059 sheep in extensive farming system (n=3964). The ratio of sampling goats and sheep was 3:1 over all and 2:1 in the pastoral area, since the small ruminant population in the pastoral area have similar ratio. The local breeds of goats in the surveyed areas include Woito-Guji, Long eared Somali, and Arsi-Balle. The sheep breed types are Fat- rumped and Black head Ogaden in the low lands and thin tailed high land breed types. Serial sero- prevalence in goats is 3.2 % (n=2905) and in sheep 1.6 %(n=1059) which indicated the higher value in goats than sheep. There is significance difference between Pastoral and mixed crop livestock production system with OR value 2.406, 95% CI of OR1.422, 4.070 and (p= 0.001).

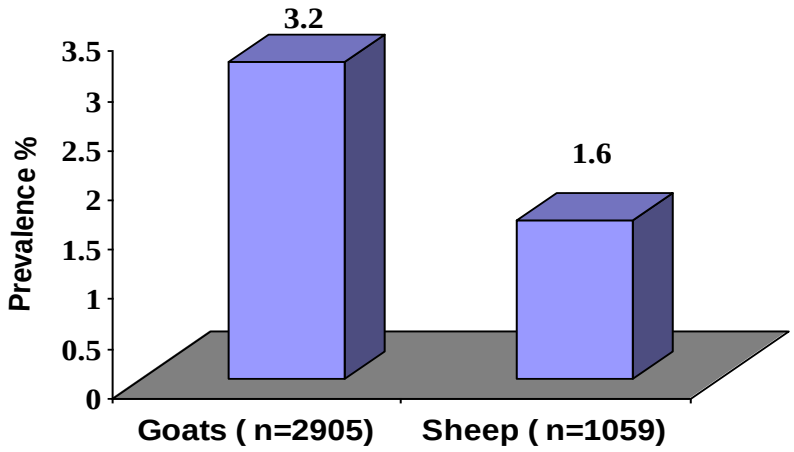

Figure 4. Species prevalence

4.3.3 Sex

In the surveyed areas samples were collected from 3393 female and 571 males in extensive farming system (n=3964). Prevalence rate of serial in females is 3.1 % (n= 3393) and in males 1.2 % (n=571) indicating higher rate in female than male. There is significance difference with OR value 2.226, (95% CI of OR 1.023, 4.843) and (P=0.044).

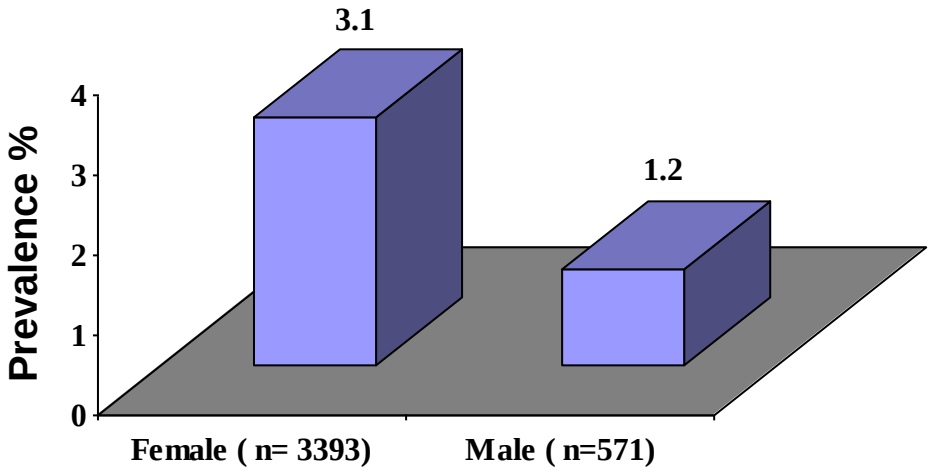

Figure 5. Sex prevalence

4.3.4 Age

Table 13. Sero- positives to age distributions

Age Years	Prevalence %	serial positives	total sample
1	0.4	1	243
2	1.2	8	635
3	3.3	32	966
4	2.6	27	1022
5	5	27	542
6	2.5	8	319
7	3.2	4	123
8	2.9	2	68
9	0	0	23
10	8.7	2	23
Total		111	3964

The degree of associations between risk factors and prevalence was measured by odd's ratio using the multi-variant logistic regression model.

Table 14. Multi-variate regression model for the risk factors analysis

		B	Sig.	Exp(B)	95.0% C.I.for EXP(B)	
					Lower	Upper
Step 1(a)	PS(1)	2.552	.000	12.831	4.682	35.164
	SP(1)	.878	.001	2.406	1.422	4.070
	SEX(1)	.800	.044	2.226	1.023	4.843
	AGE	.041	.467	1.042	.933	1.163
	Constant	-7.327	.000	.001		

a Variable(s) entered on step 1: PS, SP, SEX, A

Table risk factors Key:PS (1):

Pastoral production system, PS (2): sedentary productionsystem

SP (1): Species, goat, SP (2): sheep

SEX (1): Female, SEX (2): Male

AGE, > 1 year not categorized.

4.3.5 Parity

There were no full records regarding parity. However in the certain sampled animals the information was gathered and the relations to sero- positive rate were evaluated. For the records are not full (only 35% of the data, n=1381), significant test is not done, however, the information is presented as follows.

Table 15. Sero- positives prevalence distribution to kidding

Parity relations	Rates %	Serial positives	Total sample
Parity 1	3.1	10	302
Parity2	3.4	11	323
Parity3	4.6	11	239
Parity4	5.3	12	228
Parity 5	5.9	8	136
Parity 6	5.2	4	77
Parity 7	8.7	4	46
Parity 8	0	0	19
Parity 9	12.5	1	8
Parity 10	0	0	1
Parity 11	0	0	2
Total		61	1381

4.4 Questionnaire

4.4.1 Generality

Questioners were administrated to 24 individuals each representing a peasant association. These were 10 from Borena, and 10 from South Omo Zones, two from Konso district and each one from Awassa Zuria, Lemu and Kadida Gamella districts. The objective of the questionnaire was to understand the over all situation in regard to small ruminants production, management, prevalent diseases and to the existence of brucellosis.

4.4.2 Small ruminants management and production

The herd (flock) size, in sheep and goats observed averaged 70-80 per person in pastoral areas. The observed herd structure indicates equal male to female ratio in younger animals less than one year and in adults' ratio of 1:10. It also holds true to sheep. Small ruminants are important components of livestock production particularly in the pastoral system as a complementary use of grazing land, risk management in losses of cattle in drought other than the known benefit of meat, milk, hide and manure. Small ruminants are more resistant to drought than cattle and ranks second to camel in drought prone areas. Sheep and goats corral

together in the same pen at homesteads during the night. Both goats and sheep milk is consumed in some pastoral areas, while in others only goats milk is consumed. Goats are mostly milked twice a day and have a daily yield of 500-600 ml. The mixed crop-livestock production system is a semi-intensive farming area with small flock size 5-7 per house hold. There is shortage of grazing land, browsing trees and forage. Small ruminants may cohabite with man. Goats' milk is consumed particularly for infants.

Table 16. Questionnaires responses in small ruminants' management and production

Variable	Categories	No.of respondents' n (%)	Remark
Grazing	Mixed (goats and sheep)	22 (92)	
	Household individually	14 (58)	
	Communally	10 (42)	
Night corralling	Mixed (goats and sheep)	24 (100)	
	Cohabit with human	2 (8)	
Species milk used	Goats	24 (100)	
	Sheep	12 (50)	In pastoral areas
Milk consumption	Row	22 (92)	
	Boiled with tea	6 (25)	

4.4.3 Disease

The small ruminant diseases enumerated by the respondents during the questionnaire administration in order of importance are contagious caprine pleuro-pneumonia (CCPP), peste des petit ruminants, parasitic diarrhea, coenuruses, mange mite, pox, contagious ecthyma, pneumonia and others, but brucellosis is not mentioned.

Contagious caprine pleuro pneumonia (CCPP) is a serous and economically important disease in pastoral areas. Economical losses are incurred through high morbidity and mortality. All farmers in the pastoral area indicated CCPP to be a major problem.

Coenuruses is a disease with symptoms of central nervous system in goats and sheep. It is prevalent in Borena (Oromya), and locally called ″siergo″. It has a morbidity rate about 20% in some flocks (Personal communication of veterinary personnel). It was found to be the second important disease following CCPP.

Table 17 Questionnaire responses in small ruminants' prevalent diseases

Disease or syndrome	Species	No.of respondents' n (%)	Remark
CCPP	Goats	21 (86)	Mostly in pastoral system
PPR	Goats & sheep	7 (30)	Through out
Coenerosis	″	9 (38)	In Borena Zone
Parasitic disease	″	22 (90)	Through out
Mange	″	15 (62)	Sever in goats
Ticks	″	5 (20)	
Nervous sign	″	6 (25)	
Trypanosomosis	″	4 (17)	Mostly in goats
ORF	″	3 (13)	
Abortion	″	24 (100)	
Still birth	″	5 (20)	

4.4.4 Abortion and syndrome of brucellosis in small ruminants

The existence of brucellosis is not known and even veterinary professionals do not have any idea in the surveyed areas. The respondents mentioned the existence and syndrome of abortion in sheep and goats. The causes of abortion as told by the farmers are diseases such as CCPP, disease with nervous signs locally called ″Kendo″ in Borena and other areas. In surveyed areas aborted materials were not managed properly. The aborted materials were given to dogs or discarded outside and in a few cases were buried, hence, facilitating transmission.

There were no full records regarding abortion. However, the result of serial positive was computed to available data records in pastoral production areas where the sero-positives significantly exist. In animals with the history of abortion, 9.4% (n=502) were sero-positives,

and the other 90% was sero-negative. This indicates that other disease or factors responsible for abortion surpasses brucellosis.

Table 18. Questionnaire responses in small ruminants' abortion and syndrome of brucellosis

Variable	Categories	No.of respondents' n (%)	Remark
Gestation period	1 st	12 (50)	
	2 - 3	5 (21)	
Pregnancy month	2 -3	16 (67)	
	> 4	3 (13)	
Causes for abortion said by farmers	CCPP	8 (33)	
	Nervous sign / Kendo (local name)	4 (17)	
	Drought	3 (13)	
	Unknown	9 (38)	
Syndrome brucellosis	Orchitis	14 (60)	
	Artheritis	8 (33)	
Management of aborted material	Give to dogs	17 (70)	
	Discard outside	10 (42)	
	Bury	6 (25)	

5. DISCUSSION

5.1 Individual level brucellosis infection rate

The study concludes the existence of small ruminant's brucellosis particularly in low land pastoral areas and in some intensive farms. The disease is distributed to all pastoral production system areas and semi- intensive areas bordering with the pastoral region.

The sero- prevalence in pastoral production system is 4.7% (n=1898) in goats and 2.0% (n=846) in sheep and over all 3.9% (n =2744) in small ruminants. This is comparable to works in Afar pastoral areas that is 5.8% in goats, 3.2% in sheep and over all in small ruminants 4.8% (Ashenafi, 2006). Other previous works in pastoral area of Afar Region are 16.2% in goats and 14.6% in sheep where as in Samali Region 1.7% in goats, and 1.6% in sheep (Yibeltal, 2005). The finding in this study is also comparable to results observed in East Africa. The prevalence reported in Eritrea is 3.8% in goats and 1.4% in sheep (Omer *et al.*, 2000) and in Somali 7.2% in sheep, 5.29 % in goats (Falade and Husein, 1979).

In the high land, the mixed crop livestock production system (sedentary), the sero- positive rate is low about 0.3% (n=1220) in this survey, and the distribution in certain district is even 0%. Previous work carried out in Ethiopian high land showed a prevalence rate of 1.3% in goats and 1.5% in sheep (Takeley and Kasali, 1990). It is high compared to our results.

The over all individual prevalence is comparable and fall in the range of prevalence observed in North Africa and Middle East which is 0.3% to 21% in goats, 0.6% to 22.8% in sheep respectively. Most prevalence reports from these countries is below 10% at the individual level (Benkirane, 2005).

The disease is associated with nomadic animal husbandry, large herd size, contamination of pasture facilitating transmission and difficulty of regular immunization due to movement of animals which is related to developing countries (Banai, 2002; Blasco, 2005). This is true to our case that the disease is more prevalent in pastoral areas with nomadic animal husbandry, and large herd size.

5.2 Flock (herd) level prevalence rate

Flock level consideration was possible only in pastoral production system. As it is mentioned above, there is higher flock size in these areas. In pastoral areas samples were collected from 7 to 8 flocks from each peasant association. Flock prevalence of serial test was calculated in two zones, namely Borena and South Omo and bordering peasant associations in Konso. Herd size and animal density are directly related to prevalence of disease (Hirsh and Zee, 1999). The flock prevalence in this work can be compared to results of different countries having wide variations. In Uganda, 43% of flock prevalence was observed (Kabagambe *et al*., 2001). In West Asia and North Africa flock prevalence was 37% and 49% in Kuwait, and 12.1% in Morocco. In Tunisia herd prevalence in goats was 61%, flock prevalence in sheep 30% (Benkirane, 2005). It was also observed that increasing herd size was associated with herd prevalence (Mikolon *et al*., 1998). The association with increasing herd level may be attributed to increased contact in communal grazing areas and in Spain Reviriego *et al*, (2000) reported flock prevalence of 33.5 % which agrees with the results of this survey in the pastoral production system.

5.3 Risk factors

5.3.1 Production systems

Comparison of extensive and intensive managements in the survey was carried out. Over all prevalence in extensive farming system is 2.8% and in intensive farms 4.4% which shows significant difference ($p < 0.05$).

5.3.2 Species

In the survey goats and sheep were considered since these species are reared rose mixed, they graze and corralled together at night in the same pen. In pastoral production system sero-prevalence in goats is higher than in sheep. The observation is supported by works in Afar where sero-prevalence was 5.8% in goats and 3.2 % in sheep (Ashenafi, 2006). Goats are highly susceptible to *B. melitensis* infection, sheep less so (Radostitis *et al*., 2000). This finding agrees with other works though prevalence variations recorded and varied from country to country. In Morocco, 4.1% in goats and 1.6% in sheep, in Egypt 8.2% in goats and

2.4% in sheep, in Tunisia 18.0% in goats and 4.0% in sheep, in Sudan 16.7% in goats and 14.2% in sheep (Benkirane, 2005), in Eritrea 3.8% in goats and 1.4% in sheep (Omer *et al*., 2000) and conversely in Somali 7.2% sheep, 5.29 % (Falade and Hussein, 1979) are reported indicating clear differences in occurrence of brucellosis between goats and sheep.

5.3.3 Sex

In this work the sero - prevalence in female is higher than male though there is sample size difference in female and male.

5.3.4 Age

The prevalence in the different age groups varied from group to group. However significant difference was not observed in ten age groups except that prevalence is lowest at first year and highest in the fifth year

6. CONCLUSIONS AND RECOMMENDATION

The sero-epidemiological study has established the existence and the wide spread occurrence of small ruminant brucellosis in the low land pastoral areas of southern Ethiopia. The absence of sero-positive reactors in the highlands of mixed crop livestock farming system areas was also realized. Therefore making this work as premises recommendations are given as follows:

- Further research to establish other associated risk factors such as breed and others.

- The etiological agent of the disease, the bacteria has to be isolated.

- Zoonoses aspect of brucellosis in Ethiopia is not well known; hence it is important to know the disease situation particularly where there is high frequency of contact in farmers. Consumption of raw milk was found the common practice according to the responses of questionnaire administered. Infected animal shed the organism in the milk and can be a ready source of infection.

- Further scrutinizing is required of the economic aspect of the disease, losses in relation to abortions and aliments from export point of view.

- Finally after making study on these important issues, the control of the disease may be ensued by:

    - Awareness creation of the disease to the community about the effects of brucellosis in goats' productivity and the public health hazard.

    - The use the results by policy makers to plan different strategies of prevention and control in the country, then after the export market of small ruminants may be enhanced.

- Intensive farms require routine screening for brucellosis and cull out of positive animals to keep the flock free of the disease.

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9. ANNEXES

9.1 Annex 1 Questioner survey and sample record formats.

FORMATS.1

Date_____

1. Address: Region_____ Zone_____ District_____

PA_____ Village_____ GPS_____

2. Name of the owner_____ Age_____ Sex_____

3. No of goats' owned_____

3.1 Age and sex distribution of goats' 0wned

<1 year (1)		1-2years (2)		>2 years	
F (0)	M (1)	F (0)	M (1)	F (0)	M (1)
_____	_____	_____	_____	____	_____

4. Comparative use / importance/ keeping animal species (1 to 3)

	Cattle	Goats	Sheep	Camel
Disease resistance	_____	_____	_____	_____
Drought resistance	_____	_____	_____	_____
Market performance	_____	_____	_____	_____
Economic value	_____	_____	_____	_____
Milk use	_____	_____	_____	_____

5. What is the objective of raising goats

Dairy (1)	Cash income (2)	Meat consumption (3)	Others (4)
_____	_____	_____	_____

6. Do you consume goats milk Yes/No _____ Any milk treatment before consumption _____

6.1 The amount of milk produced per goat _____

6.2 Who is milking goats? _____

7. What kind of production system do you practice?

Sedentary (1)	Trans humans (2)	Pastoral (3)	Others specify
_____	_____	_____	_____

8 How do you manage during day? Communally, if communally

8.1. Is it will the same community and other communally or both

_____ _____

8.2 If there mixed grazing other species of animals.

_____ mixed with sheep (1)

_____ mixed with cattle (2)

_____ mixed with camel (3)

9. Did you encounter goat health problem? Yes/No

10. What are the major goat diseases in the order of importance (1-5)?

1._____ 2_____ 3._____ 4_____ and 5._____

11. Do these dieses involve sheep? Yes/No

12. Is there abortion or still birth in your herd /neighboring? Yes (1)/ No (0)

_____

12.1. Is the disease which causes abortion in goats is known and have local name?

Yes? No What is the local name_____

12.2 At which parity (pregnancy) or age abortion is frequent

_____

Possible reasons for abortion? _____

How do you manage aborted materials and fetus? _____

Any lameness and swelling of joints? _____

Testicular infection or problem in male? _____

13. How is night corralling? _____

A) Separate B) with Sheep C) with other animals

14. Do you slaughter goat for consumption? _____

15. How do you consume goat meat? _____

A) Cooked B) Raw C) other treatments

FORMAT 2 serum sampling record

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

PA---------- Date----------

Ser.No	Name of Owner	Sex	Age	Herd size	Breading Female History in the heard			Test	
					Kidding	Abortion	Stillbirth	mRBT	CFT

.

9.2 Annex 2: Procedures of Confirmatory test by Complement Fixation Test

I. Preparation of sheep RBC for the hemolytic system

Ten ml of SRBC in Alsever's solution are centrifuged at 2500 rpm for 5 minute. The supernatant is discarded and replaced by veronal buffer diluent (VBD). The red blood cells are resuspended in the diluent completely. This procedure is repeated four times. Before discarding the supernatant after the last washing, the volume of the packed cells (PCV) is measured. Using graduated conical tubes for centrifuging, the PCV can be read directly. Otherwise, an identical tube is placed next to blood containing tube and filled up to the level of the blood by a measured amount of water. By the addition of a calculated amount of diluent a 2% SRBC suspension is prepared.

II. Amboceptor's titration

1. Prepare 1: 500 amboceptor and dilute serially to 1:8000.

A/ prepare 5 test tubes.

B/ Add 1 ml of diluent (VBD) to each of the test tubes from 2-5.

C/ Mix 0.5ml VBD with 0.5 ml of 1:250 amboceptor to make dilution of 1:500 in the first tube (20µl amboceptor with 9980µl of VBD).

D/Transfer 1 ml from the first to the second up to the last tube and discard 1 ml.

1:500 1:1000 1:2000 1:4000 1:8000

2. Prepare 1:750 amboceptor and dilute serially up to 1:12000.

A/ Prepare 5 test tubes and add 1ml of VBD to test tubes 2-5.

B/ Mix 10µl amboceptor with 7490µl in the first tube.

C/ Transfer 1 ml from the first tube to the second up to fifth tube and discard 1 ml. Put the tubes in order of ascending dilution.

1:750 1:1500 1:3000 1:6000 1:12000

3. Transfer 0.5ml from each of these test tubes to a second set of ten tubes. Start with 1:12000 dilution.

4. Add 1 ml of VBD to each of the test tubes.

5. Add 0.5 ml of 2% SRBC to each of the test tubes and shake well.

6. Leave on the bench for ten minutes.

7 Add 1.0 ml of complement at working dilution.

8. Incubate tubes for 30 minutes in a water bath at 370c.

9. Read and record the last tubes showing complete hemolysis, minimum hemolytic dose

(MHD).

The working dilution of amboceptor is four times the MHD.

III. Evaluation of complement.

1. Freeze-dried complement is reconstituted according to its instructions.
2. A 1:100 complement is prepared.
3. Complement is added into 9 wells increasing by 5µl every time, starting with 10µl.
4. Diluent is added into the 9 wells in decreasing amount by 5µl, starting with 40µl.
5. 25µl of diluent is added into the wells with the cornwall syringe..
6. The plate is placed in the water bath at 370cfor 1hr.
7. 25µl of 2% SRBC is added in all wells.
8. 25µl of amboceptor at working dilution1: 1000 is added in all wells.
9. The tubes are properly mixed and put again in the water bath of 370c for another 30 minutes.
10. The test is read by reading the minimum hemolytic dose of complement (MHD), which is represented by first well showing complement hemolysis. The next well contains the full hemolytic dose (FHD).

The complement dilution =2FHD / Dilution of complement.

IV.Titration of antigen

Micro titer plate I

1. 25µl of VBD is added in all wells.
2. 25µl pre-diluted antigen is added to all wells of row A.
3. By serial doubling (two fold) dilution 25µl of antigen is transferred from row A to B, and again from row B to C, etc. until row G by multichannel pipette; 25µl mixture is discarded from row G (row H has only the diluents).

Micro plate II

1. 50µl VBD is added to all wells.
2. 50µl of pre-diluted (1:2.5) inactivated positive control serum is added to all wells of Column1
3. 50µl is serially transferred by two-fold dilution from col. 1 to col. 2, and again from col. 2 to col. 3, etc. until col. 11. 50µl is discarded from col. 11.

Mix plate I and II.

1. 25µl is transferred from plate II to plate I

2. 25µl of complement in 1:40 dilution is added to all cups of plate I.

3. Plate is added in the refrigerator, covered with second empty plate (cold fixation) or incubated in water bath at 370c for 30 minutes (hot fixation).

4. 50µl of 2% SRBC , amboceptor's pre-mixture, equal volume, i.e.25µl of SRBC and 25µl of a 1:100 working dilution of amboceptor's, is added to all cups.

5. The plates are covered with sealing tape, shecked well and kept in water bath at 370c for 30 minutes (hot fixation).

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

The test proper, multiple sera technique

1. The sera are pre-diluted to 1:2.5 and incubated at 62-630c in a water bath for 30 minutes in order to inactivate the native complement.

2. 25µl of diluted sera is placed in wells of first and second rows of U-bottom plate, and 25µl of veronal buffer is added to all wells except those of the first row.

3. Serial doubling dilutions are then will be made by transferring 25µl volume of serum from the second row onwards continuing for at least four dilutions.

4. 25µl of antigen diluted to working dilution excluding those of the anti-complementary controls, which received 25µl VBD instead.

5. 25µl of complement at working dilution (1:25) is added to all wells except control wells.

6. Control wells containing: serum control has serum + complement + diluent and antigen control has antigen + complement + diluent. Complement control has complement + diluent and hemolytic system has diluent set up to contain 75µl total volume in each case before the hemolytic system is added.

7. The plates are incubated at 370c for 30 minutes with agitations (warm fixation).

8. 25µl of volume sensitized 2% SRBC suspension is added to each wells. The plates are sealed and re-incubated at 370c for 30 minutes with agitations. Before reading the result the plates are left in the refrigerator at +40c for 1 hour in order to allow non-lysed cells to settle.

9. Plates are taken out from refrigerator and left at room temperature for ten minutes.

10. Positive reaction is indicated by the absence of hemolytic, sedimentation of SRBC and negative reactions by the hemolysis of SRBC.

Interpretation

Sera with strong reaction, more than 75% fixation of complement (3+) at dilution of 1:5 and at least with 50% fixation of complement (2+) at a dilution of 1:10 and dilution of 1:20 are classified as positive (OIE, 2004).

10. CURRICULUM VITTE

Name Mengistu Mekuria W/Tsadik

Sex Male

Date of birth June 1954
Place of birth Gamo Gofa (Birbir)

Marital Status Married

Adress SNNPRS, Wolliata Zone
Sodo, Ethiopia
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Language Gamugna, Amharic, English

Educational background

1. Elementary school (1 - 6): Ezzo elementary school
2. Junior high school (7 - 8): Dejazmach Wolde Mariam high school, Chencha.
3. High school (9 - 12): Arbaminch Comprehensive High school.

Colleges

-Animal Health Asstants School, Debre Zeit (1972 - 1974).
Diploma awarded.
-Addis Ababa University Faculty of Veterinary Medicine (1980 - 1986).
Degree awarded DVM.
-Addis Ababa University Faculty of Veterinary Medicine (2005 - 2007).
Degree awarded MSc in Tropical Veterinary Epidemiology.

Work Experience

1.Assitant Vet. In field service and as laboratory technician in NIV in MOA.

2. Field Veterinarian.

3. Research officer in Bahir Dar and Wolliata Sodo laboratories in departments of Bacteriology, Parasitology and Sero- epidemiology.

4.Laboratory manager in Sodo Veterinary Laboratory.

5.Non-Governmental Organization (NGO): FAO-FFHC/AD (Freedom From Hunger Campaign / Action in Development), in rural livestock development programme.

Research Areas:

-On etiology of bovine mastitis in Bihar Dar.

-Preliminary study on bovine dermatophilosis in Bahir Dar

-Surveys of trypanosomosis and tsetse fly in some areas of SNNPRS and control trials by the integrated use of Spot- on (deltamethrine and trypanocidal drugs).

-On sero – epidemiology of brucellosis of small ruminants in Southern Ethiopia of SNNPRS and Oromya region

11. SIGNED DECLARATION SHEET

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

Name: Mengistu Mekuria W/ Tsadik

Signature.....

Date of submission…….

Advisors

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