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Seroepidemiological Study of Brucellosis in Humans and Dairy Cattle in Addis Ababa

By

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SEROEPIDIMOLOGICAL STUDY OF BRUCELLOSIS IN HUMANS AND DAIRY CATTLE IN ADDIS ABABA

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By

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This work is dedicated to my father Ato Kassahun Sahilu, my mother W/o Sara Lemma, my sisters Bethelhem, Hiwote and Mihret, and my fiancee Frehiwot Mulu

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ABBREVIATIONS

CFT: Complement Fixation Test

CI: Confidence interval

EHNRI: Ethiopian Health and Nutrition Research Institute

FAO: Food and Agricultural Organization

FVM: Faculty of Veterinary Medicine

GITI: Gastrointestinal tract infections

HS: Hemolytic system

H_2S: Hydrogen sulphide

IgA: Immunoglobulin A

IgG: Immunoglobulin G

IgM: Immunoglobulin M

2-MET 2-MercaptoEthanol Test

NAHRC: National Animal Health Research Center

RBPT: Rose Bengale Plate Test

RTI: Respiratory tract infections

SAT: Serum Agglutination Test

SRBC: Sheep red blood cell

S19: Strain 19 vaccine

UTI: Urinary tract infections

VBD: Veronal buffered diluent

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Abstract

Sero-epidemiological study of brucellosis was conducted on occupationally exposed humans and dairy cattle in Addis Ababa. A total of 336 human individuals were screened by Rose Bengal Plate Test. Those found to be positive were further subjected to 2-MET. Similarly, 552 sexually matured dairy cattle were screened for RBPT and positives were further subjected to Serum Agglutination Test and Complement Fixation Test. A simultaneous survey was conducted in the farms and Addis Ababa abattoir enterprise to investigate epidemiological factors. An over all seroprevalence of (16/336) 4.8% in humans and 10% (57/552) in dairy cattle was found, taking $RBPT^+$ / $2MET^+$ in humans and $RBPT^+$ / CFT^+ in dairy cattle as a gold standard. A sensitivity and specificity of 88% and 94% in humans and 100% and 68.5% in dairy cattle was observed for RBPT. Where as SAT was 63.3% sensitive and 89.2% specific in dairy cattle. The risk factors contributing to the occurrence of the disease in humans include occupation, sex, raw milk and meat consumption, failure to use detergents after work, and unsafe handling of infected materials. However, herd size and lack of knowledge about brucellosis are the major factors in dairy cattle seropositive animals.

Key words: Sero-epidemiological, sensitivity, specificity and risk factors

1. Introduction

Brucellosis is a zoonotic disease, named after its discoverer, David Bruce (Evans, 1950). This disease has been recognized worldwide for its serious public health hazard and economic significance (Matyas and Fujikura 1984). It is a bacterial disease caused by organisms of the genus *Brucella* species. This disease causes undulant fever, sterility in man. However, it causes abortion, infertility and a subsequent loss or decrease in milk yield in cattle. It also affects a wide range of host animals including sheep, goats, swine, horses, fowl and others (Weidman, 1991, Radostitis *et al.*, 1994,). Even though, higher numbers of countries are declaring to be *Brucella*-free, the disease remains widespread in many parts of the world (Madkour and Gargani 1989; Mikolich and Boye 1990). According to the World Health Organization, about half a million cases of human brucellosis occur each year (FAO/WHO Expert Committee on Brucellosis, 1986)

Public health significance includes illness, physical incapacity and loss of manpower and also results in the scarcity of animal proteins due to loss meat. Occupational risk of brucellosis is important because of the high possibility of direct transmission from infected animals to the people employed in animal husbandry. This exposed group includes slaughter men, dairymen, herds men and veterinary clinicians. Herdsmen are the most exposed members. This occupational exposure is high in countries, where herding of animals is traditional and unscientific (William, 1975).

This disease is transmitted to man through contact, ingestion, inhalation or accidental inoculation. The disease is also transmitted from infected animals to other through direct contact, consumption of contaminated feed, though low through mating (Weidman, 1991).

It causes great economic losses in livestock industry and it is the most economically ravaging of the zoonoses (Weidman, 1991). Economic losses caused by bovine brucellosis are high. For instance, the losses in Latin America and U.S.A. were estimated to be 700 million dollars annually (Abdusalam and Fein, 1975 and Dajani, *et al*., 1989). This direct loss of meat (as result of abortion, infertility and weight loss) in infected herds of cattle was estimated to be 15% and for milk (reduction in milk production) at 20% per infected cow (Thimm, 1982).

The geographical distribution of human brucellosis is closely related to the endemicity of animal infection, the methods of animal husbandry, human food habits, the standard of hygiene and other socioeconomic activities (Abdusalam and Fein, 1975). Thus, it is mandatory to study and establish the occurrence of this disease in the reservoir animals including cattle, swine and other animals prior to the inquiry of human brucellosis (Alton, 1973; Abdusalam and Fein, 1975).

The global incidence of human brucellosis is unknown because of the variable quality of disease reporting and notification systems in many countries. World wide the only countries that declared to be free of brucellosis are Norway, Sweden, Finland, Denmark, Iceland, Switzerland, Czechoslovakia, Romania, the United Kingdom including the channel islands, the Netherlands, Japan, Luxembourg, Cyprus, Bulgaria and the U.S. Virgin islands. However, the prevalence is highest in the Mediterranean countries, Central and South America, the Middle East and South Asia, and also *Brucella* infections are common in the tropics including East Africa (Makarem *et*

al., 1982; Madkour *et al.,* 1985; FAO/WHO Expert Committee on Brucellosis 1986; Mousa *et al*., 1988; Dajani, *et al*, 1989). Reports indicate that even in developed nations the true incidence of brucellosis may be up to 26 times higher than official figures suggest. In the United States about 200 new cases are reported every year, however it is estimated that only 4 to 10% of cases are recognized and reported (Abdusalam and Fein, 1975 and Dajani, *et al*., 1989).

This disease is endemic in Ethiopian and researchers have established its prevalence rate in cattle in different regions of Ethiopia. Though, countrywide prevalence in cattle is not yet determined. However, with the exception of few case reports by Ephrem (1981) from Bale Administrative region and also Teshale (1982) reported cases of brucellosis on four patients from Tigrai, Sidamo, Arsi and Shoa Administrative regions by standard serum agglutination test, but the prevalence rate of the disease in human population is not studied and is unknown in Ethiopia.

2. Literature Review

2.1. Definition and Ethiology

Brucellae are small, cocco-bacillary or short rods; with a size range of 0.5 to 0.7 μm. by 0.6 to 1.5 μm. These organisms are gram-negative and frequently take the counter stain poorly and require a minimum of three minutes for a good definition. They are aerobic, non-motile, non-fermenting and non-toxigenic. They occur singly or in groups are non-sporulating, and non-encapsulated (Grimont, *et al*., 1992).

Six species of pathogens are known as causative agents of brucellosis in different animals, which include *B. abortus* that is the causative agent of brucellosis in cattle and Bang's disease in humans. *B. melitensis* is the causative agent of brucellosis in small ruminants and Undulating or Malta fever in humans. *B. ovis*, the causes brucellosis in sheep and *B. suis* the caustive agent of brucellosis in pigs which also can be transmitted to humans. *B. canis*, the causative agent of brucellosis in dogs and *B. neotomae* occurs in desert rats in the USA.

The different species cannot be distinguished from each other morphologically; however, differentiation of *B.abortus*, *B.melitensis*, and *B.suis* is based on quantitative differences in several physiologic tests. Such tests include requirement of increased CO_2 for growth, .H_2S production and growth in the presence of basic fuschin and thionin. Besides, within each of these species of *Brucella*, a number of strains have been recognized on the basis of these tests and additional biochemical properties (Grimont, *et al*., 1992).

The species can be differentiated based on susceptibility to specific phages, their metabolism and their ultrastructural differences in cell wall and envelope components, which actually is correlated with their antigenic characteristics. The biotypes are differentiated serologically (agglutination) applying specific monosera A, and M (Cherwonogradsky *et al*., 1987). There is no single test by which a species may be identified with absolute certainty. A combination of growth characteristics, colonial and cellular morphology, staining properties, agglutinating antisera and biochemical reactions allow an accurate identification (Bishop*, et al.,* 1994).

Pasteurization and the usual disinfectants chalk solution; caustic soda, formalin 2% and lysol 1% destroy the *Brucellae* Species. The *Brucellae* survive the production process of soft cheese for up to 6 months in contrast; the preparation of hard cheese destroys the organisms. Brucellae survive for up to 4 months in butter, for up to 6 weeks in milk, for 14 days in cooled meat, and up to 30 days in ice cream. In milk, the organisms are found mainly in the fatty components (Dajani *et al*, 1989).

The environmental resistance of the pathogens depends on whether they are protected against the radiation of sunlight and high temperatures. Neutral soil pH and a moist environment, which is rich in organic material, are favorable elements for survival. In liquid manure, the brucellae survive for months, for 22 weeks in humid feces, up to 4 months in aborted fetus and afterbirth, 44 days in the dust of streets, in tap water for 30 days, 51 days in sterile water, for 2-5 weeks in the soil of paddocks, up to 2 months in desert soil, and up to 2 years in frozen soil (Dajani *et al*, 1989). Contaminated straw remains infectious for longer than a month (Weidmann 1991).

2.2. Determinants of Pathogenicity in Humans and Cattle

The intracellular survival and multiplication of *Brucella* is a property associated with virulence, since it is essential to the organism's ability to gain access to nodes and tissues (Young *et al*., 1985). Normal human serum has a heat-labile bactericidal effect on *B. abortus* that is not complement dependent. Since *B melitensis* is serum resistant and is more virulent in man, serum sensitivity has been suggested as a virulence determinant (Corbeil *et al*., 1988)

B. meltensis, *B. abortus* and *B. suis* are virulent in the smooth phase but lose the ability to cause disease or establish persistent infection in the preferred natural host when they mutate to rough-phase organisms. In *B. canis* and *B. ovis*, however, where no smooth form exists, the rough phase is virulent but the organism has a narrower host range than other *Brucella* species (Robson *et al*., 1993).

Recent *in vitro* work has shown that rough strains bind immunogloublin (IgG) and other serum proteins non-specifically, whereas smooth strains do not and this idea is consistent with the hypothesis that outer membrane proteins are exposed as the organisms become rough and it is also possible that these surface changes contribute to the loss of virulence by increasing the accessibility of the organisms to specific and nonspecific IgG. Another potential virulence factor of *Brucella* is a cell wall carbohydrate that is responsible for binding to human B-lymphocytes. Although all *Brucella* can bind to lymphocytes, species pathogenic for humans exhibit greater activity. The binding appears to result from interaction between a lectin on the lymphocyte and a specific carbohydrate on the bacterial cell wall (Gazapo, *et al*, 1989).

Studies of cell envelope components of smooth *B. abortus* and attenuated rough organisms do not show any ultramicroscopically detectable differences. The Lipopolysaccharide (LPS) composition varies by the presence of phenol- and water-soluble LPS fractions in smooth-phase organisms, that is absent in rough organisms. The fatty acid composition of *Brucella* LPS is distinct from that of entrobacterial LPS (Moreno *et al*., 1981). A native hapten (NH), or second polysaccharide, is present in endotoxin preparations from smooth phase *Brucella*. Polysaccharide B, present in the cytoplasimic fraction, is also non-toxic and nonimmunogenic when extracted from rough organisms. It has been proposed that the situation in *Brucella* is analogous to that in *E.coli* and *Salmonella*, where the nontoxic hapten is thought to be an incompletely assembled cytoplasmic precursor of the O-polysaccharide chain of the smooth LPS (Madkour and Kasper, 2001).

B .abortus exhibits a characteristic predilection for fetal bovine tissue. Quantitation of the tissue distribution of organisms has shown that 60% to 85% of the organisms extracted from the tissues of infected animals are present in fetal cotyledons (Anderson *et al*., 1986). The 4-carbon polyhydric alcohol erythritol ($OHCH_2$—CHOH-CHOH—CH_2OH) appears to be a fetal product measurable in placental tissue, amniotic and allantoic fluids from normal bovine fetuses (Smith *et al*., 1962). This alcohol functions efficiently as a carbohydrate source in a basal medium for virulent *B .abortus* but not for attenuated or rough organisms. It also enhances the intracellular growth of the organisms in an *in vitro* system employing phagocytes and *B.abortus* (Anderson, *et al*., 1986). Therefore, there is a significant correlation of the organtropism in cattle, sheep and goats with the presence of erythritol. The absence of such tissue localization in human disease correlates with the absence of large amounts of erythritol in these organs (William, 1975).

2.3. Epidemiology

Brucellosis occurs worldwide in domestic and game animals and it is one of the major drug neglected diseases (Nicoletti, 1980). They create a serious economic problem for the intensive and extensive animal production system of the tropics. The disease has been eradicated in some industrial countries, especially in Europe, through intensive schemes of control and eradication. However, its occurrence is increasing in developing countries in an even aggravating epizootiological situation, which depends on the policy of many developing countries of importing exotic high production breeds without having the required veterinary infrastructure and the appropriate level of development of the socioeconomic situation of the animal holder (Seifert, 1990). Furthermore, the increasing international animal trade with increasing movements of animals and the trend towards intensification of animal production favors the spread and transmission of the infection (Nicoletti, 1980).

According to reports by Abdusalam and Fein (1975), Brucellosis has been shown to occur in 94 of 153 countries of the world; most of other countries do not know about it or do not report its presence. It is known to exist in 40 of the 49 African countries, representing a major problem in 20 of these and a moderate problem in ten others (Thimm, 1982). Even highly developed countries like the USA and France have so far not been able to eradicate brucellosis completely. In intensive dairy production systems of the tropics, an incidence of infection of up to 80% can be found. In the extensive animal production systems of the Sahel an average disease incidence of 25-30% was calculated (Seifert 1990). Likewise, an infection rate of almost 22% in cattle and 13.6% in sheep was found in eastern Sudan (Butana) (Weiser, 1995). In Central Africa, an

incidence of infection in cattle of above 30% has been found and economic losses of the yearly income of the animal holder have been calculated to be 6% (Domenech *et al*. 1982).

Surveys conducted in different countries showed that 1.0% reactors in USA were recorded in 1961, in U.K, 25% of dairy cattle herds and about 2% of individual cattle were infected in 1961, and similarly in Canada 9.5% of herds and 1.3% of cattle were infected in 1963 (William, 1975). In Chad in 1955, in southern Ghana in 1966, in southern Sudan in 1960 and in Senegal in 1967 the infection rate was 12%, 23.5% 13.9% to 18% and 9.4% in Fulani cattle herds (Zebus) respectively (Hellmann, *et al*, 1984). According to the survey conducted in Northern Nigeria, in 1970, the incidence was 8.6% in the private Fulani cattle and 3.4% in the government herds.

The endemicity of bovine brucellosis in Ethiopia has also been gradually established over the last two decades by various researchers (Bayleyegn, 1989, Endrias, 1989; Tekelaye, *et al*., 1989; Abeje, 1994; Tariku, 1994 and Yilikal 1998). Using past reports and investigations, Yilikal (1998) produced a summary of bovine brucellosis seroprevalence in Ethiopia, thereby bringing the disease into a sharper focus. A survey of 226 animals in Gobe ranch by Bayleyegn (1989) showed 16.81% of the animals to be infected with brucellosis. A study carried out in the Abernosa Ranch by Taye (1991) showed that, out of the 963 animals that had been tested for brucellosis 137 (14.2%) were infected. Endrias (1989) in his study around Sidamo Region of Ethiopia found 11.6% reactors in cattle. Muktar (1993) found a seroprevalence rate of 38.7% (57/147) at Bako Research Center, Western Ethiopia. Abeje (1994) reported a crude seroprevalence rate of 16.9% in his survey in and around Bahir Dar and Abay (1999) in Arsi Yekatit dairy farm 5?? . Thus, the disease appears to be widespread in both indigenous and

exotic crosses of cattle in the country. But concerning human brucellosis, except case reports no prevalence study have been made.

The prevalence of human brucellosis in Africa corresponds more closely to that of goats than cattle, due to a general pattern of contact between man and these two species. However, bovine infection has a wider distribution and is more important than infection of sheep and goats as far as economic losses are concerned (Abdusalam and Fein, 1976). Although the over all prevalence of bovine brucellosis in cattle have not been compiled, it's incidence has been some how summarized by Yilkal (1998).

2.4. Clinical manifestation of Brucellosis in Humans

The clinical manifestations of human and animal brucellosis mimic the features of other febrile illnesses (Corsby *et al*., 1984). In humans the most common symptoms are fever, chills, diaphoresis, headaches, myalgia, fatigue, anorexia, joint and back pain, weight loss, constipation, and dry cough. Physical examination often reveals no abnormalities and patients can look deceptively well. However, some patients in contrast are acutely ill, with pallor, lymphadenopathy, hepatosplenomegaly, arthritis, spinal tenderness, epididymoorchitis, skin rash, meningitis, cardiac murmurs or pneumonia (Young *et al*., 1983 and Corsby *et al*., 1984).

The fever of brucellosis has no distinctive pattern but may exhibit diurnal variation (undulant fever). In general, infections of bone and joints, heart (cardiovascular complications), respiratory tract, gastrointestinal tract, genitourinary tract, central nervous system complications (neurobrucellosis) and other manifestations like keratitis, corneal vices, uveititis, retinal detachment and endopthalmitis are also observed (Young, 1983).

In humans, the incubation period of brucellosis may be as short as 3 days but is sometimes several months in duration (Corsby *et al*., 1984). More commonly, there is a time period of approximately 3 weeks after known exposure to organisms before the onset of symptoms Organisms can enter through abraded skin, where they gain access to the lymphatics and lymph nodes. There is often lymphadenopathy with subsequent blood stream invasion secondary to the bacterial multiplication and dissemination from the primary node. The subsequent localization of the organisms occurs particularly in the reticuloendothelial system and intracellular organisms are protected from antibodies and antibiotics. Infected tissues may have granulomas, microabscesses and in rare cases caesation and the spleen is heavily infected and the bone marrow frequently has detectable granulomas. Hepatic involvement is very common, and bacteria may be present despite normal liver function tests and microscopic examination most often reveals granulomas, but diffuse hepatitis or microabscesses may be present. In general, infection with Brucella takes place through the mucosae or the injured skin and oral ingestion of contaminated food/feed. The invasion of the pathogen via the upper parts of the intestinal tract and infection through the mucoase of the respiratory system or the eyes occurs also frequently (Bishop *et al*., 1994).

Chills, sweats, and anorexia are seen in approximately three fourths of the patients with acute illness, and more than one half of the patients report generalized muscle aching, headache and backache. Associated mental depression and increased nervousness may accompany these nonspecific symptoms (Corsby *et al*., 1984; Madkour, and Kasper, 2001). Acute brucellosis is characterized by septicemia, oscillating fever, and sweats, fitting pains in the bones, joints and muscles, fever then plateaus at 39°c to 40 °c, tachycardia, defervesence after 10 to 14 days hepatosplenomegally and generalized adenopathy occur often with a group of nodes gathered

around a single larger one. However, in subacute stage brucellosis is manifested with focalization, localized foci of infection that evolves autonomously but mainly osteoarticular (sternocostal, knee, tibia, spine, sacro-iliac). Also meningeal and encephalitic foci occur .It is also at this stage that brucellosis can mimic osteitis or tuberculosis or meningitis. However, in chronic cases, low-grade fever, fatigue, vague pains, and sometimes-infectious foci (such as arthritis) are reported to occur in patients. Weakness is seen in the vast majority of patients and is the most outstanding complaint. Fatigue results in the inability to perform normal activities.

The findings on physical examination are minimal in contrast to the multiple complaints. More than 90% of the patients have fever, but only 10% to 20% have palpable splenomegaly or lymphadenopathy and organomegaly is more common in children. The fever tends to be intermittent, with characteristic diurnal variation. Illness may begin either insidiously or with a rather abrupt onset. The majority of patients with infection due to *B .abortus* have a self-limited disease. *B .melitensis* is the most invasive species in humans, and illness due to *B .melitensis* or *B .suis* may be more severe or chronic (Young, 1983).

Complications from Brucella infections are usually attributable to the granulomas that occur in various organs and tissues. Debilitating neuropsychiatric disorders, infection of a bone or joint (including the vertebral column), and in unusual cases endocarditis due to Brucella has been seen. Other viscera, such as spleen and liver, and bone marrow may have evidence of infection for a significant period of time (Young, 1983). Mostly, in humans organisms are acquired through contact with infected materials. For instance, within the United States 90% of brucellosis is now due to contact with infected materials rather than to ingestion of contaminated fresh milk and milk products. The occurrence of disease principally in men between the ages of 20 and 50 years reflects the occupational hazard to persons in the meat processing industry

(WHO, 1971). There is no adequate means of detecting infected meat. Prevention is possible only by elimination of the infection in cattle and swine (Alausa, 1980). Movement of animals allows the organism to be reintroduced into uninfected herds. Other workers at risk because of animal contact include veterinarians, livestock producers, farmers, dairy workers and laboratory personnel working with the organism (Wise, 1980).

2.5. Clinical Infection in Cattle

The incubation period of bovine brucellosis varies between 14 and 120 days. If an infection appears in a herd that has so far been immunologically naive, all pregnant animals will abort. However, if the infection is enzootic in a given herd, usually only firstly calving animals abort. The susceptibility of the animal depends significantly on their natural resistance (breed), their age, and their level of immunity and on environmental stress The transmission during mating is important but perhaps not as much as often supposed (Nicoletti, 1980).

After infection of the regional lymph nodes, bacteriaemia occurs which can last for 1-3 weeks and distribute the organisms to the lymphatic system, the large parenchyma and other organs and tissues. The facultative intracellular organisms may infect all organs and tissues and in pregnant animals, the uterus is a preferred site of infection where it leads to a necrotizing placentitis whereas in nonpregnant animals, the first infection often occurs in the udder followed by the infection of the uterus later after the onset of pregnancy. In cattle, the uterus is the central site of multiplication of the pathogen, the enhanced virulence of the brucellae inside the reproductive system is supposed to be the consequence of the increased level of the erythritol that is maintained in the reproductive system. A characteristic exudative and proliferative process

develops in the gravid uterus starting from the epithelium of the villus of the chorion (Radostitis, 1994).

The variable facets of clinical symptoms which are typical 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 influences as well as virulence of the pathogen (Bishop *et al*., 1994).

A few days before abortion, slimy pus like, gray whitish to reddish secretion appears in the vagina. A yellowish, slimy layer covers the aborted fetuses; the fetuses may be macerated. The afterbirth is edematous, slushy, the affected cotyledons, or parts of them are covered by a sticky, odorless, brownish exudate, and are yellowish gray as a result of necrosis. Parts of the intercotyledonary placenta are thickened, edematous, yellowish gray and may contain exudates on the surface. Large amounts of pathogens are excreted with the evil smelling, dirty gray lochiae. Retentiosecundinarum is usually the consequence of abortion and can lead to permanent sterility (Nicoletti, 1980).

2.6. Immunity

2.6.1 Cellular immunity

After natural infection in both humans and animals there is an initial IgM antibody response, followed by an IgG antibody response (Olivera, 1995). The agglutination test measures antibody directed at Brucella LPS antigens. Macrophages from immune animals more efficiently destroy the intracellular organisms and undoubtedly contribute to eradication of this infection by the host

(Madkour and Kasper, 2001). Macrophage activation occurs when T-lymphocytes of the appropriate subset are stimulated to release lymphokines (interleukins), which is dependent upon recognition of the appropriate antigen by the T-lymphocyte and is subject to regulation through the major histocompatibility complex. Nevertheless, Brucella organisms are less sensitive to killing by activated macrophages and this relative resistance to Brucella may contribute to the chronicity of infection (Tizard, 1992).

2.6.2. Humoral Immune Response

The surface antigens that result in the production of agglutinating antibodies by the host are polysaccharides, lipopolysaccharides and protein component of the cell wall. The immunoglobulin isotypes present in significant concentrations in bovine serum are IgG_1, IgG_2, IgM and IgA and similar isotypes at different relative concentration occur in milk, although most of the IgA is present in the secretory form (WHO, 1986).

The first isotype produced after an initial heavy infection or strain 19 immunization is IgM. This can usually be detected in the first or second week following the initial antigenic stimulus, but is soon followed by IgG antibodies. IgG_1 immunoglobulin is the most abundant in serum and exceeds the concentration of IgG_2. It is widely believed that sustained production of IgG_1 antibody is characteristic of chronic infections but that IgM antibody persists in animals immunized with strain 19 (WHO, 1986; William 1995). IgG_1 antibodies are non-agglutinating, have no opsonosing activity, block IgG_2 and IgM and may aid the persistence of *B. abortus* in the host. This explains why serologic evidence of high humoral antibodies does not correlate with immunity or perhaps effective immunity in clinically infected cattle. Further, protective S-

19 vaccine primarily induces IgM antibodies and lesser IgG_1 suggesting that IgG_1 non-agglutinating antibody may be harmful host responses (William, 1995).

2.7. Diagnosis

The major objective in the laboratory diagnosis of brucellosis is to identify humans/animals that are infected and potentially shading the organism and spreading the disease. There are basically three diagnostic methods for detecting brucellosis, which includes bacteriological, serological and allergic tests (Weidmann, 1991).

The clinical illness is often nonspecific when considered in the individual patient. Therefore, evaluation of patients often includes a number of tests dictated by the differential diagnosis. When a patient is suspected of having brucellosis, at least one blood specimen, bone marrow and tissues aspirates can be taken for culture. Bone marrow cultures have been positive more often than blood cultures, especially when patients have taken antibiotics (Gotuzzo *et al*., 1986). The intracellular localization of Brucella within reticuloendothelial cells may account for the positive cultures from bone marrow aspirates at a time when blood cultures from the same patient are negative. Isolation of Brucella organisms provides the definitive diagnosis and isolation of organisms from the tissues of infected animals may also be important (Alton and Jones, 1967 and Young, 1983).

2.7.1. Culture

Castaneda technique, involving a biphasic (solid and liquid) medium in the same bottle, is recommended for the culture of blood and other body fluids. For the solid phase, trypticase soy agar, tryptose agar, or *Brucella* agar may be used: the liquid phase consists of the same basal

medium without agar, and also Serum Dextrose Agar can also be used to culture *Brucellae*. Incubation should be in an atmosphere of 5% to 10% CO_2 at 35^{o}C to 37^{o} C .*Brucella* colonies usually appear after 4 to 5 days, but cultures should be kept for 30 days before they are discarded as negative. *In vitro* growth of *Brucella* from patient specimens requires careful and informed laboratory processing of materials. For primary culture, direct inoculation of materials on solid media is recommended to facilitate recognition and isolation of the developing colonies and to limit the establishment of non-smooth mutants. Delicate translucent colonies of 2-3 mm in diameter grow on blood- or glucose- agar. *Brucella ovis* grows always in the M (mucoid) from, *B. abortus* and *B. melitensis* grow at the beginning in the S (smooth) form and later dissociate into the R (rough) and the M (mucoid) form (Cherwonogradsky *et al*., 1987). Commercial blood culture bottles may also be used for culturing *Brucella;* however, subcultures must be made every 4 to 5 days. Laboratory processing of materials, such as animal tissue or milk that may be heavily contaminated with other microorganisms, requires either animal inoculation or the use of selective media containing antibiotics (Merck and Co, 1991)

2.7.2. Serologic Diagnosis

Bacteriologic examinations are of less practical use in determining the prevalence rate of brucellosis on humans and cattle population. This is due to the long incubation period, higher cost and usual negative results of culture. Thus, the use of various standard serological testing methods like Rose Bengal Plate Test (RBPT), 2-mercaptoethanol Test (2ME), and Dipstick Assay (DSA) in humans and also Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT), and Complement Fixation Test (CFT) are methods of choice in testing of animals (MacMillan, 1990). However, the serological tests should be accomplished in combined and justifiable manner to avoid false positive or negative results.

Rose Bengal Plate test (RBPT) is a qualitative, simple and fast screening test that classifies animals as positive or negative and it is valuable test in developing countries like Ethiopia where laboratory facilities are lacking. Thus, the usefulness of such test in under equipped health institutions is well accepted. It is the most sensitive test with 98.3% sensitivity. Serologic diagnosis of infection by the standard agglutination test is sensitive and reproducible. The success of agglutination test has depended largely on the selection and standardization of the antigen. The antigen from *B. abortus* (strain 1119) has been used to diagnose disease with any one of the three commonly encountered species of Brucella (*B. abortus, B. suis*, and *B. melitensis*). For the diagnosis of disease caused by *B. canis*, it is necessary to use either specific antigen or antigen from *B .ovis*.

The serum agglutination test (SAT) reveals both M and G immunoglobulins. SAT may yield both false negative or false positive result; however, it effectively detects brucellosis on a herd basis. (Corbel *et al*., 1984). It is generally accepted that in active stage of Brucellosis, IgG is always present. However, in agglutination tests, IgM reaction usually predominates (Levieux, 1974). Comparative serologic studies by Buchanan (1980) indicated that a single titer of greater than 1:160 by the standard tube agglutination test is presumptive evidence of current or recent infection with Brucella organisms and a four-fold rise in agglutinins is seen in the first 3 months of infection in more than 90% of patients with cultures positive for *Brucella* (Neilson, 1990).

However, the complete absence of agglutination does not rule out the possibility of infection. Serum agglutination test in case of acute infection is usually positive where as in cases of chronic and past infection this test results in weak or negative reaction. However, in sub acute or chronic phase of brucellosis serum agglutination test (SAT) may be difficult to interpret and other tests are needed to be included. As the disease progresses from the acute to the chronic

form and organisms become localized intracellularly in various parts of the body, IgM antibodies decrease so that the agglutination titer falls to a low level and may finally be absent even when patient is still ill.

Thus, when low serum agglutination titres are found, tests that reveal the presence of IgG must be carried out. Such tests are the 2- Mercaptoethanol Test (2-MET) and the Complement Fixation Test (CFT), the IgG's fix complement but often lack agglutinating power. Since, the antibody response to brucellosis is characterized by the production of both IgG and IgM, the persistence of higher IgG is taken as indicative of active infection. 2-MET reveals the presence of IgG antibodies by elimination of IgM and thus persistence of IgG antibodies at a positive titre of 1:40 or more is taken as an indication of active infection (Buchanan & Faber 1980; Lopez-Merino & Lopez Santiago 1989).

2-MET is a confirmatory test, and it is a slight modification of SAT, which facilitates the diagnosis of past or chronic infections. As it eliminates the phenol saline sensitive non-persistent IgM antibodies which otherwise would interfere in the agglutination process. This test is done in the same manner as (SAT) except that the saline diluent contains 0.05M –2-mercaptoethanol i.e. (14ul per 1000 ml of phenol saline) and used to detect human infection. And therefore agglutination in this test is indicative of the continuing presence of IgG and the likelihood of continuing infection. Persistence of IgM agglutinating antibody titers for year's in-patients without active infection confuses serologic evaluation (Diaz *et al*., 1976).

There is a low level of cross-reactivity between the serum agglutinins of *Brucella* Species. and those of *F .tularensis*, *V .cholerae*, and *Yersinia* species. There is also prozone phenomenon in the measurement of agglutinating antibodies to Brucella organisms, which apparently is due to

the presence of blocking antibodies. It is easy to detect agglutination, during the acute phase of illness, when IgM agglutinating antibodies predominate. However, IgG antibodies are formed during the course of the infection, and some of them bind with antigen, thus preventing agglutination by the larger IgM molecule. A modified coombs (antiglobulin) test has been used to increase the efficiency of serologic antibody and antigen agglutination (Gazapo *et al*., 1989).

2.7.3. Skin Test

The *Brucella* skin test is measurement of sensitization to the antigens of the organisms at an undetermined time. It is not used for diagnosis of acute infection because it may remain positive for years after infection, even after the agglutination test becomes negative. Pouillot and his colleagues (1997) made an assessment of the diagnostic value of the Brucellin allergic skin test (AST) in a brucellosis false positive serological reaction and reported that allergenic skin test is to be more specific than Rose Bengal and complement fixation test. Therefore, this test could be used as a confirmatory test on cattle non-vaccinated against brucellosis. The clearest proof of Brucella infection is isolation of the organism by culture from blood, bone marrow, cerebrospinal fluid or abscesses in acute stage. In chronic & sub acute cases, however, this is of limited practical use. Thus, serological tests are alternatives in prevalence study and according to the Joint FAO/WHO expert committee on brucellosis; a high or rising agglutination titer is presumptive evidence of Brucella infection (WHO, 1971).

Highly sensitive and specific methods are also in use these days by developed nations. Such tests include ELISA, PCR, and PCR-ELISA. These tests help to over come some of the limitations of conventional serodiagnostic methods and culture (Morata, *et al*., 2003)

2.8. TREATMENT, PREVENTION AND CONTROL

Brucellosis is one of the drug-neglected diseases and treatment of brucellosis in domestic animals is not indicated. However, humans are usually treated with the following antibiotics, doxicycline with rifampicine. Relapses are, however, possible. Single agent therapy for brucellosis has now been abandoned because of the high rates of failure and relapse and the potential development of antibiotic resistance. Relatively short courses (less than 8 weeks) of treatment with antibiotic combinations have similarly been associated with high rates of relapse (Luzzi *et al*., 1993).

The combination doxycycline and an aminoglycoside (gentamicin, streptomycin, or netlimicin) for 4 weeks followed by the combination of doxycycline and rifampin for 4 to 8 weekdays is the most effective regimen (Solera, 1995). The doxycycline /aminoglycoside combination is more effective than the doxycycline/rifampin combination in that rifampin reduces levels of doxcycline in plasma (Corbel, 1997).

Within 4 to 14 days after the initiation of therapy, patients become afebrile and constitutional symptoms disappear. The enlarged liver and spleen return to their normal size within 2 to 4 weeks. An acute, intense flare-up of symptoms may follow the start of treatment, especially with that of tetracyclines. This reaction is transient and does not necessitate the discontinuation of therapy. In endemic areas the coexistence of brucellosis and tuberculous spondlylitis may result in a failure to respond to appropriate treatment. Treated patients whose infections are apparently cured should be followed clinically and serologically, with repeated blood cultures, every 3 to 6 months for 2 years (Solera, 1995).

The strategies for preventing brucellosis have to be adapted to the kind of animal production system. Failures of disease control are mostly due to the absence of efficient scheme for which the veterinary infrastructure exists, the low socioeconomic status of the animal holder, and also the lack of reliable serological laboratories and respective techniques should be adopted to develop good control and eradication programs (Saravi *et al*., 1995). There are two alternatives;

1. Test and slaughter (t+s) i.e., recognition of all animals which have responded immunolgocially to a *Brucella* infection and subsequent culling of the reactors. Part of the scheme has to be a careful control of all animals, which will be newly added to the herds as well as a production system, which prevents contact with infected neighboring farms and/or contaminated feed or pastures (Radostitis, *et al*., 1994)

2. Vaccination of exposed herds with inactivated or live vaccines (Weidman, 1991). Workers in the meat and dairy industries in the former Soviet Union, China, and France have been vaccinated; the vaccine (two injections given 2 weeks apart, each containing 1 mg of an insoluble fraction of phenol-extracted bacteria) markedly reduces the rate of infection. However, the vaccine induces fever in 6% of recipients and severe pain at the injection site in 16%. Moreover, immunity is short-lived, and vaccination should be repeated every 2 years. This vaccine is not used in the United States (Hellmann, *et al.,* 1984).

Prevention of human brucellosis is primarily dependent on control of animal sources of infection. Modifications in processing of milk and dairy milk products, as well as animal surveillance and animal immunization, have greatly reduced the dangers of this disease within the United States. The population at risk consists almost exclusively of persons in contact with animals or their contaminated products. Available vaccines are suitable only for animals. The

disease remains one of economic importance in many countries of the world, where control of infected animal herds has not been readily accomplished (Alton and Jones, 1967).

Thus, this project was aimed to determine brucellosis occurrence in Addis Ababa that is actually part of an extensive project undertaken by Zoonoses research team at The Ethiopian Health and Nutrition Research Institute (EHNRI).

This Project was primarily aimed at investigating the prevalence rate of the neglected, under diagnosed and one of the drug neglected diseases human and bovine brucellosis. Which, is generally assumed to be due to the diagnosis difficulties leading to misdiagnosing the disease as it mimics other febrile infections such as typhoid fever, typhus, and pyrexia of unknown origin.

Objectives

1. The general objective:

This project was intended to undertake a seroprevalence study of brucellosis on humans and sexually matured dairy cattle from selected private dairy farms, veterinary clinics and abattoir.

2. Specific objectives

( ) To determine the prevalence of brucellosis on humans and dairy cattle in Addis Ababa.

( ) To assess some of the epidemiological factors, which contribute for maintenance and spread of brucellosis in the study area.

(c) To evaluate the performances of different serological tests at local conditions.

3. Materials and Methods

3.1. Study area and Sample collection

The present study was conducted from December 2002 to April 2003 in different private dairy farms, veterinary clinics and Addis Ababa abattoir enterprise. Blood specimen from dairy cattle and humans, and data on some epidemiological variables were collected from various farms and veterinary clinics that are found in the six zones of Addis Ababa. Blood specimens only from workers in Addis Ababa abattoir were collected from in Addis Ababa, Region 14. Informed and written consents were used before serum samples and data from human subjects were collected. The address of the study areas and the number of humans and animals selected in the respective farms are indicated in the **(Appendix-1).**

Shola regional veterinary laboratory routinely under takes surveillance in some diseases including brucellosis, mastitis and other endoparasitic diseases, and also provides veterinary assistance to the health problem of dairy animals in Addis Ababa. They collaborate with EHNRI, Zoonoses research team in research mainly in humans and in dairy cattle. Besides, the farm owners and management bodies in the farms have made cooperation at all levels particularly by making animal records available, restraining of the animals and in the collecting of samples.

3.2. Human subjects

A cross sectional study was undertaken in individuals, who were presumed to be exposed to brucellosis (occupationally risk groups). These individuals were selected by random sampling method from various dairy farms and slaughterhouse, since prevalence study has not yet been

done equal proportion of infected (P1) and non-infected (P2) individuals presence in a population was assumed i.e., $H_0 = 0.5$ and $H_A \neq 0.5$. A sample size of 384 was calculated at 95% CI, however due to the time limitation the sample size taken was 336. These were all volunteer individuals that agreed to participate by filling a consent form. It is these same individuals that were subjected to standard serological tests like RBPT and 2- mercaptoethanol by drawing about 10 ml of blood sample each. Data on epidemiological factors was also generated by interviewing these subjects through filling questionnaire.

3.3. Dairy cattle

The sample size of the dairy cattle was calculated on the basis of the prevalence of bovine brucellosis in Addis Ababa region i.e. 5.2% Yilkal (1998) computed with a worst acceptable error of 2.4 and at 95% confidence interval. It was approximately 300. However, a total of 552 dairy animals were taken as a sample and individual animals were selected from the total population by systematic random sampling method. Moreover, the data was analyzed using statistical softwares SPSS and Minitab.

3.4. Blood samples and data Collection

Approximately 10 ml of venous blood sample was obtained from human and animals, using either plain vacutainner tubes or sterile disposable syringe and needle. After identifying each individual human and animal, vaccutainer tubes were labeled with the names of individual human subjects or ear tag of the animals, or owner's name of the dairy animals. The tubes were set tilted overnight at a room temperature to allow clotting. Sera were separated from the clot next. They were then stored at $-20^{o}C$ until used for serological tests.

3.5. The serological Tests

Three serological tests Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT) and 2-Mercapto Ethanol Test (2-MET) were done at the EHNRI laboratory. The Complement Fixation Test (CFT) was done at the National Animal Health Research Center, Sebeta. In the present study, RBPT was used to screen the 552 dairy animals sera and 336 human individual subjects. The sera of dairy cattle found positive for RBPT, were subjected to SAT and CFT. RBPT, 2-MET and dipstick assays were utilized for detection of *Brucellae* antibodies from human samples. To check cross-reaction with *Salmonella,* salmonellosis test was undertaken using *salmonella* antigens 'H 'and 'O'.

3.6. Questionnaire survey

The questionnaire was designed to obtain information from the dairymen in the farms and slaughter men in the abattoir and veterinary clinicians. Data on sex, age, work experience, previous exposure to diseases for humans, and type of breed, abortion history, herd sizes and other epidemiological factors for dairy cattle were collected. The managerial practices that affect the prevalence of brucellosis in the farms and slaughterhouse based on Kellar (1976) and Young (1983) was also enquired.

The factors considered of potential biological importance in the epidemiological of human and bovine brucellosis were history of reactors, exposure level, and use of maternity pen, breeding method, disposal of aborted materials, replacement strategies, quarantine usage and measures on reactors. The knowledge and awareness of cattle attendants for brucellosis in the study farm and

the way they consume milk and meat were formatted and included in the questionnaire (**Appendix-2**).

3.7. Rose Bengal plate test

25 ul of test serum was dispensed on the plate and then, 25 ul of RBPT antigen was dropped along side the serum. For each plate negative and positive controls were included. Using new applicator stick each serum was mixed thoroughly with the antigen. The plates were rocked by hand for about 4 minutes. Then results were interpreted according to Nielson and Punkan (1990), "0" as negative (No agglutination), "+" (Barely perceptible agglutination), "++" (Fine agglutination and some clearing), "+++" (Course clumping, definite with clearing)

3.7. Serum agglutination test

50 ul of phenol saline was added to each well of the plate. 30 ul of phenol saline was added in the first column of the wells (1A-8A). Then 20 ul of control and test sera were added in the in the first column of the plate making a final volume of 100 ul. A serial dilution ranging from 1/5 to 1/10240 was done by transferring 50 ul from column 1 to column 2 until column 12. 50 ul of a 1:10 dilution of Brucella antigen with phenol saline was prepared and added to each well and incubated over night at 37 ^{o}C in humid chamber. A sample was taken as positive if there is 50% or more agglutination in 1/20 or 25 percent of 1/40 (Alton, and Jones, 1967.

3.8. 2 –mercaptoethanol test (MET)

This test is the same as **(SAT)** except that the saline diluent contains 2-mercaptoethanol i.e.14ul of 2-ME in 1litre of phenol saline. This test detects IgG and helps to diagnose sub-acute and chronic cases (Buchnan and Faber, 1980).

3.9. Dipstick Assay

The detection reagent was prepared by mixing it with a 250 ul of reconstituting fluid. Then this reconstituted detection reagent was mixed in 5 ul of serum sample to have a dilution of (1: 50). This mix was incubated for 3h at 37^0c, the dipstick was rinsed with tap water to remove excess detection reagent. Then the wetted dipstick was air dried at ambient temperature. The results were interpreted as a positive, if reddish stain antigen band was seen. Then, the staining of the antigen band was scored as "1+" through "4+" by comparison with a colored reference strip. When no coloring is observed, the test was considered as negative with '0' scoring (Smith, 1999).

3.10. Complement Fixation Test (CFT)

For CFT a 3% sheep red blood cell suspension was prepared before being used in the test proper. Sheep red blood cells (SRBC) were prepared as follows; approximately 130 ml of blood was drawn from a sheep into 270 ml of Alsever's solution. After it was stored at +4 ^{0}C for 5 days, 20 ml of SRBC in Alsever's solution, it was centrifuged at 2500 rpm for 5 minutes. Then the supernatant was discarded and the SRBC was resuspended in VBD and centrifuged again. This procedure was repeated 4 times and finally a 3% suspension of SRBC was prepared. The preparation of reagents and CFT procedure were according to the protocols of (Nielsen and Dunkan, 1990). The CFT was done at Sebeta National Animal Health Research Center (NAHRC).

Sera to be tested were thawed and inactivated for 30 minutes at 58^0C in water bath. 80 µ1 of VBD was added to column 1 and column 7 of the U-bottom microtitre plate. Except for column 2 and column 8, 25µ1 of VBD was added to the rest of the wells. 20 µ1 of the positive control

sera was dispensed in A1 and A7 and the same volume of negative control sera was dispensed in B1 and B7. 20 µ1 of test sera was dispensed to column 1 and 7. After mixing well, 25 ul was transferred from column 1 and 7 to column 2 and 8, respectively. 25 µ1 was discarded from column 1 and 7. Again 25 µ1 was transferred from column 1 and 7 to column 3 and 9, respectively.

This mixture was thoroughly mixed and serially diluted in 25 µ1 amounts until column 6 and 12 from where 25 µ1 was discarded. 25 µ1 of antigen in working dilution was added to all columns except column 1 and 7 (to these, 25 µ1 of VBD was added (serum control). 50 µ1 complement in working dilution was added to all wells. Plates were covered with other empty plates and placed in a refrigerator for 18-24 hours. The following day, 50 µ1 of equal volume of premixed 3% SRBC amboceptor (at working dilution) were added. Plates were sealed with other empty plates and placed on a shaker (working) housed in an incubator (37^0C) for 30 minutes. Plates were taken out from an incubator and results read after being left on the table for 10 minutes. Interpretation, sera with at least 50% fixation of the complement at a dilution of 1:10 were taken as positive. A hemolytic reaction of 50% or less at a dilution of 1:5 was considered as the minimum seropositive threshold (Dohoo *et al*., 1986).

3.11. Data Analysis

The prevalence rate was determined by dividing the respective number of $RBPT^+/2\text{-}MET^+$ or $RBPT^+/CFT^+$ positive humans and animals by the total number of humans or animals tested. The percentage of infected animals was calculated by dividing the number of humans or animals infected by brucellosis to the number tested. For assessing the trend between the proportions of

Brucella infected animals and epidemiological factors the Chi-squares test for trend was employed (Mentel, 1963) cited by Thrusfield, (1995).

The Chi-square, t-test, and ANOVA were used to investigate association between seropositive reaction and epidemiological factors. (OR), were also used. Odds ratio is the ratio of the odds of disease occurring among animals exposed to a factor and the odds of disease occurring among animals not exposed to a factor (OR= a X b/c X d) (Thrusfield, 1995).

	Exposed	Non-exposed
Disease present	a	b
Disease absent	c	d

The performance characteristics of the serological tests were estimated using a two-by-two table as shown below.

RBPT/CFT				
SAT		+	-	Total
	+	a	b	a+b
	-	c	d	c+d
	Total	a+c	b+d	a+b+c+d

RBPT/CFT				
RBPT		+	-	Total
	+	a	b	a+b
	-	c	d	c+d
	Total	a+c	b+d	a+b+c+d

Where a = true positives, b = false positives, c = false negatives and d = true negatives. $RBPT^{+}/CFT^{+}$ was taken as "gold standard" test and the values were defined as follows. Sensitivity (a/a+c): the ability of a serological test to give a positive result when the sera contain *Brucella* antibodies. Specificity (d/b+d): the ability of a serological test to give a negative result when the sera contain no *Brucella* antibodies. Predictive values were calculated to estimate the probability of an animal to be truly infected when its test is positive (PPV) (a/a+b) and to estimate the probability of an animal to be truly negative when its test is negative (NPV) (d/c+d). Efficiency (a+d/a+b+c+d) is the ability of a serological test to classify non-infected animals as test negative and infected animals as test positive (Thrusfield, 1995).

The kappa statistic was used to test the agreement between the different serological tests. K-measures if the result agrees to an extent significantly in excess of "chance agreement" and was calculated as: K=% agreement beyond chance / maximum % improvement (>0.81) = almost perfect agreement, 0.61-0.80 = substantial agreement, 0.41-0.60= moderate agreement, 0.21-0.40 fair agreement, 0-0.2 slight agreement and 0 = poor agreement) (Thrusfield, 1995). Percent agreement was determined by taking the proportion of sera that gave the same result in each test.

4. Results

4.1. Seroprevalence of Human Brucellosis

As shown in Table-1, a total of 336 occupationally exposed human sera were subjected to, Rose Bengal plate Test (RBPT) and 2-Mercaptoethanol Test (2-MET). Using RBPT a 10.4% seroprevalence was observed for *B. abortus* (35/336). On the other hand, less seroprevalence was found using the 2-MET (4.8 %) (16/336). However, two individuals negative to RBPT were found to be positive for 2-MET. Moreover, individuals that were reactive to *brucellae* antigens were further checked for salmonellosis ''H'' and ''O'' antigens to check false positive results, and 2 individuals for ''H' 'and 7 individuals for 'O'' were positive, and a total of 9 subjects were found to be salmoellae positive. Proven positive cases were also subjected for acute brucellosis test by using a dipstick assay method. However, all these positive cases yield negative result even if the internal control was working properly.

Table 11. Apparent prevalence rate and relative percentage of brucellosis infected individuals.

	RBPT	$RBPT^+/2\text{-}MET^+$
Negative %	301 89.6	320 95.2
Positive %	35 10.4	16 4.8

Table 2. RBPT Degree of reaction in human brucellosis detection

Occupation	0	+	%	++	%	+++	%	Total
Dairy men	43	5	71	2	28	-	-	7
Slaughtermen	228	5	19	13	50	8	30	26
Vet.clinician	22	-	-	-	-	-	-	-
Other	8	1	50	-	50	1	50	2
Total	301	10	28	15	42	9	25	35

Table 3: Antibody titer of individual humans tested for brucellosis

	2-MET					
Dilution Rate	1:5∗	1:10∗	1:20∗	1:40	1:80	1:160
No of individuals	14	13	15	10	4	2

Key: ∗ negative

4.2. Performance evaluation of various serological tests

Sensitivity and specificity of RBPT was computed by taking 2MET, as a confirmatory test and a respective value 88% and 94% were observed and also a positive predictive value of 45.7%, Negative predictive value of 99.4% and generally test efficiency of 94% was observed. The agreement between the two tests has already been indicated to be moderate. The Kappa test has shown that there is moderate agreement between RBPT and 2-MET at 95% confidence interval ($P< 0.05$). This is an indication that most positive cases that have been identified by RBPT has also been identified by 2-MET. These two tests have a K- 0.416, indicating a 41.6% agreement between the two tests and this was a significant ($P< 0.05$).

4.3. Human brucellosis, seroprevalence and epidemiological factors

Some of the major factors contributing to the prevalence of human brucellosis in occupationally exposed individuals were assessed and among the main predisposing factors studied age, sex, working year, habit to raw milk and meat consumption, Unsafe handling of aborted fetuses and cutoffs and other infected materials, the use of detergents after work, and prompt treatment of sores and openings were factors compared with seropositive brucellosis cases.

4.4. Seroprevalence and age

The association between seroprevalence and the age of infected individual subjects was measured. The odds ratio computed was 1.05 however it was not significant (P=0.145). These results indicated that there is equal risk of getting the disease when comparing the younger and older age groups.

4.5. Seroprevalence and sex

The association between seroprevalence and sex of infected human individuals was measured. The odds ratio computed was (3.05) this value was not significant (P>0.05). These results indicate that there is a three times higher risk for males to be infected by brucellosis than females.

4.6. Seroprevalence and consumption of raw milk

The association between seroprevalence and consumption of raw milk was measured, and odds ratio of (0.25) was observed (P = 0.086). This indicates that there is an inverse association for

consumption of raw milk and seropositivity, which implies consumption of raw milk to be rather a non-predisposing (even protective) factor. However, this inverse association was not significant. Two (20%) of the 10 positive cases were found out to consume raw milk.

4.7. Seroprevalence and consumption of raw meat

The association between seroprevalence and consumption of raw meat was measured; and an odds ratio of (0.24) was observed (P >0.05), however, this was not a statistically significant value. This result indicates that there is an inverse association between seropositivity and consumption of raw meat, which implies consumption of raw meat to be rather a non-predisposing (even protective). Among the 10 seropositive individuals, six (60%) were found to consume raw meat.

4.8. Seroprevalence and unsafe handling of potentially infected materials

The association between seroprevalence and unsafe handling of potentially infected materials was measured, and an odds ratio shows that there is a 3.4 times increase in seropositivity cases for every decrease in safe handling of infected materials. This was statistically significant value (P < 0.05).

4.9. Seroprevalence and use of detergents after work

The association between seroprevalence and use of detergents after work was measured, odds ratio of 2.66 was observed (P = 0.233) this value was not significant. The result indicates that there is a 2.66 times risk of seropositivity for workers, who do not use detergents after work as compared to those who used detergents.

4.10. Seroprevalence and prompt sore treatment

The association between seroprevalence and sore treatment was measured and odds ratio of (0.24) was observed. This value was not significant (P = 0.527), indicating that prompt treatment of sores to have protective value against brucellosis than workers, which do not treat sores promptly.

4.11. Seroprevalence and other common diseases

To assess the occurrence of various clinical signs and symptoms of brucellosis and their similarity with symptoms of other common febrile diseases, a total of 290 volunteer individuals were interviewed. These variables and their respective proportion is presented and summarized in (Table 4) below.

Table 4. The frequency of different complaints and epidemiological factors in occupationally exposed human individuals

S/N	Complaints/Epidemiological factors	Frequency	% Proportion
1	Sex	Male	96.6
2	Age	28	5.9
3	Working year	35	12.1
4	Malaria	39	13.4
5	Typhoid	45	15.5
6	Fever	83	26.6
7	Headache	98	33.8
8	Sweating	56	19.3
9	Arthritis	87	30.0
10	Back pain	90	31.0
11	Consumption of raw milk	140	48.3
12	Consumption of raw meat	247	85.2
13	GITI	148	51.0
14	RTI	97	33.4
15	Diaphoresis	26	9.0
16	UTI	43	14.8
17	Pain on testes	32	11.0
18	Myalgia	25	8.6
19	Fatigue	50	17.2
20	Nervous disorder and insomnia	28	9.7
21	Ability to give birth	184	63.4
22	Use of detergents after work	26	9.0
23	Prompt treatment of sores	246	85.1
24	Abortion case encountered	19	6.6
25	Infertility case encountered	7	2.4
26	Unsafe handling of aborted fetuses and cut offs	288	97.6

4.12. Seropositive cases and complaints/diseases

Out of the 336 occupationally exposed human subjects 10 (4.8%) of individuals were found to be serologically positive. For these 10 seropositive individuals a Chi-square value was computed and also the proportion of various variables was calculated and the proportion in percentage was determined and summarized in (Table 5).

Table 5: Chi-square values and proportion of various variables in human brucellosis

S/No	Variables	Presence	Absence	% Occurrence	Total	P-value.
1	Sex	(M) 9	(F) 1	(M) 90	10	0.01*
2	Malaria	2	8	20	10	0.058
3	Typhoid	2	8	20	10	0.058
4	Fever	3	7	30	10	0.206
5	Headache	3	7	30	10	0.206
6	Sweating	3	7	30	10	0.206
7	Arthritis	2	8	20	10	0.058
8	Back pain	3	7	30	10	0.206
9	Consumption of raw milk	2	8	20	10	0.527
10	Consumption of raw meat	6	4	60	10	0.058
11	GITI	4	6	40	10	0.527
12	RTI	3	7	30	10	0.206
13	Diaphoresis	1	9	10	10	0.011*
14	Pain on testes	2	8	20	10	0.058
15	Myalgia	1	9	10	10	0.011*
16	Fatigue	2	8	20	10	0.058
17	Nervous disorder and insomnia	3	7	30	10	0.206
18	Inability to give birth	1	9	10	10	1.000
19	Use of detergents after work	2	8	20	10	0.058
20	Prompt treatment of sores	6	4	60	10	0.527
21	Abortion case encountered	2	4	33.33	6	0.414
22	Unsafe handling of fetuses and cutoffs	9	1	10	10	0.011*

❖ Key = * Significant

In general a Chi- square statistic (χ^2) was computed for the following variables sex, diaphoresis, myalgia, and unsafe handling of aborted fetus and offal /cutoffs, and a similar p-value was observed (P = 0.011) these observations were significant, which shows that most seropositive individuals were males with less complaint of diaphoresis and myalgia. But they had a history of unsafe handling of aborted fetuses and offal.

However, for variables like malaria, typhoid, and the use of detergents after work, a similar P-value was observed (P = 0.58) and besides for other variables like fever, headache, sweating, back pain, RTI, nervous disorder and insomnia a similar P-value was observed (P=0.206). For variables like for consumption of raw meat, GITI, and prompt sore treatment a similar P-value was observed (P = 0.527) was observed. Finally, for abortion case encountered around the worker locality (P = 0.414) was observed. The P-values are not significant meaning there is a less association between seropositivity and the above variables (Table 6).

Table 6: Percentage distribution of exposed group

S/No.	Occupation	Total	% Positive	RBPT		2MET	
				Negative	Positive	Negative	Positive
1	Dairy men	50	8	43	7	6	4
2	Slaughter men	256	5.46	228	28	12	14
3	Vet clinician	22	-	22	-	-	-
4	Other	8	-	-	-	-	-
5	Total	336	4.76	301	35	18	18

Key: - refers to negative

4.13. Seroprevalence of bovine brucellosis

Using RBPT 48% (265/552) prevalence was determined. On the other hand, the animals were subjected to SAT, in which 15%(81/552) seroprevalence was observed Finally subjects that were found to be positive both to the RBPT and SAT were further tested by CFT and 10% (57/265) seroprevalence was observed (Table-7). In general, there is no significant difference observed for RBPT positive and negative cases 0.48 and 0.52 are respective proportions the difference between the two proportions is found to be not significant (P>0.05). However, for SAT and CFT (P > 0.05) obtained was observed, with a respective positive and negative proportion of 0.15 and 0.85 respectively for SAT, and with a relative proportion of 0.10 and 0.90 respective positive and negative cases for CFT and these values were significantly different (P<0.05).

Table 7. Apparent seroprevalence rate and relative percentage of infected animals

	RBPT	%	SAT	%	CFT	%
gative	287	52	471	85.3	495	89.7
Positive	265	48	81	14.7	57	10.3
Total	552	100	552	100	552	100

Table 8. Percentage distribution of RBPT degree of reactions in cattle

Dairy farms	RBPT degree of reaction							
	0	+	%	++	%	+++	%	Total pos
Zone 1	9	1	11.11	5	5.55	3	33.33	9
Zone 2	144	49	44.54	43	39.09	18	16.36	110
Zone 3	23	14	51.85	8	29.62	5	18.51	27
Zone 4	26	7	24.13	12	41.37	10	37.03	29
Zone 5	36	25	34.68	23	36.50	15	23.80	63
Zone 6	49	14	51.85	9	33.33	4	14.81	27

Table 9. Antibody titre result of SAT and CFT tested dairy animals

	Dilution rate	Pos/Neg.
SAT	1:10	175 *
	1:20	156 *
	1:40	60
	1:80	14
	1:160	4
	1:320	3
CFT	1:10	34
	1:20	16
	1:40	2
	1:80	5
	1:160	-

Key: inconclusive (negative) *

4.14. Performance evaluation of serological tests in bovine brucellosis

The kappa statistic had also been computed between RBPT, SAT and CFT and the result has shown that there is a significant agreement between the three tests (P< 0.05). Kappa statistic had been computed, and a Kappa value of K= 0.184 and K=. 0.208 and a K= 0.143 were observed respectively for the following paired tests RBPT and CFT, SAT and CFT and RBPT and SAT.

Table 10. Estimates of sensitivity, specificity, predictive values s and efficiency of SAT and RBPT

Serological test	Sensitivity%	Specificity %	PPV %	NPV %	Efficiency
RBPT	100	68.5	20.51	100	70.76
SAT	63.3	89.2	48.71	93.75	82.6

4.15. Bovine Brucellosis, seroprevalence and epidemiological factors

Different epidemiological factors known to contribute to the prevalence of bovine brucellosis have been studied and a comparison of mean values of such variables was made among the sixth zones in Addis Ababa using a one-way analysis of variance (One way ANOVA). Farm size and herd size were among the parameters studied (Table-11).

Table 11. Seropositive animals and zonal distribution

Zone	Number of farms studied	Number of cattle tested	Number of Positive Cattle	%
1	3	16	3	18.75
2	6	262	20	7.6
3	7	55	4	7.2
4	5	50	6	12
5	7	100	16	16
6	5	69	8	11.5
Total	33	552	57	10.32

4.16. Seroprevalence and abortion

The mean values of two proportions, brucellosis and abortion were computed and as a result, a respective mean value of 0.26 and 0.46 was observed. The significance between the two proportions was measured by a paired t-test statistic and it was not significant (P = 0.08), which implies that the concurrence of abortion and brucellosis to be not highly associated.

4.17. Seropositive and knowledge about brucellosis

Odds ratio of two was observed but this value was not significant (P >0.05). This results show that there is a 2 times higher risk of seropositivity in farms with farm attendants and management personnel, a lack of knowledge about brucellosis as compared to those with knowledge about brucellosis.

4.18. Seropositive, parturition pen and bedding/cleanliness of cow stalls

Odds ratio of 0.862 was observed but this value was not significant (P>0.05). This result shows that seropositivity to be favored by separate parturition pen, which is not the case in reality. Odds ratio of 1.24 was observed at (P >0.05), this was not significant. This indicates that there is a smaller chance of being seropositive for farms with no bedding and unclean cow stalls as compared to farms with good bedding and clean cow stalls.

4.19. Seropositive and milk reduction

Odds ratio of 1.36 was observed at (P< 0.05), this was significant. This indicates that seropositive farms to have higher risk of milk reduction as compared to seronegative farms, and the reason for milk reduction odds ratio of 3.8 was observed (P > 0.05), which indicates that there is a 3.8 times higher risk of milk reduction due to diseases like abortion as compared to other factors like low quantity and quality of feed and low genetic potential of the dairy animals.

4.20. Seroprevalence and frequent contact between animals

A comparison between the mean values of two proportions was made between the mean values obtained for brucellosis and frequent contact between animals and as a result a respective mean value of 0.26 and 0.38 was observed. The association between the two proportions was measured by a paired t-test statistic ($t = -1.07$), but was not significant ($P = 0.28$). Which indicates that the occurrence of brucellosis not to be associated with frequent contact between animals.

4.21. Epidemiological factors and their variability

Analysis of Variance (ANOVA) was computed in order to assess the variability of various epidemiological factors, presumed to contribute for the prevalence of bovine brucellosis and the comparison was made along the six zones. Variables like farm size, total herd size, lactating cows, dry cows, heifer, calf, bull, and others the mean value for such variables compared was tested with respective ($P < 0.001$) indicating that there is highly significant difference in the proportions of variables mentioned above among the six zones of Addis Ababa.

4.22. Seroprevalence and the number of lactating cows, dry cows, heifers, calves, bull, total herd size and average milk yield

Mean values of the above mentioned variables were compared with seroprevalence cases, and the number of lactating cows was compared with seroprevalence, using independent t-test. The result showed that there is no significant difference between seroprevalence and the number of lactating cows ($P = 0.40$). Negative t-value (-1.23) indicates higher prevalence rate observed in farms with larger number of lactating cows as compared to farms with a smaller number of lactating cows.

Similarly, the number of dry cows and seroprevalence cases were compared, by using independent t-test. The independent t-test showed that there is significant difference between these variables (P = 0.05). Negative t = -1.9 indicates that higher prevalence rate observed in farms with larger number of dry cows as compared to farms with a smaller number of dry cows.

The number of heifers and seroprevalence cases were compared using independent t-test. The independent t-test showed that there is significant difference between these variables (P < 0.05). The negative t = -2.38 indicates higher prevalence rate observed in farms with larger number of heifers as compared to farms with a smaller number of heifers.

Seroprevalence and the number of calves were compared by using independent t-test. The independent t-test showed that there is no significant difference between the two above-mentioned variables (P = 0.54). A positive, t = 0.61 indicates that farms with lower seropositive cases had larger number calves as compared to farms with smaller number of calves.

Seroprevalence and the number of bulls were compared, by using independent t-test. The independent t-test showed that there is significant difference between the two variables studied (P < 0.05). The positive t- value (3.85) indicates that farms with lower seropositive cases had larger number of bulls as compared to farms with seronegative cases.

Seroprevalence and total number of herd size were compared, by using independent t-test (P > 0.05). To that effect, the independent t-test showed that there is no significant difference between the two variables (P = 0.22). A negative t-value (-1.23) shows that higher prevalence rate observed in farms with larger number of total herd size as compared to farms with smaller number of total herd size.

Seroprevalence and quantity of average milk yield were compared, by using independent t-test. (P= .082). The independent t-test showed that there is no significant difference between the two variables observed (P = 0.22). The positive t = 2.8 indicates that farms with lower seropositive cases had higher average milk yield as compared to farms with higher seropositive cases.

4.23. Homogeneity of zones with respect to farm size, total herd size, number of lactating cows, bulls, heifers and calves

The mean values of various parametric variables were compared with respect to the six zones. For farm size zones 1, 4, 5 and 6 are grouped into homogeneous subsets, however, zones 2 and 3 each were assigned in independent groups (P > 0.05). In terms of total herd size zones 1 and 4 were homogeneous, however zone 4 was again assigned in another group with zone 2, and again zone 2 was assigned in another group with zone 3 and 5, however zone 6 was assigned in independent group (P > 0.05). In terms of the herd composition of lactating cows, zone 1 and 4 were grouped into homogeneous subsets (P > 0.05), on the other hand zones 2, 3 and 5 were also assigned into another homogeneous group (P = 0.27), nevertheless zone 6 had the largest number of lactating cows (P > 0.05).

Pertaining to the mean value for dry cows zones 1, 3, 4 and 5 were assigned into a given homogeneous subsets (P = 0.45). Zone 2, 4 and 5 were also assigned in the same group though zone 4 was assigned twice in two groups (P = 0.77). Zone six is representing the largest herd size as well has the largest number of dry cows and was assigned in distinct group.

For heifers that are considered (the most replacement herds) zones 1, 2, and 4 were assigned in the same group (P = 0.10) though zone 2 was assigned twice. Zone 2, 3, 5, and 6 were assigned

in another group (P = 0.10). For the mean values of the number of calves in the sixth zones 1, 2, 4 and 5 were assigned in the same group (P = 0.082). However, zone 3 and 6 were assigned each in two different groups with a respective (P>0.05). The maximum and minimum values of the different variables in the six zones is summarized and presented in the (Table. 12).

Table 12. Maximum and minimum mean values of herd size and some epidemiological factors across the six zones of Addis Ababa

Frequency					
S/No	Variables	Maximum value		Minimum value	
		Zone	Mean	Zone	Mean
1	Farm size	2	21.4	1	0.458
2	Abortion	2	2.67	1	1
3	Lactating cows	2	22.8	6	4.6
4	Dry cows	2	11.8	5	1.857
5	Heifer	2	13.8	4	3.00
6	Calf	6	13.8	1	1.4
7	Bull	2	0.4	1	0.000*
8	Total herd size	2	55.8**	1	10.60

Key = **Max, *Min value

5. Discussion

In countries like Ethiopia, where there is a large cattle population, the prevalence of zoonotic diseases such as brucellosis should be investigated both in humans and in reservoir animals. Though the incidence of human brucellosis in Ethiopia is unknown, a bacteriologically proven case of brucellosis had been reported by (Ephrem, 1981). Besides Teshale (1982) reported about a veterinarian from Mekele and also found out other seropositive cases.

Sixteen individuals in this study had brucellosis diagnosed on the basis of their occupation, significant agglutinin titers, and the fact that brucellosis is endemic in the cattle population. There is a large amount of cattle population incoming and raised in Addis Ababa, to satisfy the highly needed livestock products mainly milk and meat. Following this demand, there are more than 500 private dairy farms and some slaughterhouses but these farms cannot fully satisfy these need. This is due to a lack of adequate health service, poor management practices and other factors. This leads to the free spread and transmission of the disease to humans and dairy animals.

Shola Regional Laboratory is a branch of Region 14 Agricultural bureau, a governmental institution, entitled to make a routine surveillance of the common diseases including mastitis, brucellosis, and endoparasitic diseases on dairy animals in various dairy farms. They usually undertake serological tests like RBPT and California Mastitis Test (CMT) to detect brucellosis and mastitis respectively.

So far a number of mastitis cases have been found. However, according to a casebook study only a single seroreactive animal with abortion history was associated with brucellosis even if several

cases of abortion are being reported from various farms. Such picture about the status of the disease in Addis Ababa could be due to the use of expired antigen.

In this study the only kind of production systems are intensive and semi intensive, which are characterized by a relatively large/medium size of landholding, the use of improved inputs like forages, agricultural by products, better veterinary services including vaccination for common diseases like black leg and pasteurellosis and the size of these farms were small, medium and large. Such intensive dairy production systems of the tropics, report an incidence of infection of up to 80%, but the Sahel extensive animal production systems reported an average disease incidence of 25-30% was reported (Seifert 1990).

Among a total of 336 occupationally exposed individuals initially screened by RBPT, all positive cases were subjected to 2- MET that is a confirmatory test. An over all prevalence rate of 4.8 % was observed in occupationally exposed individuals including slaughter men and dairymen but no seropositive case was found in veterinary clinicians. The proportion of infected slaughter men was higher as compared to infection rate observed in dairymen. Endrias (1989) reported a seroprevalence of 8% in dairymen in Abernossa ranch, a relatively similar prevalence as compared to the present study, though his study was limited to farm attendants. On the other hand, in a study carried out in Saudi Arabia different results were reported by (Young, 1983 and Mousa, *et al* 1988) however the sample size taken and the study area were not similar in which a higher seroprevalence (46.7%) was observed as compared to the present study.

According to the present study, subjects found to be positive were those with complaints of active / chronic disease or those with sub acute cases, as shown by their clinical history. The same individuals were tested for fear of cross reactivity with Salmonellae species and checked

for ‘H’ and ‘O’ antigens. A total of 9 individuals (2 for ‘H’ and 7 for ‘O’ antigens) were observed to be seroreactive to salmonellosis and this could be due to co-infection as the individuals are already exposed occupationally. In order to check whether the individuals were acutely, sub acutely, or chronically sick a dipstick assay was performed (Smith *et al*., 1999). All the sera checked were negative indicating that they are not acutely sick while the seropositive individuals represent sub acute and chronic cases. This result is backed up by the fact that dipstick assay having the ability to detect IgM antibodies, which also helps to discriminate patients in the early phase of the disease and those with chronic brucellosis (Smith, *et al*., 1999; Buchnan, *et al* 1980 and Young, 1991).

Out of the 336 individuals tested, 35 were found to be RBPT positive and 16 of them were confirmed to be positive by 2-MET. However, two individuals were found to be RBPT negative but positive to 2-MET. Sensitivity (88.8%) and specificity (94.3%) RBPT were shown to be good and NPV and efficiency of this test was also found to be high (Smith, 1999). As indicated by K- value a moderate agreement was observed between RBPT and 2-MET positive results, this actually goes in line with the fact that both tests detect IgG though RBPT detects IgM in addition. Thus, it is accepted to find more or less similar results in these tests than other serum agglutination tests (Staak, 1990).

It was observed that seropositivity and age to have less association as indicated by OR = 1.05, (P = 0.145). However, this small OR shows that, the older the age of individual workers the more the exposure for brucellosis. This fact was also indicated by the result observed to study the association between seropositivity and work experience in which most seropositive individuals were the ones with a long-time work experience (Suliman, 1995)

A Chi-square value computed showed that there is a significant difference between the proportions of seropositivity and the sex of individuals (P = 0.01), also OR=3.05, (P = 0.275) showed that most seropositive individuals were males; this is due to the culture of male individuals to be more involved in energy intensive works like slaughtering as compared to females. 90% of the seropositive individuals were males indicating that, males are the most occupationally exposed individuals working in slaughterhouses and dairy farms in Addis Ababa. Slaughtering is a common practice of male individuals in Addis Ababa and other cities and also rural areas in Ethiopia, the same is true for dairying in Addis Ababa. However, this is not the case in rural areas in which females are more involved in dairying. Though, this result is similar with those of Buchanan, *et al*., (1976), Endrias, (1989) and Spink, (1956) a different result was observed in the study carried out in Saudi Arabia where females were the ones culturally and socially involved in caring for domestic animals (Yong, 1983 and Mousa, *et al*., 1988). This is in agreement with fact that, the disease affects primarily men and individuals who are usually involved in animal husbandry (Champys, 1950; Kelly, *et al.,* 1960; Schaller and Kus, 1972). The occurrence of disease principally in men between the ages of 20 and 50 years reflects the occupational hazard to persons in the meat processing industry (WHO, 1971).

A Chi-square value computed showed that there is no significant difference between the proportions of seropositivity and consumption of raw milk (P = 0.58). Among ten positive individuals 2 (20%) were found to be seropositive such proportion of brucellosis-infected individuals who ingest raw milk cannot be under estimated, besides, OR = 0.25, (P = 0.086) indicates that raw milk consumption to be protective against brucellosis. However, this is not the case in reality, since it is known that infected cows excrete a large amount of brucellae along with the milk. Thus, individuals who consume unpasturized milk from such infected cows and

those associated closely with infected calving cows are more susceptible to be infected (Champys, 1950; Kelly, *et al.,* 1960; Schaller and Kus, 1972). The findings of this result were not also consistent with those reported by (Abay, 2000; Suliman, 1995; Yilkal, 1998), who showed that individuals who are directly or indirectly exposed to unpasteurized milk and milk products had higher risk of infection.

A Chi-square value computed showed that there is no significant difference between the proportions of seropositivity and raw meat consumption (P = 0.53), however, 6(60%) proportion of seropositive individuals were notified to consume raw meat out of the ten, which is a higher proportion, this variable is quite big enough to be significant for identification of epidemiological factor, and also OR = 0.24, (P = 0.086) indicates that raw meat consumption to be protective against brucellosis. However, this is not the case in reality, since there is substantial amount of brucellae in infected animals meat and also the associated appreciation of raw meat consumption by most workers in this study. Such low association between the two factors may be due to a smaller sample size or false history. In Ethiopia raw meat consumption is a well-appreciated habit by most people. During slaughtering there is a tendency of consuming raw meat and eating raw liver is also a habitual exercise. Teklu (1999) indicated that most cows coming to the Addis Ababa Abattoir for slaughtering are old aged, and with a reproductive failure. It is known that brucellosis is among a major disease of cattle, which brings milk reduction and reproductive failure. Thus, consumption of raw meat from either infected cows or oxen could be one of the reasons as hepatosplenomegally observed in acutely or untreated brucella infected cattle. This fact is the same to the behavior of pastoral cattle owners who usually send female animals for slaughter only if they are only of no further use regarding reproduction (Yong 1983; Al-Fraihi *et al.,* 1986). According to study in Sudan, Nicoletti, (1980),

concluded that 9.4% female Dinka cattle have been slaughtered because of infertility caused by brucellosis. There is no report about habit of raw meat consumption in other countries; however, transmission of the disease from infected animals through abraded skin or wound during slaughtering is reported (Buchnan, *et al*., 1980).

A Chi-square value computed showed that there is a significant difference between the proportions of seropositivity and individuals who unsafely handled infected materials like aborted fetus, retained placenta, and offal from slaughter houses (P = 0.01), also OR indicated that there is a 3.4 times higher risk of brucellosis for individuals who unsafely handling of such infected materials for every seropositive case at (P<0.05). This factor is assumed to be one of a major factor responsible to the disease transmission, which could be either due to a lack of awareness about the disease or the traditional practice of slaughtering or dairying (Mousa *et al*, 1988). In humans organisms could also be acquired through direct contact with infected materials. Within the United States, 90% of brucellosis is now due to contact with infected materials rather than to ingestion of contaminated fresh milk and milk products (WHO, 1971).

A Chi-square value computed showed that there is a significant difference between the proportions of seropositivity and diaphoresis and myalgia with a similar P-value (P = 0.011). Only 10% of seropositive individuals had these complaints. Though, there is no statistically significant difference, it seems as if such complaints have lesser association with seropositivity. However, for complaints like malaria, typhoid, and consumption of raw milk and use of detergents after work was observed which is statistically a non-significant value (P = 0.58). For these cases the same proportion of 20% was observed. These 20% proportion of such brucellosis-infected individuals indicated that they had been mis-diagnosed as either malaria or typhoid with subsequent mistreatment. This aspect should not be under estimated (Ephrem,

1981; Teshale 1982). This fact is also explained by (Champneys, 1950), who showed brucellosis to be a disease that mimics various febrile conditions, which can easily be missed, particularly in those areas where its prevalence is unknown and diagnostic facilities are lacking. The long and continuous disability caused by this infection, which then results in losing one's job, is not only a matter of health importance but it is also of an economic importance.

Pertaining to the use of detergents after work, only 20% of individuals were using detergents. This value is statistically non significant that most infected individuals were not in a position to use detergents after work which could either be due to lack of awareness or low economic status of the farms or slaughter houses. For variables like fever, sweating back pain, and nervous disorder, insomnia and headache a non-significant value was observed (P = 0.21). But a substancial proportion of 30% of seropositive cases had such complaints. Though, there is no statistical significance it is not simply possible to overlook the misleading effect of such generalized and neuropsychiatric signs and symptoms. In human brucellosis complaints of such non-specific symptoms like fever, sweats, fatigue, anorexia, and muscle or joint aches and neuropsyschiatric symptoms, notably depression, headache, and irritability occur frequently (Crosby *et al*., 1984).

For variables like occurrence of GITI, and prompt treatment of sores (P = 0.527) was observed, which is statistically non significant. The proportion of respective variables in the infected individuals is 40%. Though, the difference indicated by P-value is not significant, such proportion of variables is quite big enough to be significant in epidemiological studies (Yong 1983; Al-Fraihi *et al.,* 1986).

For the factor i.e. prompt treatment of sores by the workers, which can occur due to finger cuts or other reasons, it was found to be statistically non- significant. Though, highest proportion of seropositive individuals, i.e. 60%, had a practice of treating sores promptly. The infected individuals better manage this factor, as compared to other predisposing factors. However, it is difficult to exclude the possibility that the rest 40% of infected individuals, were not exposed due to untreated wounds or sores. It has been shown in this study that the slaughter men encounter finger cuts at least 5-8 times during their stay in the work, such traditional and manual system of slaughtering is considered to be one of the most important predisposing factors (Alausa, 1980).

Similarly, for infected individuals encountering abortion cases around their locality or farm (P = 0.41) was observed. This was not statistically significant value, however, among the four individuals asked, i.e. dairy men, nearly two, i.e. 50%, had encountered cattle abortion cases, but the rest of six individuals were slaughter men with less access of abortion cases (Dajani *et al*., 1989)

In general with the exception of few complaints most results were not significant (P>0.05). Though, the proportions observed were not small for medical aspect, such non-significant result could either be due to a smaller sample size used in this study, incomplete survey under taken or false history.

Pertaining to the seroprevalence of bovine brucellosis, in the earlier times neither proper quarantine and control measures or vaccination program was practiced to combat brucellosis. In general, by taking $RBPT^{+}$ /CFT^{+}, as a Gold standard an apparent prevalence rate of 10% was determined in this study area showing the status of the dairy farms and these farms were declared as moderately infected areas.

The results observed are similar with previous studies by Yilkal (1998) who reported an infection rate of 5.1% and 8.1% in and around Addis Ababa respectively. However, an increase in the seroreactors can be either due to the absence of proper quarantine and control measures, or the low intensity of the study However, as compared to the seroprevalence study of cattle in other regions of Ethiopia by several researchers relatively a comparable result was observed with that of Endrias (1989) in Sidamo Region of Ethiopia found 11.6%, Taye (1991) in the Abernosa Ranch 137/963(14.2%), Bayleyegn (1989) out of 226 animals in Gobe ranch showed 16.81%, Abeje (1994) reported a crude seroprevalence rate of 16.9% in and around Bahir Dar and Abay (2000) reported 3.6 % prevalence in Yekatit dairy farm that is found in Arsi, however, Muktar (1993) found a higher seroprevalence rate of 38.7% (57/147) at Bako Research Center, Western Ethiopia.

Agreement between RBPT, SAT and CFT were indicated to be significantly different ($P<0.05$). The agreement between positive and negative results for RBPT against SAT and CFT was K = 0.265, and K = 0.129, i.e. 26.5% and 12.9% agreement. The agreement between RBPT and SAT is said to have moderate agreement, however the agreement between RBPT and CFT is a fair agreement (Morgan *et al* (1967); Thrusfield, (1995). This percentage agreement and kappa statistic for the pair wise comparison of the above-mentioned tests is not similar with that observed by Abay (2000) where RBPT and CFT had almost perfect agreement K=0.98. However, Yilkal (1998) reported a moderate agreement K=0.44 between $RBPT^+$ and CFT^+. The higher agreement observed between RBPT and CFT in this study is in line with the fact that both tests detects mainly IgG antibodies as compared to that of SAT which detects the phenol saline sensitive IgM agglutinating antibody such test agreement was also shown in studies by (Yilkal, 1998, Abay, 2000).

The relative sensitivity and specificity of RBPT was 100% and 68.46% respectively. This result is similar in terms of its sensitivity as compared to that reported by Abay (2000) that is 100% and it is also comparable with the results reported by Nielson and Duncan (1990) and Yilkal (1998) in terms of sensitivity. However, less similarity was observed in its specificity as compared to those reported by Stemshome *et al.,* (1985); Abay (2000)

The relative sensitivity and specificity rate of SAT were 63.3%, and 89.2% and this result is not consistent with findings of (Nielson and Duncan, 1990) and also it was not also consistent to that reported by Dahoo, *et al.,* (1986) i.e. 98.3% sensitivity and 99.5% specificity. Such variations in test results among reports of SAT and the finding of this study suggests that the incidence and the magnitude of the known limitations of SAT (vaccination status of, type of vaccine, stage of infection, and local conditions, etc) might be different or a given local condition (Radostitis *et al.*, 1994). The positive predictive value (20%) and negative predictive value (100%) and (70%) efficiency of RBPT was lower as compared to that reported by Abay (1999). For SAT positive predictive value of (48.71%) and negative predictive value of (93.75%) and (82.51%) efficiency showed that, it has a better positive predictive value and efficiency, but less negative predictive value meaning has more false positive result.

Association between the proportions of brucellosis and abortion was indicated by 26% and 44.7% mean value, which indicated that there is no significant difference between the two mean values ($P > 0.05$). These findings do not correspond with that of previous reports (Hellman, 1984; Abeje, 1994; Yilkal, 1998). However, this is not the case in reality since most of abortion cases observed during this study were found to occur at trimester gestation period, and cows were also with a history of infertility problems such as, retained placenta, prolonged calving

interval and subsequent milk yield reduction. Moreover, seropositivity of the animals showed the higher association of brucellosis and abortion, however, the less association observed between the two factors could be due to poor recording system about the history of cows as a result of frequent release of farm attendants from their work or other reasons (Nicoletti, 1980). The direct loss of meat as result of abortion, infertility and weight loss in infected herds of cattle was estimated to be 15% thus it is difficult to rule out the possibility of higher association between the two variables (Thimm, 1982)

Association between seroprevalence 26.3% and frequent contact between animals 38.2% was indicated to be not significant ($P > 0.05$). This value indicates that there is a lower probability by which frequent contact between animals corresponds to the occurrence of brucellosis. It is only for some semi-intensive farms, which have inadequate space, that there is a frequent contact between animals. However, most of the farms have adequate land holding size and are also well fenced, thus less frequency of contact within the animals in the same farm or from other farms attributes to the less effect of this epidemiological factor but this findings are not in agreement with Yilkal (1998), in which intensive dairy farms due to frequent contact between animals enhance the exposure potential especially following an abortion through increased contact and common feeding, which promotes brucellosis transmission.

Odds ratio of 2 was observed between seropositivity and knowledge about brucellosis but this value was not significant ($P > 0.05$). However, this result showed that there is a 2 times higher risk of seropositivity in farms with a lack of knowledge about brucellosis as compared to those with knowledge about brucellosis. Besides, odds ratio of 0.862 was observed for seropositivity and parturition pen but this value was not significant ($P > 0.05$). This result shows that seropositivity to be favored by separate parturition pen. This is not in line with the fact that

separate parturition pen to be one of a preventative method to avoid or minimize a within herd infection or cross contamination rate. Odds ratio of 1.36 was observed between seropositivity and milk reduction ($P < 0.05$), this was significant. This indicates that seropositive farms to have a relatively higher risk of milk reduction as compared to seronegative farms, and reason for milk reduction ($P>0.05$). Higher risk of milk reduction due to diseases like brucellosis as compared to other factors like low quantity and quality of feed and/or low genetic potential of the dairy animals was observed ($P > 0.05$), this was similar with the studies of Kellar *et al*, (1976) and Nicoletti, (1980). The economic significance of reduction in milk yield was indicated to be 20% per infected cow (Thimm, 1982; Yilkal, 1998).

Significant variability among variables like farm size, the number of lactating cows, dry cows, heifer, calf, bull, and total herd size was observed ($P < 0.05$). This result showed that there is diversity in the general set up of the farms in terms of the above-mentioned variables, which contributes to the variability in brucellosis predisposing factors, which could emanate from different economic status of farm owners, different production and managemental system of the farms this also makes implementation of various control or eradication systems difficult (Nicoletti, 1980).

For the different zones test of homogeneity was computed for some variables like farm size, and herd size and other variables. Different zones were assigned into homogeneous subsets for some variables and others were not aligned to any of the groups showing high variability ($P > 0.05$). However, this result is not in line with the results of ANOVA, which shows a significant variability among the zones in the variables. Mean values of the above mentioned variables were compared with seroprevalence cases, and the numbers of lactating cows were also compared with seroprevalence, using independent t-test. The result showed that there is no significant

difference between seroprevalence and the number of lactating cows (P = 0.40). Negative t-value indicates higher prevalence rate observed in farms with larger number of lactating cows as compared to farms with a smaller number of lactating cows.

The difference between seroprevalence and the number of lactating cows was found to be not significant (P > 0.05). A negative (t- 0.837) indicates that farms, which have a larger number of lactating cows, are those with higher seropositive cases and the same was true for the number of dry cows, and number of heifers with a respective negative (t-0.839) and (t 0.001), (P < 0.05). However, for the, number of bulls there was a significant difference between seropositive and seronegative cases (P < 0.05). These results showed that farms with larger number of bulls are those with seronegative cases, the reason behind is that big farms that follow intensive system of production mostly rely on A.I than Natural service by bulls for breeding purpose. Pertaining to the number of calves no significant difference was observed between seropositive and seronegative cases (P>0.05). However, a positive t- value indicates that farms with seropositive cases to have a relatively similar number of calves, even if the seropositive farms have a higher number of both lactating cows and heifers that are sexually matured animals. These shows the possible economic loss incurred by the farms due to abortion by brucellosis that resulted in unexpected similar number of calves as compared to farms with a lower number of sexually matured animals (Rankin, 1965).

In general there is no significant difference observed in the total number of herd size (P > 0.05) between seropositive and seronegative farms. A negative t-value indicates that farms with a larger herd size are those with seropositive cases. Large herd size dairy farms are often maintained by the replacement of cattle from outside source, these animals may have originated from multiple sources increasing the probability of introducing infected cattle. Thus, as the herd

size increases management problems in controlling brucellosis and other related livestock diseases also increase. This is consistent with findings of Abay (1999) and Kellar, *et al*., (1976) that indicate dairy farms with more than 100 animals had more infection rate of brucellosis than farmers that keep less than 50 animals (Nicoletti, 1980).

No significant difference was observed in average milk yield between seropositive and seronegative cases ($P > 0.05$). However, a positive t-value indicates that farms with seropositive cases have higher quantity of average milk yield as compared to farms with seronegative cases. This is not to mean that seropositivity increases milk yield, but the result shows that as it is already observed that seropositive farms were with larger herd size, the pooled milk yield of the cows is higher as compared to the seronegative farms. Though, the milk yield was not as expected for cross breeds usually less than 12/ litre/ day. Thus, the economic loss incurred as a result of brucellosis is high (Alausa, 1980).

6. Conclusion and Recommendations

A (16/336) 4.8% human and (57/552) 10 % dairy cattle seroprevalence of brucellosis observed in Addis Ababa in this study is an indicative of the presence of previously unknown human brucellosis in Ethiopia. However, the extent of the prevalence of the disease in the countryside is expected to be more than the lower human brucellosis observed in Addis Ababa. This is due to the relatively smaller sample size considered in this study as compared to studies in other countries, and the different socio-economic set up as compared to that found in rural areas. The less association observed between the seroprevalence and epidemiological factors and moderate seroprevalence observed in this study seems to be due to the cattle rearing system that is intensive and semi-intensive, the smaller, number of cattle population, and individuals employed in this economic sector, as compared to the extensive system of production and larger number of cattle expected to be found in country sides.

Brucellosis should be considered as one of the most important zoonotic disease which necessities keen attention. Especially, in cases of fever of unknown origin, physicians must investigate it as a differential diagnosis. There is a need for adaptation of highly sensitive and specific serodiagnostic methods as this disease is usually misdiagnosed and as its endemicity in cattle population is already established.

In Ethiopia cattle rearing system still remains unscientific and traditional, thus it is expected that the status of the disease both in humans and reservoir animals is expected to be more than the existing information. It is even difficult to control the disease without having good information about the disease in reservoir animals. As far as the management of cattle in traditionally held stock continues as in its present manner without formulating quarantine, controls and/ or

preventative measures, the spread of the disease covering a wide range of area is inevitable and will result in, significantly, high health damage and economic loss.

If the management and sanitary practices in the intensive and semi-intensive holdings is not improved the spread of the disease among housed herd will attribute to the public health problem and additional economic loss. On the other hand, poor milking hygiene, poor sanitary conditions in the abattoir could be considered as major reasons for the maintenance and spread of the disease.

Recommendation

Proper disposal of fetuses and fetal membranes and offal from slaughterhouses, also personal hygiene of the workers through the use of detergents, safe handling of potentially infected materials, prompt sore treatment and creation of awareness among workers, if possible wearing of protective cloths is an important measure for the prevention of brucellosis the use of cooked meat and milk should also be given due attention. Animals from infected farms must be slaughtered separately in the Abattoirs with much care to discontinue the existence of the pathogen and as well its possible transmission to occupationally exposed groups.

Milking should be in separate tanks and by separate personnel with the proper sanitary care from infected and non-infected animals. Managemental practices should include keeping the newly introduced animals in an isolation pen, and an establishment of parturition pen to avoid further cross contamination among non-infected animals is also mandatory.

Routine screening of animals for brucellosis is very important in that it helps to detect positive cases as early as possible so as to reduce the risk of contamination to other animals within the

herd in the farm and take proper measures on time. Routine screening tests should be introduced in health institutions, since human animal/products contact is high.

- In the infected farms owners should be advised so that milk and milk products from such farms have to pass through heat treatment (pasteurization) before consumption and carcass from clinically, pathogenically and serologically positive animals must be boiled, stewed and roasted before consumption.
- Compulsory strain 19 vaccination of heifer calves between 4 to 8 months of age and adult vaccination with a smaller dose of strain 19 vaccine or killed vaccine such as 45/20 has to be practiced in animals under intensive and semi intensive management systems.
- Health education and publicity campaigns are necessary parts of control programs and all accessible means of public information should be utilized. Besides teaching about the nature of the disease, public health importance and its economic loss the need of self-contained herds and the danger of buying cows in open market must be stressed as well.
- Formulation of legislation concerning testing of animals and animal products, which are both imported and exported, as well as on movement of animals within the country is a necessary measure to control the disease.

In general, it is difficult to create brucella free animals in Addis Ababa or a given locality thus country wide implementation of quarantine and preventative policies shall be exercised soon, as control of brucellosis in humans is largely a matter of disease control in reservoir animals.

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Declaration

I, the undersigned, declare that the information provided in this work is an original work, and that it has not been presented in other Universities, colleges or institutions, seeking for a similar degree or other purpose.

Jiksa Kassahun Sahilu _____ _____

Name Date Signature

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8. APPENDICES

(**Appendix-1**). List of farms and number of animals tested

S/No	Farm name	Address				No of animals
		Zone	Wereda	Kebele	H/no	
1	Mohamed	3	17	-	-	2
2	Girma	1	4	39	262	4
3	Tesfaye	1	4	29	677	11
4	K/selasse	6	27	-	-	9
5	Tilahun	2	24	16	1301	23
6	Markos	4	11	17	909	7
7	Assefa	3	-	-	-	2
8	Ethegenet	6	27	11	-	21
9	Getachew	5	17	25	-	1
10	Silesh	1	-	-	-	5
11	Berta	5	25	01	1417	24
12	Mereta	4	11	03	480	2
13	Wubalem	3	28	03	-	10
14	Repi	2	28	P/A	79	28
15	Stella	2	24	16	1837	108
16	Abrham	1	06	25	005	6
17	Geleta	5	8	02	548	4
18	Worknesh	4	13	15	767	13

S/no	Farm name	Adress				No of animals tested
		Zone	Wereda	Kebele	H/no	
19	Adugna	3	19	60	445	7
20	Alula	6	27	11	-	33
21	G/Fanta	2	24	15	930	26
22	Keirga	5	10	22	218	7
23	Likyelesh	5	8	10	541	9
24	Hamid	5	-	-	-	38
25	Almaz	4	13	03	1900	7
26	Kelemuwa	3	28	2	1792	14
27	Alula	6	27	11	-	24
28	Askale	2	23	16	1853	11
29	Gelaw	2	23	16	393	1
30	Yishak	4	9	14	846	11
31	Selam	3	28	1	1039	30
32	Hailu	5	8	06	523	7
33	Zeritu	5	71	28	520	1

(Appendix-2) List of farms, vet. Clinics, and abattoir house and the number of humans tested

Sno	Sampling site/Farm	Address				No of humans
		Zone	Wereda	Kebele	H/no	
1	Gossaye	1	3	47	643	1
2	Selam	3	28	1	1039	9
3	Akaka Bolele	6	-	-	-	14
4	Berta	5	25	01	1417	9
5	Repi	2	28	P/A	79	5
6	Zeritu	5	71	28	520	2
7	Hailu	5	08	6	523	7
8	Nigist	4	13	06	282	3
9	Taffesech	4	6	13	949	3
10	Yonas	3	-	-	-	1
12	Addis Abatoir Enterprize	2				262
14	Vet.clinic	5				2
15	Vet.clinic	3				8
16	Others	In farms, clinics,Lab.				8

(Appendix-3) Questionnaire format

Date----------

Name----------Sex----------Age-------Region----------Zone------

Wereda-------Kebele/P/A---------H/no-------Occupation----------

Occurrence of brucellosis in humans

1.The number of milker /slaughter men/herdsmen/veterinary clininicians in the farm/clinic-----

2.For how long have you been working in this slaughterhouse /farm /clinic ----------Has any of you shown symptoms of prolonged fever since starting keeping dairy cattle/ working in abattoir or farm.

1. Yes----------2.No----------. If yes, for how long ----------.

3.Have any of you visited a health institution in the last six months?

1.Yes----------2.No----------. If yes, health problems include----------.-

4.Has any of you shown the following generalized sign and symptoms since starting keeping dairy cattle or working in abattoir or in the farm ?

1.Headache 1.Yes ----------2.No----------.

2.Weight loss 1.yes----------2. No----------.

3.Conistipation 1.yes--------2. No----------.

4.Dry cough (pneumonia) 1.yes--------2. No----------.

5.Insomina 1.Yes------- 2.No----------.

6. Fatigue 1.Yes----------2.No----------.

5.Differencial symptoms

1.Undulant fever 1.Yes ----------2.No----------.

2.Joints and back pain 1.Yes----------2.No----------.

3.Pain on testes 1.Yes----------2.No----------.

4. Chills 1.Yes----------2.No----------.

5.Diaphoresis 1.Yes----------2.No----------.

6.Myalgia 1.Yes----------2.No----------.

7.Sore throat 1.Yes----------2.No----------.

8.Sweats with no coughing 1.Yes----------2.No----------.

9.Nervous disorders 1.Yes----------2.No----------.

6 Have you ever consumed raw milk? 1.Yes----------2.No----------..If yes, for how long? ----------Years/month.

If yes, what problem did you encounter in the past? ----------.

7.Have you observed abortion 1.Yes----------2.No----------.

Sterility 1.Yes----------2.No----------. Infertility 1.Yes----------2.No----------in your locality or farm ever before, while working associated with cattle?

8. Have you had any close association with cattle during parturition or slaughtering?

.

1.Yes----------2.No----------.If yes explain----------.

9. Have you ever consumed raw meat? 1. Yes----------2.No----------.

10. How do you handle cutoffs/offal during and after slaughtering/aborted fetuses? ----------
.

11. Do you wash your hands with detergents after slaughtering and /or assisting parturition?

12. Do you treat sores and openings on the skin promptly while damaged during slaughtering or after assisting dystocia cases? 1. Yes----------2. No----------.If yes, explain
.

13. Social factors

What was the last school you attended?

1. Elementary 1.Yes----------2.No----------.

2. Junior high school 1.Yes----------2.No----------.

3. College/University 1.Yes----------2.No----------.

14. Economic factors

What are your reasons for working in this abattoir /farm and what amount of money do you get per month /day? ----------.

Do you have a family to support 1.Yes----------2.No----------.

If yes how many children Male----------Female---------- Total----------Adult relatives-------.

.

(Appendix-4)

Brucellosis in dairy cattle

Name of the dairy farm /Farm owner ----------.

Sex----------Age ----------Occupation----------.

Region ---------- Zone------Wereda------- Kebele (Village) ---------- House Number ----------

1.Year dairy farming started---------- E.C.

2.Is the farm fenced? Yes----------No----------.

3. Is there frequent contact between your animals with other herds? Yes----------No----------

4. Breed of dairy animals

1. Zebu 1.Yes----------2.No----------.

2. Cross breed75% or greater 1.Yes--------2.No------25%-50%1.Yes-------2.No----------3.Pure exotic 1.Yes----------2.No----------

5. What type of insemination do you use for your animals?

1. Artificial insemination 1.Yes----------2.No----------.

2. Natural service 1.Yes----------2.No----------.

3. Both methods are used 1.Yes----------2.No----------.

If natural services have you observed sterility in your breeding bulls (entire) animals. Yes----------No----. If yes, what measures have you taken What symptoms had the animals shown?

6. Are there separate parturition pens? Yes----------No----------

.

.

7.What do you do to the calving pen after the cow and calf left? ----------

8.Herd size

1. Lact.cows ----------.
2. Dry cows ----------.
3. Heifer----------.
4. Calf (T)----------M---------F----------.
5. Bull----------.
6. Others----------.

9. How much milk /liter/day/Lact.cows do you get, on average? ----------.

10. Have you observed milk reduction? Yes----------No----------.

11. What diseases are commonly occurring in your herds?

1. Mastitis 1.Yes----------2.No----------.
2. C.B.P.P. 1. Yes----------2.No----------.
3. Rinderpest 1.Yes----------2.No----------.
4. Brucellosis 1.Yes----------2.No----------.
5. Tuberculosis 1.Yes----------2.No----------.
6. Endoparasites 1.Yes----------2.No----------.
7. Ectoparasites 1.Yes----------2.No----------.
8. Foot and mouth disease 1.Yes----------2.No----------.

12. Is there any abortion in your herd /flock? Yes----------No----------.

If yes, how frequent was it? And at what month of the gestation period ---------- month.

13. How did you handle the aborted fetuses and /or afterbirth?

1. Buried 1.Yes----------2.No----------.

2. Burned 1.Yes----------2.No----------.

.

3. Left simply 1.Yes----------2.No----------.

4. Others 1.Yes----------2.No----------.

14. How many calves /replacement herds have you lost so far? ----------

1. Yes----------2.No----------.

Is hair clipped from the hair? 1. Yes----------2.No----------.

15. What are your reasons for producing milk?

1. To earn a living 1Yes----------2.No----------.
2. To supplement family food 1.Yes----------2.No----------.
3. To supplement family income 1.Yes----------2.No----------.
4. As a hobby 1.Yes----------2.No----------.
5. Other

Do you have another job? 1. Yes----------2.No----------.