ADDIS ABABA UNIVERSITY
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THE EPIDEMIOLOGY OF BOVINE BRUCELLOSIS IN INTRA- AND PERI-URBAN DAIRY PRODUCTION SYSTEMS IN & AROUND ADDIS ABABA

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A thesis submitted in partial fulfilment for the degree of
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ABBREVIATIONS

ANOVA	Analysis of Variance
BgVV	Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin
BPAT	Buffered Plate Antigen Test
CFT	Complement Fixation Test
CI	Confidence Interval
CV	Coefficient of Variation
DDE	Dairy Development Enterprise
DRDP	Dairy Rehabilitation and Development Project
EDTA	Ethylene Diaminetetraacetic Acid
EIA	Enzyme Immuno Assay
FAO	Food and Agricultural Organization of the United Nations
FHD	Full Hemolytic Dose of complement
GDP	Gross Domestic Product
HIGT	Hemolysis in Gel Test
I-ELISA	*Indirect*-Enzyme Linked ImmunoSorbent Assay
ICFTU	International Complement Fixation Test Units
ISABS	International Standard for Anti-*Brucella* Abortus Serum
ILRI	International Livestock Research Institute
IU	International Unit
MHD	Minimum Hemolytic Dose of complement
MOA	Ministry of Agriculture
MRT	Milk Ring Test
OR	Odds Ratio
PPV	Positive Predictive Value
RBPT	Rose Bengal Plate Test
SAT	Serum Agglutination Test
SD	Standard Deviation
SE	Standard Error
Se	Sensitivity
Sp	Specificity
USDA	United States Department of Agriculture
VBD	Veronal Buffer Diluent
WHO	World Health Organization of the United Nations

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ABSTRACT

Bovine brucellosis is an infectious disease of economic importance by causing abortions and infertility. Bovine brucellosis is considered a zoonosis. Information on the extent of bovine brucellosis, however, is scarce for the expanding intra- and peri- urban dairy enterprise in Ethiopia. The purpose of this study was to investigate the status of bovine brucellosis and its impact on reproduction in some intra- and peri-urban dairy production systems in Ethiopia. A cross-sectional investigation of bovine brucellosis was carried out from June to October 1997 on 42 dairy farms located in Debre Zeit, Kaliti, Sebeta, and Addis Ababa. The farms represent peri-urban systems, intra-urban systems in secondary towns and intra-urban production systems. Bulk milk samples were collected from each farm (herd) in June, August, and October. Blood samples from 950 non-vaccinated breeding animals above six months of age were collected once in June. The Milk Ring Test (MRT), the Rose Bengal Plate Test (RBPT), and the Complement Fixation Test (CFT) were carried out on milk and serum samples. Farm management parameters and zoonotic aspects were collected by administering a questionnaire.

An overall herd infection rate of 33.3% was determined. With respect to the production systems 100%, 30%, and 12.5% of the farms were infected in peri-urban, intra-urban secondary town, and intra-urban systems, respectively. The within herd prevalence ranged from 0-16.7%. At least one or two reactors were identified in 64.3% of the farms. An overall prevalence rate of 8.11% was found on individual animals. A prevalence rate of 9.8% was observed in the age group 2 to 4 years. A prevalence rate of 9.1% and 3.3% was identified in farms that keep more than 100 animals, and less than 50 animals, respectively. Significant difference in prevalence rate was observed in animals aged below and above 2 years and between no parturition and at least one parturition. A significant difference in prevalence rate was also observed between farms that keep below 50 and above 100 animals. Odds ratios (OR) of 2.14 (1.04-4.83) between age and brucellosis infection and 2.99 (1.29-8.06) between herd size and brucellosis infection were determined. Significant differences in prevalence rates were not observed among the production systems and districts in individual animals.

Agreement (kappa values) between RBPT+/CFT+ and MRT tests were 0.31 (first MRT result), 0.43 (first and second MRT results pooled), and 0.58 (first, second and third MRT results pooled). Moderate agreement (k=0.44) was found between RBPT+ and CFT+, and good agreement (k=0.74) was found between RBPT+ and RBPT+/CFT+ testing procedure.

Considering positive results in both RBPT and CFT as gold standard the sensitivity (SE) and specificity (SP) of the pooled MRT were 57.1% and 89.3%, respectively. Sensitivity and specificity of RBPT were 100% and 94.6%, respectively.

Associations between reproductive and production parameters and brucellosis infection could not be established for individual animals. However, on the basis of questionnaire answers, previous abortions and use of natural service were associated with *Brucella* infection; OR of 15.58 (1.72-79.16) and 0.06 (0-0.61) were calculated, respectively. Abortions were recorded in 56.1% of the farms. Lack of knowledge of brucellosis was recorded in 87.8% of the farms. Cleaning and disinfection was done regularly in 29.3%, removal of infected animals was farm practice in 14.6%, provision of maternity pens was found in 4.9% of the farms, and 21.9% of the farms obtained replacement stock from outside source.

Prolonged fever was observed in nine persons interviewed. They were attending cows for at least six months.

Absence of vaccination, herd size, cattle density, lack of knowledge of brucellosis, and poor management and husbandry practices are likely factors responsible to explain the relatively high prevalence rate observed in this study. Due to its impact on production and for its zoonotic significance, awareness of brucellosis in urban farming should be given priority. Conclusions and recommendations in this regard, emphasizing control of brucellosis are given.

1. INTRODUCTION AND OBJECTIVES

Brucellosis is an infectious disease produced by bacterial species of the genus *Brucella*. Infections of domestic animals such as cattle, goats, sheep, equines, camels, pigs, and dogs leads particularly to abortions and infertility; decreases in production cause significant economic losses (Radostits *et al.*, 1994; Sewell *et al.*, 1990; Weidmann, 1991). Man can be infected by contact with diseased animals and their excretions and by ingesting infected animal products.

Brucellosis in cattle, previously called "contagious abortion", has been recognized since ancient times. Brucellosis in cattle is caused by *Brucella abortus* of which nine serovars are recognized (Bisping and Amtsberg, 1988). The first member of the genus *Brucella* was isolated in 1887 in Malta by David Bruce from the spleens of patients who died of Mediterranean fever. The organism was named *Brucella melitensis*. Ten years later a Danish veterinarian, Fredrich Bang, isolated a similar organism from an aborted bovine fetus and named it Bacillus (*Brucella*) *abortus* (Gillesepie and Timoney, 1982).

Cattle in Africa are a source of hard currency through the export of hides, packed meat and other products. Domestic cattle serve as a source of food, energy, fertilizer and cash income. In sub-Saharan Africa, with increasing population growth and a parallel decrease in the availability of the production factor land, demand and supply for food is not always reconciled. People are migrating to cities in search of jobs or looking for better services. Economically-well-to-do people prefer to live in cities than in the country side. These factors still create an ever higher demand for food of animal origin. With, having the limited factor land, to match demand and supply, increasing productivity seems to be the logical step. This implies intensification of animal production.

At the end of the second millennium, the total earth population will reach 5.5 billion inhabitants, of which approximately three quarters are living in developing countries. The FAO estimated in 1983-85 that undernourishment affected 512 million persons in developing market economies, 10% of the earth's total, or 20% of the population living in these countries. The estimates for the amount of undernutrition per region are: Africa 32%, Far East 22%, Latin America 14%, Near East 11% (cited by Zessin, 1996). During the Eighties global food production has increased by 24%, the increase in developing countries being 39%. Among developing countries, the Far East region has performed better with an increase of 47%, the other three regions reaching 27%, with great year-to-year variations in countries of the Near East (cited by Zessin, 1996). However, when calculated per caput, food production remained constant or even has experienced a slight decrease. In developing countries the share of livestock to agricultural production is 51.4%. In

sub-Saharan Africa livestock contributes 18% to the agricultural GDP; still the net trade balance of meat and milk in the developing world is -17.9 and -14.0 million tons (cited by Zessin, 1996). During the same period, livestock production has been increased by 25% worldwide, with a limited increase in developed countries (12%), but a 53% rise in developing countries. As for total food production, animal production has risen faster in the Far East region (+86%) than in the three other regions (+27%) (cited by Zessin, 1996). Again, as with total food production, when calculated per caput, livestock production has stagnated in Africa, Latin America and Near East, while a 54% increase has been observed in the Far East. One major difficulty faced by developing countries is that the relative increase of production has generally been achieved mainly through an increase in animal numbers rather than through a real improvement of performance.

In the year 2000, 45% of the world population will live in cities (39% in developing countries), 60% in 2025 (56.5% in developing countries) and 75-89% in 2100 (cited by Zessin, 1996).

Over the past two decades sub-Saharan Africa experienced relatively low growth rates in production of dairy products compared to the average production level for all developing countries (Mbogoh, 1984). Total consumption of dairy products, however, grew relatively faster during the same period. During the 1970's the population of sub-Saharan Africa grew at a rate of 2.9% per annum. Over the same period dairy production only grew at a rate of about 1.9% per annum, while the consumption of dairy products increased at a rate of 2.1% per annum (Mbogoh, 1984). However, available data suggest that the consumption of goat and sheep milk declined in East Africa between 1963 and 1980 and that of camels milk stagnated. Only the consumption of cows milk increased fairly rapidly in the whole of sub-Saharan Africa.

Africa has 12% of the world's total cattle population but produces only 3% of the world milk output (cited by Doepmann *et al.*, 1997). The World Bank goal of a 4% annual increase in food production requires local milk production in Africa to increase from 8.2. million to 35.6 million tonnes by 2025. This increase will only be achieved if good progress is made in increasing feed supplies, in genetic improvement, in improving technology transfer, and in improving reproductive efficiency and controlling diseases.

Bovine brucellosis is one of the diseases hampering cattle productivity.

According to Nicoleti *et al.* (1984) and Staak (1990) brucellosis is perhaps the most widespread and economically important disease in tropical and sub-tropical regions. The direct loss of meat (as a 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. Human brucellosis, as a geomedical survey has shown, is known to exist in 37 of the 49 (75.5%) African countries (Thimm, 1981). Keeping in mind the large number of animal foci present in Africa one should expect most of its countries to be ridden with brucellosis. Human prevalence rates of 0.6% and 6.4% were reported in Somalia and Uganda, respectively (Chukwu, 1987). Chukwu (1987) also indicated a prevalence rate of 22.6% in adults and 20% in school children in Tanzania. In southern Saudi Arabia, 19.2% of the population showed serological evidence of exposure to brucellosis, and 2.3% had active disease (Suliman, 1995).

Many countries have made considerable progress in limiting the occurrence of brucellosis but in East Africa the disease is still a serious cattle health problem. The sero-prevalence ranges from 1.8%-34.9%. The prevalence in cattle in Djibouti was 4% (Chantl *et al.*, 1994), in Somalia 11.9% (Hussein *et al.*, 1978), in Kenya 10% (Kagumba and Nandoka, 1978), in Rwanda 34.9%

(Akakpo *et al.*, 1978), in Sudan 6.5-22.5% (Hellmann *et al.*, 1984), in Uganda 1.8% (Oloffs, 1994).
The situation in Ethiopia is not much different. The prevalence in the central highlands in zebu cattle was found to be 4.2% (Tekeleye *et al.*, 1989). Prevalence rates of 22% (Tariku, 1994) and as high as 38.7% (Muktar, 1993) were recorded in cross-bred cows.

In Ethiopia a growing tendency for intensification of dairy cattle around major cities is observed. This is particularly evident around Addis Ababa, the capital city. These intensive dairy units constitute urban and peri-urban livestock production systems according to Ethui *et al.* (1995) and Nauheimer *et al.* (1995). The farms contain upgraded Holstein-Zebu crosses. Most of the milk consumed in the major cities comes directly from these farms.

Some of the major health problems recorded in many of the farms in Africa are abortions, infertility and sub-fertility (Chukwu, 1987). Cows usually require more than two services to conceive, and some have remained sterile for years. Very few produce calves regularly at annual intervals. In Nigeria at Potola ranch, calf losses due to abortion were in the order of 29.39% in N'dama breeds (Esuruoso, 1980). Muktar (1993) in a mixed herd of Friesian-zebu crosses and zebu found a 54.2% non-conception rate. In Chaffa state dairy farm, a significantly higher life birth rate and milk yield per breeding cow/day was observed in brucellosis-free groups when compared with brucellosis-positive cohorts (Tariku, 1994).

As the disease is hardly spectacular in its chronic stage and indeed, despite the losses and yield decreases it causes, often goes undetected (in contrast, for example, to rinderpest), its negative effects on the profitability of cattle production are extremely underestimated, particularly in tropical areas (Weidmann, 1991).

Information on the status of brucellosis and its impact on production and reproduction in intra- and peri-urban dairy production systems in improved cattle is scant and incomplete. The objectives of this study, therefore, were:

- to establish the prevalence of brucellosis in intra- and peri-urban dairy production systems in and around Addis Ababa, Ethiopia.
- to estimate the effect of the level of intensification on brucellosis prevalence.
- to investigate agent-host-environment-and management risk factors.
- to asses the effect of brucellosis on reproductive wastage.
- to evaluate agreement among serological tests used, and
- to gain an overview of the presence of the disease in humans.

The study is a component of ILRI's project on: *Impact of diseases of intensification and reproductive wastage on the efficiency of African peri-urban milk production systems.* The objectives of which are to:

- assess the importance of diseases of intensification in peri-urban dairy farms versus major epidemic diseases.
- develop methodologies and models to investigate the biological and economic impact of these constraints to peri-urban milk production.
- study the epidemiology of and risk factors associated with diseases of intensification.
- evaluate cost and benefits of alternative control measures for diseases of intensification.

The study was carried out under the auspices of the International Livestock Research Institute (ILRI). It was conducted as a requirement for the partial fullfilment for a Master's of Science degree in Veterinary Epidemiology under the joint postgraduate program between the Freie Universität Berlin (Germany) and the Addis Ababa University (Ethiopia).

2. LITERATURE REVIEW

2.1. Prevalence

2.1.1. Prevalence of bovine brucellosis in East Africa

Brucellosis is a prevalent disease in many countries of Africa. Chukwu (1985) has summarized the prevalence of the disease in East Africa (Table 1). The prevalence in most of these countries is quite high. The reasons are lack of consorted plans and national policies in the control and prevention of the disease. Even the disease is not recognized in many places. In a study conducted in Uganda, Oloffs (1994) found that half of the farms questioned did not have knowledge about brucellosis.

Table 1: Prevalence of bovine brucellosis in East Africa

Country	Prevalence(%)	Breed	No. animals examined	Tests	Reference
Djibouti	4	Zebu	499	SAT, CFT	Chantl *et al.* (1994)
Rwanda	35.1	Ankole	510	RBPT, CFT	Akakpo *et al.* (1984)
	33.3	Mitis	141	"	"
Sudan	6.5	Dinka	5982 (30 herds)	SAT, CFT	Hellman *et al.* (1984)
	22.5	Felata	1228 (72 herds)	"	"
Somalia	2.7	Crosses	902	SAT	Hussein *et al.* (1978)
	11.9	Zebu	2184	"	"
Kenya	10	"	10361(total)	RBPT	Kagumba and
	3.76	"	"	SAT	Nandoka
	9.02	"	"	(1978) CFT	"
Uganda	5	"	1739 (total)	RBPT	"
	4	"		SAT	"
	4.6	"		CFT	"
	1.8	Crosses	1359 (83 herds)	MRT, RB, CFT	Oloffs (1994)
Tanzania	5.8	Zebu	23017	RBPT	Kagumba and
4.8	"		SAT	Nandoka	
				(1978)	
	5:0	"		CFT	"
	10.8	Mixed	13078	SAT	Jiwa *et al.* (1995)

2.1.2. Prevalence of bovine brucellosis in Ethiopia

Some studies conducted on the prevalence of brucellosis in Ethiopia are presented in Table 2. It can be seen that the prevalence is higher in improved breeds of cattle than in local breeds. The majority of improved cattle breeds are located inside or near big cities, and the rest in ranches, state farms or cooperatives.

Table 2: Prevalence of bovine brucellosis in Ethiopia

Year	Prevalence (%)	No. examined	Breed	Tests	Reference
1989	4.2	1609	Zebu	RBPT	Tekelye *et al.* (1989)
1989	8.26	2178 (28 herds)	Mixed	RBPT	Bayleyegn (1989)
	7.62	"	"	SAT	"
1989	15	n	Crosses	RBPT; SAT	Abebe (1989)
	3	n	Zebu	"	"
1989	15.8	734 (5 herds)	Crosses	RBPT	Endrias (1989)
	11.6		"	SAT	"
1991	38.7	n	Crosses	RBPT, SAT	Muktar (1993)
1989	22.0	182 (1 herd)	Crosses	RBPT, SAT	Tariku (1994)

n = not available

2.2. Epidemiology

2.2.1. Etiology

Brucella abortus is the causative organism of bovine brucellosis. *Brucellae* are small non-motile, gram negative cocci, coccobacilli, and short rods with straight or slightly convex sides. The micro-organisms are usually arranged singly, or less commonly in pairs, short chains, or small clusters. They do not produce capsules, spores or flagella. They are not acid-fast but may resist decolorization by weak acids or alkalis. *B. abortus* usually requires supplementary CO_2 for growth, especially on primary isolation, and usually produces H_2S from sulfur-containing amino acids or proteins. In addition they hydrolyze urea but some strains may not. Usually growth occurs in the presence of basic fuchsin, methyl violet, pyronin and safranin O but not thionin at standard concentrations (Gillespie and Timoney, 1981; Buxton and Fraser, 1977).

Nine biotypes have been recognized as well as a number of strain variants (Nicoletti, 1980). About 85% of infections are from biotype 1. Biotypes 1, 2, 3, 4, 6, 7, 8, and 9 are recognized in Africa (Chukwu, 1985). There are no proven differences in the pathogenicity or antigenicity among the field strain biotypes. Natural infections in cattle with *Brucella* species other than *B. abortus* are rare. Infection of cattle with *B. melitensis* occurs in regions where the organism is enzootic in sheep and goats (Waghela *et al.*, 1980). However, abortion in cattle due to *B.melitensis* is rare although some may become carriers and excrete organisms in their milk (Nicoletti, 1980).

Cattle are the main reservoir of *B. abortus*. Water buffalo, yak, and American bison are also highly susceptible to infection. Sheep, goats, pigs, equines, camels, and dogs are occasionally

infected but rarely act as a source of infection for cattle. *Brucella* organisms or antibodies have been detected in several species of wild animals, rodents and birds but these are also not regarded as true reservoirs of infection for cattle. Man is susceptible (Sewell *et al.*, 1990). *Brucella* organisms have been isolated from cetaceans, seals, and an otter (Foster *et al.*, 1996)

2.2.2. Transmission

In most circumstances the primary escape route of *B. abortus* is the uterine fluid and placenta expelled by infected cows when they abort or have a full term parturition. The risk posed to susceptible animals following parturition of infected cattle depends on the number of *Brucella* excreted, the survival of these organisms under prevailing environmental conditions, and the probability of susceptible animals being exposed to enough organisms to establish infection (Crawford *et al.*,1990; Radostits *et al.*, 1994).

Brucella abortus achieves its greatest concentration in the contents of the pregnant uterus, the fetus and fetal membranes, and these must be considered as major sources of infection. These tissues infected with *B. abortus* contain between 10^{12}-10^{14} organisms (Sewell *et al.*, 1990). The excretion of organisms, however, is affected by a number of factors. It has been asserted that strain 19-vaccinated and subsequently infected cows excreted fewer organisms at parturition than non-vaccinated infected cows (Crawford *et al.*, 1990). Based on experimental cases, there is evidence of decreasing numbers of organisms when uterine discharges are cultured at sequential parturitions and a substancial proportion of the uterine samples from infected cows are culture-negative at the second and third parturition following challenge (Crawford *et al.*, 1990). The other source of infection is the infected bull. When used as semen donor for artificial insemination there is a considerable risk of inseminated cows becoming infected (Sewell *et al.*, 1990; Crawford *et al.*, 1990). The transmission during natural mating is also important and brucellosis is an important venereal disease (Seifert, 1996). Horses and dogs also may be the source of *Brucella* organisms (Crawford *et al.*, 1990).

It is generally accepted that the growth of *B. abortus* outside the mammalian host plays no part in the epidemiology of the disease, i.e., it does not multiply but merely persists (Crawford *et al.*, 1990). The viability of *B. abortus* outside the host is influenced by prevailing environmental conditions. In general, viability is enhanced by cool temperatures and moisture and limited by high temperatures, direct sunlight, and dryness.

The oral route is considered the most common means by which *B. abortus* reaches a susceptible host (Crawford *et al.*, 1990). It has been suggested that the conjunctival route is also important in intensively housed cattle. Congenital infection is of major epidemiological significance. As high as 20% of heifer calves born by infected cows are persistently infected with *Brucella*. Although feeding *Brucella*-contaminated milk to young calves from *Brucella*-free dams has not been shown to result commonly in persistent infection, the authors have observed pregnant heifers nursing other heifers in the same pen, and this could result in transmisson of *B. abortus*.

The risk that susceptible animals will be exposed to an infective dose of *B. abortus* depends largely on husbandry practices under which the cattle are managed (Radostits *et al.*, 1994). Factors contributing to this risk can be classified into inter- and intraherd transmission. Factors influencing interherd transmission include the purchase of infected replacement animals which are in turn influenced by frequency of purchase, source of purchase, and brucellosis test history of purchased animals. The proximity of infected herds to clean herds is a further important risk factor. Cattle contacts at fence lines, sharing of pastures and strays of infected animals into clean

herds are common methods by which transmission occurs to adjacent herds. The risk factors which are associated with the spread of the disease within a herd include unvaccinated animals in infected herds, herd size, population density, method of housing, and use of maternity pens. Large herd sizes are often maintained by the purchase of replacement cattle which may be infected. It is also more difficult to manage large herds which may lead to managerial mistakes which allow the disease to spread. There is a positive association between population density (number of cattle to land area) and disease prevalence which is attributed to increased contact between susceptible and infected animals (Radostitis *et al.*, 1994). The use of maternity pens at calving is associated with a decrease in the prevalence of infection, presumably due to decreasing the exposure of infected and susceptible animals.

2.2.3. Risk factors

2.2.3.1. Age

Sexually mature, pregnant cattle are more susceptible to infection with the organism than sexually immature cattle of either sex (Enright, 1990). Young cattle are less susceptible to *B. abortus* than older sexually mature cattle (Crawford *et al.*, 1990). Susceptibility appears to be more commonly associated with sexual maturity than age. Young sexually immature cattle generally do not become infected following exposure or recover quickly. Susceptibility increases with pregnancy and as the stage of gestation increases (Crawford *et al.*, 1987). After sexual maturity there apparently are no differences in susceptibility among non-vaccinated pregnant cattle (Goode *et al.*, 1957). Rankin (1965) concluded that there is a tendency for bulls to become infected at a younger age than females and they may acquire infection during calfhood and retain it into adult life. Calves may acquire infection *in utero* or by ingestion of contaminated milk. Until recent years, it was believed that the infection was always temporary. Nicoletti (1980) quoted various reports stating that a small but important percentage of heifer calves which were infected in early life were negative to serologic tests, and abort or have an infected calving due to *B. abortus* during the first pregnancy. These are referred to as latent infections. No field data, however, have been published on the extent of risk of latent infections until Wilesmith (1978) conducted a thorough epidemiological study. He estimated that 2.52% of heifer calves born to serologically positive dams reacted positive in early adulthood and constituted a risk of re-established herds.

2.2.3.2. Sex

It appears that no control studies have been conducted on the relative susceptibility of female and male cattle to brucellosis. Based on prevalence rates, it is probable that bulls are more resistant than sexually mature heifers (Nicoletti, 1980). The role of the bull in the transmission of brucellosis has been studied for over 50 years. Plackett *et al.* (1976) and Manthei and Carter (1950) reviewed literature, control experiments and field studies on natural service and artificial insemination. They concluded that transmission to susceptible cows by natural service with infected bulls has not been demonstrated in controlled experiments. Placket *et al.* (1976) and Manthei and Carter (1950) infected 8 of 12 *Brucella*-free cattle by intra-uterine insemination using contaminated semen from an infected bull.

2.2.3.3. Host

Brucellae are intracellular parasites. In this environment, they have protection from the innate host defenses and from therapeutic agents. When in the quiescent state, they do not cause

formation of humoral antibodies. It is unknown if aberrant forms of the bacteria are produced *in vivo* and if they play a role in the host-parasitic relationship. Nevertheless, this cytoplasmal survival results in many epidemiological problems. There are varying degrees of individual resistance to infection which are dependent upon gestation, exposure dose, age, vaccination, and other unknown host-resistant factors. The effects of nutrition and heredity are largely unknown.

Natural or artificial infections usually persist indefinitely although some cattle recover spontaneously. Recovery rates are probably in the range from 10-15% (Nicoletti, 1980).

The influence of antibiotic therapy has been variable and is affected by choice of compounds and post-exposure time of administration (Nicoletti, 1980).

2.2.3.4. Agent

B. abortus is a facultative intracellular parasite which is capable of multiplication and survival within host phagocytes (Radostits *et al.*, 1994). The organisms are phagocytosed by polymorphonuclear leukocytes in which some survive and multiply. These are then transported to lymphoid tissues and to the fetal placenta. The inability of the leukocytes to effectively kill virulent *B. abortus* at the primary site of infection is a key factor in the dissemination to regional lymphnodes, other sites such as the reticulo-endothelial system, and organs such as the uterus and the udder. The organism is also able to survive within macrophages because it has the ability to survive phagolysosome. *Brucella* are able to survive within host leukocytes and may utilize both neutrophils and macrophages for protection from humoral and cellular bactericidal mechanisms during the periods of haematogenous spread (Enright, 1990).

The survival of *B. abortus* under field conditions or in laboratories is influenced by the size of the inoculum, temperature, pH of medium, nutrition, action of autolytic enzymes, sunlight, and presence of other micro-organisms (Nicoletti, 1980). *Brucella* species are sensitive to pasteurization temperatures and to common disinfectants. It is difficult to ascertain the effects of environmental contamination and resistance of the organisms to natural conditions on the epidemiology of brucellosis. The bacteria can be quite fragile or survive for long periods.

2.2.3.5. Management

The factors of importance in the epidemiology of brucellosis have been summarized by Nicoletti (1980). The spread of the disease from one herd to another and from one area to another is almost always due to the movement of an infected animal from an infected herd into a non-infected susceptible herd. The trend towards larger herd size results not only in a greater probability of infection but also in a higher prevalence (Christie, 1969). The increase in herd size is usually accompanied by an increase in cattle density in housed dairy cattle. These intensive units cause serious problems in regards to exposure potential, especially following an abortion. It is practically impossible to isolate individual cows at calving or to detect imminent abortion. The failure of many infected cows to have serologic evidence of infection until after parturition or abortion nearly always assures perpetuation of the disease inspite of hygienic or other control measures. Kerr (1968) observed that infection with brucellosis was greater in herds with more than 25 cows than in those with fewer numbers. Large herd size is often maintained by introduction of replacement cattle from outside sources. Replacements may originate from multiple sources, increasing the probability of introducing cattle with incubative infections. The purchase of infected replacement animals, thus, is the major factor responsible for introducing brucellosis into previoulsy free herds (Nicoletti, 1980). Other management factors influencing

interherd transmission are proximity to infected herds, water ways, and scavengers (Crawford *et al.*,1990). A variety of cattle husbandry practices also have been shown to be associated with the spread of *B. abortus* infection within herds. Vaccination level, herd size, population density, method of housing, and use of maternity pens influence the probability of exposure to infection (Crawford *et al.*, 1990). Infection was greater in mixed herds (56%) than in single-breed herds (39%) (Kerr, 1968).

2.2.3.6. Topography and climate

Atmospheric conditions and season of the year may have several influences on the management and on contacts between infected and susceptible cattle. The types of housing and methods of feeding are often determined by climate. The survival of the organisms is also affected by the environment. The physical features of a farm or ranch and the proximity to other units furtheron must be considered in the epidemiology of brucellosis. Vectors, especially carrion feeders, are considered means of spread of infection. Physical cattle contacts at fences or confinement failures allow herd-to-herd transmission.

Brucellosis has a marked seasonal character and is most frequent in spring and summer, in temperate and cold climates, when as a result of abortion and parturition, acute cases of the disease may appear among animals. In tropical and subtropical areas where births occur throughout the year, there is no seasonal character to brucellosis. The seasonal nature of the disease is more marked in foci of ovine-caprine brucellosis than in foci of bovine brucellosis. This is possibly accounted for in part by the longer lactation period, six to seven months, in cows (WHO, 1983).

2.3. Diagnosis

The many aspects of the diagnosis of bovine brucellosis have been the subject of many scientific papers, manuals, and monographs. The FAO-WHO monograph 55 (Alton *et al.*, 1975) and the 6th report of the Joint FAO-WHO expert committee on brucellosis (Technical report series 740, 1986) provide excellent references.

2.3.1. Demonstration by microscopic examination

This is a useful procedure for the examination of abortion material. Smears of placental cotyledons, fetal stomach contents, or uterine exsudate should be heat-fixed and stained by a differential method such as Köster's, Machiavello's, or Stamps modification of the Ziehl-Neelsen stain. *Brucella* organisms resist decolorization by weak acids and a diagnosis can often be based solely on microscopic examination. The method will not differentiate between *Brucella* and *Coxiella burnetti* or *Chlamydia*, however. The fluorescent antibody method has been advocated to increase specificity. In practice, interpretation of the results of this test is difficult and it offers few advantages over the modified acid-fast stains.

2.3.2. Isolation of *Brucella*

2.3.2.1. From milk and other liquids

Udder secretions are good sources for isolating *Brucella*. Solid and biphasic selective media are used. Milk is collected from each teat (about 20ml), centrifuged at 3000g for 10 minutes, and a

mixture of cream and sediment is cultured. Samples that are likely to be heavily contaminated can be inoculated into guinea-pigs. Other fluids such as fetal stomach contents, semen and fluid from hygroma, etc., may be cultured directly or after centrifugation, when appropriate.

2.3.2.2. From tissues

The specimen is sectioned with sterile instruments and macerated with a stomacher or tissue grinder with a small amount of diluent before being streaked on the surface of agar medium or added to biphasic medium. If tissues can not be obtained aseptically, the surface should be seared in a flame before being processed for culture. Culture in selective biphasic medium will improve the isolation rate from lightly infected samples. Fetal membranes are often grossly contaminated and a fragment may be washed in successive quantities of sterile saline before being processed for culture. The wash liquid must be carefully disposed off.

2.3.2.3. From genital discharges

A vaginal swab taken after parturition or abortion is an excellent source for the recovery of *Brucella* in cows, sheep, and goats; the use of a solid selective medium is recommended.

2.3.2.4. Guinea-pig inoculation

This technique has value for the isolation of *Brucella* when specimens are derived from potentially contaminated sources, such as milk, cheese, semen, or genital discharges. Direct culture techniques are superior for uncontaminated materials. Solid specimens need to be homogenized before injection. Inoculations should be made subcutaneously and two guinea-pigs have to be used per sample. With milk samples, all the cream-sediment-mixture obtained from a 20ml sample should be used, one half being injected into each guinea-pig. For heavily contaminated material not suitable for parenteral injection, infection of guinea-pigs can be achieved by oral application. One guinea-pig is killed after 3 weeks, and the second 6 weeks after inoculation. A blood sample for serological examination is taken at the time of killing; macroscopic lesions are recorded and the spleen is cultured. Either a positive serological result or the isolation of *Brucella* warrants a diagnosis of brucellosis.

2.3.2.5. Mouse inoculation

For this technique, specimens such as placenta or milk should be washed in sterile saline solution and ground to make a homogenous suspension. The inoculum should be injected intravenously (0.1ml), or subcutaneously, if the material is heavily contaminated, into 2-4 mice. The mice are killed 7 days after inoculation and the spleen and liver are removed for culture on nutrient medium.

2.3.3. Serological tests

Results of cultural examinations are very important in the diagnosis of bovine brucellosis. Positive results are conclusive and should be the basis of evaluation of all other diagnostic methods. There are, however, many situations where bacteriological diagnosis is not practicable and diagnosis has to be based on serological methods, e.g. in surveys or eradication programs. Immunological reactions of brucellosis has been the basis for the development of serologic tests and is directly applicable to diagnosis.

2.3.3.1. Milk Ring Test

The Milk Ring Test is the most practical and economical method for locating infected dairy herds and for surveillance of brucellosis-free herds. If performed on pooled milk 3 or 4 times a year on each herd, it will detect the majority of infected herds. Modifications to the original procedures are now available to increase the sensitivity of the test for use on large herds. It can also be used to detect herd infection in nomadic or semi-nomadic herds. Herds with a positive milk ring test can then be examined by individual serum or milk tests to identify the infected individuals. Milk from individual animals can be serially diluted in *Brucella*-free milk to determine the end titre of the Milk Ring test reaction. Titres above 1:10 are suggestive of infection. The Enzyme Immunoassay has been suggested as a more sensitive and specific alternative for detecting *Brucella* antibodies in milk but requires further evaluation.

2.3.3.2. Tube Agglutination Test

The agglutination test performed by the tube method is the most widely used procedure for the measurement of anti-*Brucella* antibodies for purposes of international trade. A *B. abortus* antigen is used and results are expressed in international units (I.U.). The procedures vary but usually follow the United States Department of Agriculture (USDA) or European methods. The tube test measures the total quantity of agglutinating antibodies and has the disadvantage of reacting to postimmunization agglutinins and sometimes to those caused by heterospecific antigens. Many studies have shown that other tests are more sensitive and specific for diagnosing infected cattle. A modification of this test involved the introduction of EDTA which reduces non-specific reactions.

2.3.3.3. Plate Agglutination Test

This is a modification of the USDA tube test adapted for detection of rapid agglutination on a glass plate. The technique and interpretations have been described by Alton *et al.* (1975). It has the advantage of being simple and more rapid than the tube agglutination test, but is affected by environmental conditions. The sensitivity and specificity are similar to those of the tube test. The use of the plate agglutination test should be discouraged, because other tests are superior, except when serum quality is inferior and its use is essential.

2.3.3.4. Buffered *Brucella* Antigen Test

These are simple spot agglutination tests using stained antigens buffered to a low pH, usually 3.65 or 4.0. The Card and Rose Bengal tests are best known; the antigen is stained with Rose Bengal solution. In North America, the antigen is stained with brilliant green and gentian violet. The Rose Bengal test has found wide application as a screening test for individual diagnosis in herds of cattle. It is generally considered to be oversensitive, especially in cattle immunized with S 19. For this reason, sera positive in the Rose Bengal test are usually retested by a definitive test, such as the Complement Fixation Test. The Rose Bengal test has also been used in surveys and surveillance. The Buffered Plate Agglutination test has a similar role as the Rose Bengal Test.

2.3.3.5. The Complement Fixation Test

The Complement Fixation test (CFT) is recognized as the most reliable diagnostic test now in routine use for individual animals. It is relatively insensitive to antibodies resulting from S 19 immunization.

The work load resulting from the technical complexity of the CFT can be greatly reduced by using it only as a definitive test on samples that have been found positive in a preliminary screening test, usually one of the buffered antigen tests. Either warm or cold fixation may be used for the reaction between test serum, antigen and complement. In warm fixation, the mixture is held at 37°C for half an hour. In cold fixation, the mixture is held at approximately 4°C for 14-18 hours. A number of factors affect the choice of the method, like:

1. Anticomplementary activity in serum samples of poor quality is more evident with cold fixation.
2. Fixation at 37°C increases the frequency and intensity of prozones and several dilutions must be tested for each sample.
3. Fixation in the cold produces higher titres in positive sera.
4. With cold fixation the work is increased by one day.

When the CFT has been used as the principal definitive diagnostic test in eradication campaigns, warm fixation has generally been used. Bovine serum is usually inactivated at 58°C for 30 minutes. Higher temperatures reduce anticomplementary activity but also reduce the complement-fixing activity of IgM.

Standardization of the CFT: A unitage system has been recommended, based on the second International Standard for Anti-*Brucella abortus* Serum (ISABS). The second ISABS is taken to contain 1000 international complement-fixation test units (ICFTU) in a given method and if this serum is tested in a given method and gives a titre of say 500, then the factor for an unknown serum tested by that method can be found by the formula:

1000/500 x titre of test serum = no. of ICFTU of antibody in the test serum.

The European Union has adopted this unit as EU unit. The ISABS contains only IgG; National standard sera should be also calibrated on this isotype for their specific complement-fixing activity. It is recommended that any country using the CFT on a national scale should obtain agreement on the unitage system, fixation method and laboratory test procedures between the different laboratories performing the test by a standard method. This allows the same level of sensitivity to be obtained.

2.3.3.6. Supplementary Tests

i) Ethacridine (Rivanol) Agglutination Test

The principle of this test is to eliminate the reactions caused by IgM antibodies that persist following S 19 immunization. In the USA, the Ethacridine Test is often performed on sera that are positive in screening procedures, such as in the buffered antigen test. Equal quantities of serum and ethacridine solution are mixed in a tube. A precipitate is formed after which charcoal is added and the tube is centrifuged. The supernatant is then tested with the ethacridine plate test

antigen. Different dilutions are tested on a glass plate. The ethacridine agglutination test has been extensively evaluated and can often replace more difficult procedures, such as the CFT.

ii) Enzyme Immunoassay

The Enzyme Immunoassay (EIA) has been extensively investigated as a definitive test for the detection of antibody to *Brucella* in bovine sera. The test shows great promise of increased sensitivity and specificity. Both whole-cell and purified lipopolysaccharide antigens have been used and a variety of antiglobulin conjugates and substrates. A great deal of work is still required on the standardization of reagents. The EIA is a valuable research tool that can employ purified *Brucella* antigens and specific and sensitive anti-immunoglobulin reagents, thus permitting the measurement of the immunoglobulin subclasses of *Brucella* antibodies to defined antigens.

The Enzyme Immunoassay test promises to provide a basis for the development of a new generation of simple tests that can be conducted with inexpensive equipment and reagents, for example as dot immunoassays, using antigen adsorbed to paper. Australia and Canada have adopted EIA for use in their national brucellosis program (Alton *et al.*, 1975).

iii) The Anamnestic Test

This test has been developed to detect latent infection in cattle in eradication programs. It is based on the principle that it is possible to distinguish serologically between a primary and a secondary response to the injection of a *Brucella* antigen. In practice, cattle are tested by the routine serological method, usually CFT, and reactors are removed. The negative cattle are immunized, usually with *B. abortus* 45/20 vaccine. Approximately 6 weeks later they are again tested and further reactors are removed. The anamnestic test can not be used in cattle previously immunized with S 19. This test has been used with success to establish brucellosis-free groups of weaned heifers in range cattle that can not be mustered regularly by serological testing. It is necessary to ensure that the batch of vaccine to be used does not produce positive complement-fixation reactions in negative animals.

Various other serological tests have been used experimentally without so far being accepted for routine use. These tests include the Indirect Hemolysis Test, a sensitive and specific procedure that gives little reaction with antibodies resulting from S 19 immunization. The Hemolysis in Gel Test is a similar procedure in gel, but is considerably more sensitive to vaccinal antibodies than the Indirect Hemolysis Test. The Radial Immunodiffusion Test is a simple gel-diffusion test utilizing a polysaccharide (Poly B) hapten. It is especially insensitive to vaccinal antibodies, and detects a high proportion of cattle exerting *B. abortus* in milk. The Semen Plasma Agglutination Test is useful in diagnosing localized genital infection in bulls, since results of blood serum tests may be misleading. In allergic tests, a protein allergen is extracted from a rough strain of *B. melitensis* in cattle of various breeds. The use of allergic tests was not compatible with vaccines H 38 or 45/20 but could be used in cattle that had been vaccinated with S 19 at least 2 years previously. In newly infected herds, the skin test gave a more complete and earlier response than serologic tests. Lymphocyte stimulation used an *in-vitro* blastocyte transformation system. Using modification of this technique it was found that cell-mediated immune responses appeared earlier than antibodies, were higher in infected than in vaccinated cattle, were poorly correlated with serologic tests in vaccinated cattle, and were useful in differentiating vaccinal from infection serologic titres. Nicoletti (1980) described the stage of infection, vaccination, heterospecific antibodies/antigens, and the herd status as epidemiologic factors which affect herd and individual animal test results.

2.3.4. The sensitivity, specificity and agreement of serological tests

The Buffered Plate Antigen Test, the Standard Tube Agglutination test, the Complement Fixation test, the Hemolysis in Gel test and the Indirect Enzyme Immunoassay test were evaluated (Dohoo *et al.*, 1986). SAT I (≥1/25), EIA I (≥ 0.220) HIGT and BPAT all had actual sensitivities over 95%. The CFT had no false positive results among samples from negative herds (specificity = 100%). With the exception of SAT I (≥ 1/25) all tests had relatively high (≥ 98.8%) specificities among non-vaccinated cattle. Nielsen *et al.* (1996) established the sensitivities and specificities respectively: BPAT 97.9% and 98.6%, CFT (anticomplementary sera taken as positive) 97.1% and 93.1%, I-ELISA (≥ 0.460 OD cut off) 100% and 96%. Dohoo *et al.* (1986) have found that the percent agreement ranged from 69.3% to 98.6% and was generally highest for pairs of tests with a high level of specficity. The *kappa* statistic revealed BPAT and SAT II (≥ 1/50) to have a high level of agreement, as did the CFT with EIA III (≥ 0.300) and EIA IV (≥ 0.340). All other combinations of tests had relatively poor agreement (*kappa* < 0.4). However, all of the *kappa* statistics were significantly different from zero.

2.4. Immunology

There have been many advances in recent years in the study of immunoglobulins. This information is summarized in the joint FAO-WHO 6th expert committee report on brucellosis (1986). The following text on the immunology subtopic is largely borrowed from this report.

2.4.1. Humoral immune response

The immunoglobulin isotypes present in serologically significant concentrations in bovine serum are IgG_1, IgG_2, IgM and IgA. IgA concentrations in bovine serum are usually very low and the role of this isotype in the various serological tests has not been clearly defined. Secretory IgA in milk does play an important role in the Milk Ring test, IgM also participates in this reaction (Sutra *et al.*, 1986). IgG_1 will produce an agglutinate at the bottom of the tube and may interfere with ring formation by the other isotypes.

The first isotype produced after an initial heavy infection or S 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 antibody.

IgG_1 is the most abundant in serum and exceeds the concentration of IgG_2. The magnitude and duration of the antibody response following immunization is directly related to the age at immunization and the number of organisms administered. Following exposure to virulent *B. abortus*, antibody may appear in 4-10 weeks or longer, depending on the size and the route of entry of the inoculum and the stage of pregnancy; but even under controlled experimental conditions there is great variation in response from animal to animal. A disturbing number of infected animals do not develop antibodies of the IgG class until parturition, or 1-3 weeks after parturition.

Antibodies of the IgA, IgM, IgG_1, IgG_2 isotypes can all react in the Tube-Agglutination test, but those of the IgM class are by far the most efficient. The agglutinating and precipitating activities of IgG_1 antibodies are enhanced at high salt concentrations or under acid conditions and this

isotype is reactive in the Card and Rose Bengal tests. The reactivity of IgM in this type of test is dependent on the precise method of preparation of the antigen and the procedures used.

Treatment of serum with ethacridine dye selectively precipitates out more IgM than IgG. Treatment of serum with sulfhydryl reducing agents, such as 2-mercapthoethanol or didithiothreitol, dissociates the IgM pentamer and reduces its agglutinating activity without affecting that of the IgG isotype.

When the CFT is performed by the warm fixation plate method, effectively only antibodies of the IgG_1 isotype are detected. Using modification of the test procedure, IgM antibodies may react in the CFT and this may account for differences in results produced between laboratories. It has been suggested that, as the relative complement-fixing efficiency of IgM compared with IgG is greater at 37°C than at 4°C, the CFT should be less sensitive to IgM with cold fixation than warm fixation. Observations from the field suggest, however, that the reverse is the case.

The CFT fails to measure the non-complement fixing isotypes IgG_2 and IgA. The IgG_2 isotype can interfere with CFT by IgG_1 and in sufficient proportion, causes prozones, atypical reactions, and false negatives. These effects depend on the antigen concentration used, and are greater with warm fixation than cold fixation.

Antibodies of all isotypes except IgM may participate in the Coombs Antiglobulin test, but IgG_1 and IgG_2 are quantitatively the most important. Reactions in the EIA test depend upon the specificity of the enzyme labeled antiglobulin reagent used for the secondary stage.

It is widely believed that sustained production of IgG_1 antibody is characteristic of chronic infection but that IgM antibody persists in animals immunized with S 19. The CFT is superior to the Tube Agglutination test in detecting chronic infection, whereas the agglutination test is more subject to persistent reaction after S 19 immunization. This is believed to be because the Tube Agglutination test is more sensitive to IgM than to other antibody isotypes, whereas the CFT is particularly sensitive to IgG_1.

In all the standard tests mentioned above, the serum antibody measured is primarily directed to the smooth lipopolysaccharide (S-LPS) antigen of *Brucella*. Infected cattle also produce antibodies, predominantly IgG_1 to Native Hapten (NH) or to poly B hapten. Precipitating antibody to those is only transiently produced by vaccinated cattle. It can be assayed by means of a rapid Radial Immunodiffusion test and by EIA.

2.4.2. Cell mediated immunity

Brucella species are facultative intracellular pathogens. They are readily phagocytosed by macrophages and polymorphonuclear leukocytes, and in the case of virulent strains, are capable of surviving within these cells (Tizard, 1992). Not all *Brucella* organisms are to be found within cells, and phagocytosis is promoted by antibody. However, since virulent *Brucella* can survive within normal macrophages for long periods, recovery from infection is likely to be dependent upon the acquisition of increased bactericidal activity by those phagocytic cells.

Macrophage activation occurs when T-lymphocytes of the appropriate subset are stimulated to release lymphokines. The release of these activating factors is dependent upon recognition of the appropriate antigen by the T-lymphocyte and is subject to regulation through the major histocompatibility complex. Live organisms capable of establishing persistent intracellular

infection and certain types of antigen, with or without adjuvant, are the most effective inducers of cell-mediated immunity. Cell-mediated immunity is associated with the delayed hypersensitivity reaction, which can often be elicited in infected animals by the intracutaneous injection of antigen. Some *Brucella* vaccines, like *B. abortus* S 19 and *B. melitensis* strain Rev. 1 and adjuvant vaccines H38 and 45/20, are effective inducers of delayed hypersensitivity to *Brucella* antigens but this is not always accompanied by the development of protective immunity.

Pre-existing or passively transferred antibodies can protect against subsequent infection with *Brucella*. However, in experimental infection in mice, antibody production can be artificially suppressed without affecting the outcome of infection, suggesting that cell-mediated immunity is of major importance in recovery. Nevertheless, *Brucella* organisms are less sensitive to killing by activated macrophages than e. g. *Listeria monocytogenes*. This relative resistance to killing of *Brucella* may contribute to the chronicity of infection.

2.5. Control and prevention

The justifications for the control and prevention of bovine brucellosis are usually divided into two major categories: economic and public health. Three major forms of control and prevention are usually recommended (Nicoletti, 1980). These are vaccination, test-and-isolation or slaughter of seropositive cattle, and management practices.

2.5.1. Vaccination

2.5.1.1. Strain 19

This vaccine remains the most widely accepted immunizing agent against bovine brucellosis. Adult animals vaccinated with S 19 develop a better immunity than calves (Weidmann, 1991). However, due to the danger of abortion in pregnant animals, vaccination has thus usually been performed above all in calves, resulting in an average protection from infection of about 70%. Also, this vaccination scheme reduces the formation of completely agglutinating antibodies to a minimum. A reduced dose of strain 19 administered subcutaneously (Nicoletti *et al.*, 1978) enhances the practicality of the use of the vaccine, regardless of the age of cattle. In general, there have been few differences in protection regardless of the route of administration but large differences in serologic responses (Nicoletti, 1980). The commonly recommended age for strain 19 vaccination is 2-10 months, depending upon breed. This procedure was adopted primarily to avoid persistent agglutinins and to protect young cattle against brucellosis. While it is desirable to reduce postvaccinal diagnostic problems, restricting vaccination to calfhood results in many impractical applications of the vaccine and, therefore, in a reduced usage and in highly susceptible herds (Nicoletti, 1980). The postvaccinal titre problems can be largely overcome through a reduced dose and application of supplemental test procedures.

The strategy in S 19 usage should be towards whole herd immunization, using a reduced dose with subsequent vaccination of replacement cattle without much regard to age. The often quoted protection rate of 65-70% is largely based upon individual cattle challenge with standardized strains and doses. The protection on herd basis is much greater due to increased herd resistance.

2.5.1.2. Strain 45/20

The rough strain 45/20 is inactivated and incorporated with an adjuvant. It causes few diagnostic problems except with the CFT. Results of studies on the effectiveness have varied but have generally shown that two doses administered 6-12 weeks apart engender resistance comparable to S 19 (Nicoletti, 1980). Latently infected animals react to a vaccination with 45/20 with an immune response, which differs from that of non-infected animals if no vaccination has been administered beforehand, e.g. with S 19.

2.5.1.3. H 38 and *Brucella suis* S_2

The H 38 vaccine provides a higher level of protection after a single application than the 45/20 vaccine, but it produces very high titres in all immunoglobulin classes. Animals in all age groups and thus entire herds can be vaccinated with it at one time without risk. The protective effect is quickly reached.

Brucella suis S_2 is a live vaccine developed in China which is administered orally, and a relatively new choice in the means of combatting brucellosis. For beef cattle, sheep, goats and pigs rates of protection of 75%, 83%, 82%, and 72%, respectively, are attained with a single application (Weidmann, 1991). Booster vaccinations scarcely increase the level of immunity obtained. Within a few months after immunization, the titres of the most important immunological tests sink to a level allowing test and slaughter methods. These properties make the vaccine seem suitable for mass immunization in extensive husbandry systems.

Nicoletti (1984) and Weidmann (1991) assert that reduction of brucellosis in high prevalent areas is best accomplished by mass immunization.

2.5.2. Test-and-slaughter

When the rate of infection is reduced to an acceptable level, about 1-2%, test-and-slaughter methods can be started (Weidmann, 1991). This step is to be rigorously carried out until two successive precise whole herd tests, conducted six weeks apart, yield negative results. Then the herd in question can be given a brucellosis free status, but this still requires further regular herd tests and further prophylactic vaccinations if the disease is present in the environment. Contact with still infected stock should be prevented, or it should be limited to likewise diseases-free stock.

2.5.3. Management practices

From the epidemiology of the disease, important steps to be implemented at an early stage include the isolation of calving animals in separate calving pens which are subsequently disinfected, the burning or burial of placentas, aborted fetuses, etc., testing and quarantine of newly arrived animals, regular examination of the herd, and slaughtering of animals with positive reactions. If ones own herd is still disease-free, contact with neighbouring herds must be prevented. If the disease is enzootic, certain group sizes should not be exceeded and unnecessary new groupings should be avoided.

Many modifications of the environment, livestock management practices, social customs and resources will be necessary before many countries can consider eradication programs (Nicoletti,

1980). Only in the more prosperous industrial countries with superior infrastructure the goal can be the eradication of brucellosis at the national level (Weidemann, 1991).

There exists a general consensus though that brucellosis must be combatted everywhere, so as to keep the disease occurrence which has increased in recent years, at least at its present level.

2.6. Importance of bovine brucellosis

2.6.1. Veterinary importance

The disease is characterized primarily by abortion and infertility in cows. It is frequently associated with retention of the placenta after calving and metritis may develop. Brucellosis is responsible for many cases of hygromas in cattle and abortions in cows are associated with swellings (Akakpo *et al.*, 1978). In French West Africa, it is known that brucellosis is characterized by bursitis, arthritis, and subcutaneous abscessation (Chukwu, 1987). In the bull, *B. abortus* is known to produce orchitis and sterility. In addition, it may cause enlargement, purulent abscessation and focal necrosis of the seminal vesicles (Radostits *et al.*, 1994).

B. abortus has been found to cause natural infection in horses including occasional abortion in mares (Chukwu, 1987). It has sometimes been associated with poll-evil and fistulous withers.

2.6.2. Economic importance

Chukwu (1987) has discussed the economic importance of brucellosis. The disease causes losses due to abortion, diminished milk production, and condemnation of animals due to breeding failure, and endangers the animal export trade of a nation. Human brucellosis causes loss of man-hours and medical costs. There are losses in financial investments and government costs on research and eradication schemes.

Tariku (1994) estimated in the Chaffa State Farm in Ethiopia, consisting of 193 breeding females with a sero-prevalence of 22% annual losses to be about Birr 88,941.96. Financial losses due to Brucellosis in some African countries are shown by Chukwu (1987) (Table 3).

Table 3: Annual financial losses due to brucellosis in some countries in Africa

Country	Species	Financial loss (in million)
Côte d'Ivore	Cattle	French Franc 150
Kenya, Tanzania, Uganda	Cattle & Sheep	US$ 33.4
Nigeria	Cattle	US$ 233.88

2.6.3. Public health importance

Human brucellosis is primarily due to one of three species: *B. melitensis* (goats), *B. suis* (hogs), and *B. abortus* (cattle). *Brucella canis* has caused illness in few humans, and no human disease has been ascribed to *B. ovis* (Isselbacher *et al.*, 1980).

B. abortus causes undulant fever in man (Thimm, 1982). *Brucella*-infected cows excrete *Brucellae* in their milk sporadically throughout almost their entire period of lactation. Hence, the consumption of untreated milk and milk products from infected animals exposes man to serious risk of infection by *B. abortus*. *Brucella* remain viable for a long time in refrigerated meat. They also remain viable through the pickling process (over three months) (WHO, 1983). Meat and meat products obtained from *Brucella*-infected animals are the sources of infection in those working in the meat processing industry, in livestock owners when such livestock is slaughtered, and its meat eaten at home and in those who buy their meat in markets. Infection occurs by ingestion of infected raw milk, cream, and cheese, however, most cases are occupational hazards acquired by direct contact with infected cattle. The incidence in humans is directly related to that in cattle (Nicoletti, 1980). The prevalence in humans in some African countries is shown by Chukwu (1987) (Table 4). Literature on the occurrence of brucellosis in humans in Ethiopia is not available.

Table 4: Prevalence of human brucellosis in some countries in Africa

Country	Prevalence (%)	No. tested	Test	Year	Reference
Nigeria	7.6-29.8	13999	SAT	1962	cited by Chukwu (1987)
	5.55	738	SAT	1974	"
Somalia	0.6	353	SAT	1978	"
Tanzania	22.6	540	SAT	1967	"
	20	80		1968	"
Uganda	6.4	3164	SAT	1972	"

2.7. Brucellosis and reproductive performance of cows

Different studies showed a link between infection with brucellosis and poor reproductive performance. Conception rate in herds with no reactors was 62% versus 55% in herds with at least one positive animal (Kerr, 1968). Kerr (1968) also observed 12-14% abortions in infected animals, and 3-4% in noninfected animals using the Milk Ring test.

In the Chaffa dairy farm a significantly higher life birth rate and milk yield was observed in brucellosis-free groups when compared with brucellosis-positive cohorts with a brucellosis infection rate of 22% (Tariku, 1994). Muktar (1993) found a 54.2% non-conception rate in a mixed herd of Friesian-Zebu crosses and Zebu with a brucellosis prevalence of 38.7%.

2.8. Dairy production in Ethiopia

Ethiopia has the largest livestock population of any country in Africa. According to the FAO Livestock Sector Development Project Preparation Report (1993), livestock production is estimated to contribute around one third of agricultural GDP and 15% of total GDP. Total national milk production is assessed at 780,000 to 830,000 tons of raw milk equivalents. Of the total national production between 85 and 95% comes from cattle, the balance from the national goat flock and camel herd. This report indicated that production from the national herd - estimated at between 26 and 29 million heads - is assessed at 1.5 l per day over a 150

day lactation. The MOA estimates that 0.3% of the national herd are upgraded animals. Production from improved animals varies from 6 to 10 l per day over a 270 to 300 day lactation with calving intervals of 15 to 18 months.

The marketing of fresh milk in Addis Ababa is undertaken through five major channels: 71% is sold directly by producers to consumers, 15% is sold through producer associations or government outlets, 10% is through private sector outlets, and 4% through itinerant traders. The role of the Dairy Development Enterprise (DDE), once dominating the formal marketing channel, is declining due to the recent government policy of privatization. Recent analysis showed small peri- and intra-urban producers with an average production of 3 l per day suppling the major share of the direct producer consumers sales market (FAO, 1993).

The Dairy Rehabilitation and Development Project (DRDP) had undertaken an extensive livestock sub-sector review in 1985-86. The focus was a shift from state farm and service cooperatives development to producer cooperative development to small holder development. The FAO/TCP smallholder dairy development project subsequently e. g. assisted 40 dairy farmers in the Sidamo region. Assistance was provided by the establishment of improved fodder, provision of 2-3 crossbred cows per farmer, and installing a small scale butter plant. It was recommended that future dairy development should be built on this integrated approach.

2.9. Urban and peri-urban livestock production systems

The expression peri-urban production points at farm enterprises that are situated in the vicinity of towns or that are geared towards urban markets (Nauheimer *et al.*, 1995). Intra-urban productions often are a result of poverty. Peri-urban, modern units, supply commercial urban markets with high quality products (eggs, milk, and meat). They depend mainly on hired labour and have full access to inputs such as concentrates, artificial insemination and veterinary services. Most intra-urban and the small scale peri-urban farms concentrate on subsistence production for providing animal protein for the families. In addition, these small-scale farms generate income through sale of products to local markets, through the provision of services (transport etc.), and of animal manure for intra-and peri-urban cropping (Nauheimer *et al.* 1995; Ethui *et al.*, 1995).

One of the necessary preconditions to improve urban and peri-urban livestock production systems is that people engaged in these systems need to be educated and trained on issues concerning animal and human health as well as environmental hygiene in order to alleviate the possibility of zoonoses. Research in zoonotic diseases is often recommended (see e. g. Nauheimer *et al.*, 1995; Ethui *et al.*, 1995).

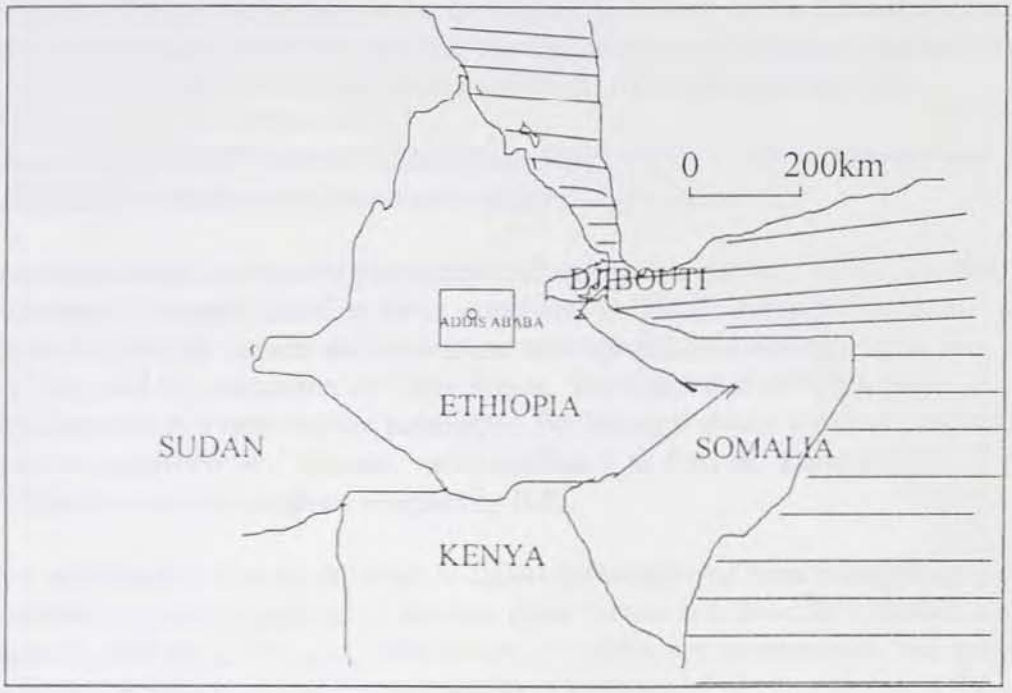
3. MATERIALS AND METHODS

3.1. Study area and population

The study area is located in central Ethiopia around Addis Ababa, the capital city. Four study sites were distinguished. These are Addis Ababa, Debre Zeit, Kaliti, and Sebeta. Kaliti, Sebeta, and Debre Zeit are located 10, 25, and 45 km, respectively, from the center of Addis Ababa. Alemgena is a district (Woreda) in which Sebeta is situated. Sebeta is the main town of

Alemgena. Adaa is the district in which Debre Zeit is situated. Debre Zeit is the main town of Adaa. The cattle population of Addis Ababa is estimated at 58,568. The figures for Kaliti, Alemgena, and Adaa are 3,569, 75,193, and 142,574, respectively (Report of Region 14 Bureau of Agriculture, Alemgena Bureau of Agriculture, Adaa Bureau of Agriculture, 1997). In Addis Ababa, Kaliti, Sebeta, and Debre Zeit 14,045 crossbred cows, 4720 heifers, 4404 female calves and 568 bulls are found.

Addis Ababa, Sebeta, and Kaliti are located at an altitude of 2,048 meters above sea level; the annual average temperature is 16.1°C. Debre Zeit is located at an altitude of 1,900 meters above sea level and has an annual average temperature of 23°C.



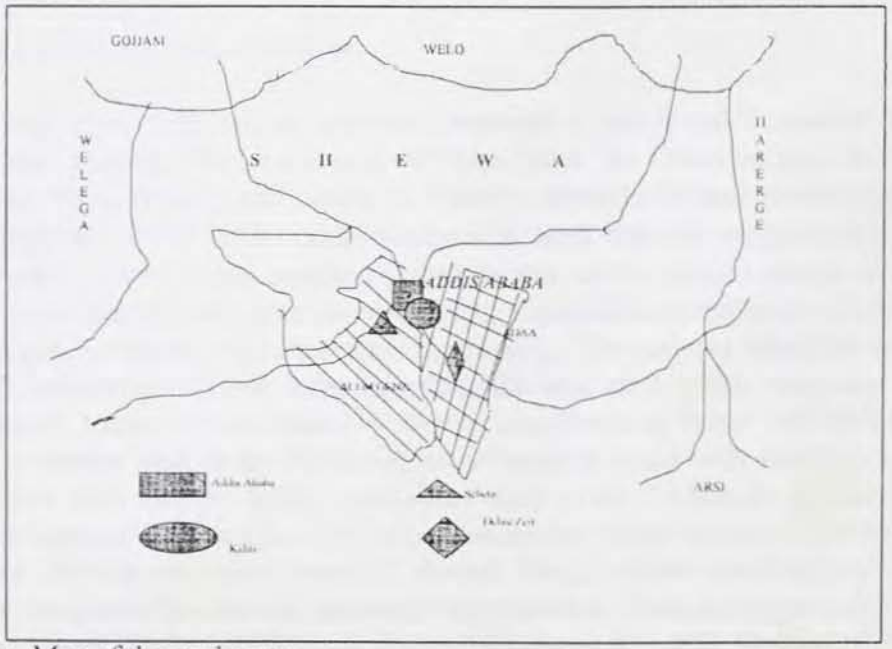


Figure 1:. Map of the study area

3.2. Study design

Investigations on brucellosis were conducted between June and October 1997. For this, 42 dairy farms keeping Holstein-Zebu crosses were sampled from the four districts. A bulk milk sample from each herd was collected in 14-16 week intervals. The Milk Ring Test (MRT) was conducted on these bulk milk samples. At the beginning of the study a blood sample was collected from all breeding animals above six months of age. Screening of serum samples was done by using the Rose Bengal Plate Test (RBPT); sera testing positive were further tested by the Complement Fixation Test (CFT) as confirmatory test. For parallel testing and to determine agreement, however, CFT was done on all serum samples. This cross-sectional epidemiological study was undertaken to estimate total prevalence, herd prevalence, and within-herd prevalence of brucellosis. The prevalence rates were determined in respect to risk factors: age, sex, parity, herd size, district, and production systems. For the analyses of infection rates and herd sizes, herds were stratified into three herd size strata: 1-50, 51-100, and more than 100.

Agreement between RBPT and CFT, RBPT and RBPT+/CFT+, MRT (different time samples) and RBPT+/CFT+ results were determined using the kappa statistic.

Cow reproductive and productive parameters like age at first calving, calving interval, number of services per conception, lactation status, abortions, life births, and pregnancy state were taken from individual animal records and association with the *Brucella*-infection status was analysed using χ^2 test and by calculation of Odds Ratios. The *t*-test and ANOVA were used to test differences among cow reproductive parameters. For this card sheets to record production yield, reproductive parameters and diseases were distributed to farmers. Recording was done on a weekly basis by research assistants assigned by ILRI.

Finally a questionnaire was administered to collect information on farm management parameters and husbandry practices; association between these factors and *Brucella* infection status were investigated using the χ^2 test and Odds Ratios. An additional questionnaire was employed to collect information on the occurrence of brucellosis in humans.

3.3. Sampling procedure

In 1992 and 1993, ILRI and its partners developed a conceptual framework for research in small-holder dairying that provides a common basis for characterizing and understanding smallholder dairy systems and assists to identify researchable and development issues. The methodology was tested in the Addis Ababa milk shed, the area supplying milk to the Addis Ababa market. A multi-stage sampling procedure was used to select a sample of 147 farmers in the area; these farmers were then surveyed using a questionnaire covering 45 measures of farm resources and parameters reflecting farm functioning. The analyses identified seven categories of market-oriented smallholder dairy farmers in the milk shed. These categories are: traditional crop-livestock farms in rural areas (10 farms), crop-livestock farms with an intensified dairy element in remote rural areas (30 farms), crop-livestock farms with intensive cropping in the Addis Ababa dairy belt (23 farms), specialized dairy farms (19 farms), peri-urban producers in secondary towns of the dairy belt (20 farms), intra-urban farms in Addis Ababa (24 farms), and intra-urban farms in secondary towns (31 farms). Next, a cluster sampling procedure was used and three categories (production systems) were selected. These were: peri-urban producers in secondary towns (Kaliti and Sebeta), intra-urban farms in Addis Ababa (Addis Ababa), and intra-urban farms in secondary towns (Debre Zeit). Using a random sampling procedure 8, 17,

and 20 farms were selected, respectively (ILRI, 1993/94). All breeding animals above six months of age were used for the study proper. Two farms were closed before the study began in Kaliti. One farm in Addis Ababa dropped out of the study at the beginning and one other at the middle of the study. All animals in the farms were correctly identified by ear tags.

3.4. Serological tests

3.4.1. Milk Ring Test

Bulk milk from a maximum of eight animals per farm was sampled (Staak, 1996). 10 ml were collected after the bulk tank milk had been agitated for 3 minutes. The sample was identified, including date of collection and was transported to the laboratory at the Department of Microbiology, Faculty of Veterinary Medicine. Milk samples were stored for at least 18 hrs at +2 - +8°C, then were warmed to room temperature and mixed thoroughly. The MRT-test method of MacMillan (1990) was followed. The MRT antigen was obtained from the BgVV, Berlin, Germany:

1) Gently the milk in the sample tube was mixed to ensure even distribution of the cream.
2) 1.0ml of milk was placed in a 1cm diameter test tube and mixed well.
3) 0.03ml of MRT antigen was added by holding the dropping pipette vertically.
4) Gently the mixture was mixed for 1 min. after adding the antigen.
5) The tube was placed in a 37°C incubator for 1 hr.

Interpretation:

A positive reaction was indicated by the appearance of a colored blue cream ring on the top layer of the milk column.

3.4.2. Rose Bengal Plate Test (RBPT)

Blood samples were collected from the jugular or coccygeal veins using plain vacutainer tubes. The tubes in the laboratory were set tilted on a table for one hour at room temperature. The clotted blood in the tubes was centrifuged to obtain a clear serum (Salchow, 1996). The serum was used for both RBPT and CFT. RBPT was done at the laboratory of the Department of Microbiology, Faculty of Veterinary Medicine, Debre Zeit. The RBPT antigen was obtained from the BgVV, Berlin, Germany. The RBPT-test method of MacMillan (1990) was followed. Sera and antigen for use were left at room temperature for half an hour before the test:

1) Holding the antigen dispenser upright, 30µl of RBPT antigen was placed on each circle on the plate.
2) 30µl of test serum was placed along side, but not into the antigen.
3) With a plastic applicator stick, the antigen and serum were mixed thoroughly.
4) The plate was placed on a rocking machine and mixed for 4 minutes.

Interpretation:

Results were read after 4 min. of contact of serum and antigen. Any observed agglutination was regarded as positive. Reactions were distinguished into 4+, 3+, 2+ 1+ and doubtful.

3.4.3. Complement Fixation Test (CFT)

In the CFT, all reagents were evaluated by titration. The preparation of sheep red blood cells, the method of the CFT test, and preparation of reagents were according to protocols of BgVV Service Laboratory (1994). The CFT test was conducted at the National Veterinary Institute, Department of Immunology, Debre Zeit. All control sera, antigen, complement and amboceptor were obtained from the BgVV, Berlin, Germany. The CFT antigen had a titre of 1:640++ against ISABS.

i) Preparation of sheep red blood cells for the hemolytic system:

Ten (10) µl of sheep red blood cells in Alsever's solution were centrifuged at 2500 rpm for 5 min. The supernatant was discarded and replaced by veronal buffer diluent (VBD). The sheep red blood cells were resuspended in the diluent completely. This procedure was repeated 4 times. Before discarding the supernatant after the last washing the volume of the packed cells was measured. The volume of the packed cells was read by placing an identical tube next to the blood containing tube and filled up to the level of the blood by a measured amount of water. By addition of a calculated amount of a diluent, a 2% sheep red blood cell suspension was prepared.

ii) Amboceptor titration:

1) Predilution of amboceptor in jumping dilution.
2) Prepared 1:500 dilution up to 1:8000.
   Prepared 1:750 dilution up to 1:12,000 dilution.
3) From these dilutions 500µl in order were transferred to a set of tubes, always starting with the 1:12000 dilution.
4) 1µl of a diluent was added to each of the test tubes.
5) 0.5µl of 2% sheep red blood cells was added, and shaken well.
6) The tubes were left on the bench for 10 min.
7) 1µl of complement at a dilution of 1:40 was added.
8) The last tube showing complete hemolysis, minimum hemolytic dose (MHD) was read and recorded. The working dilution of amboceptor was 4 times the MHD (BgVV Service Laboratory, 1994).

iii) Evaluation of complement (BgVV Service Laboratory, 1994):

1) Set up 3 rows of 9 tubes each.
2) A 1:40 complement dilution was prepared.
3) Complement was added into the 9 tubes increasing by 0.05ml every time, starting with 0.1ml.
4) Diluent was added into the 9 tubes in decreasing amounts by 0.05ml, starting with 0.4ml.
5) 1.5ml of a diluent was added into the tubes with a Cornwall Syringe.
6) The set of tubes were placed in a 37°C water bath for 1hr.
7) 0.5ml of 2% sheep red blood cells was added to all tubes.
8) 0.5ml of amboceptor at a working dilution 1:1000 was added to all tubes.
9) The tubes were properly mixed and put again into the water bath of 37°C for another 30 min.
10) The test was read by recording the minimum hemolytic dose of complement (MHD) which was represented by the first tube showing complete hemolysis. The next tube contained the full hemolytic dose (FHD).

The complement dilution = 2 FHD / Dilution of complement

v) Titration of antigen (BgVV Service Laboratory, 1994):

Microtitre plate I

1) 25µl of VBD were added to all cups (Wells).
2) 25µl of pre-diluted antigen were added to all cups of row A.
3) By serial doubling (two fold) dilutions 25µl of antigen were transferred from row A to B, and again from row B to C, etc. until row G by a multichannel pipette. 25µl mixture were discarded from row G (row H will contain only the diluent).

Microtitre plate II

1) 50µl of VBD were added to all cups.
2) 50µl of pre-diluted (1:2.5) inactivated positive control serum were added to all wells of col. 1
3) 50µl were serially transferred by two-fold dilutions, from col.1 to col. 2, and again from col. 2 to col. 3 etc. until col. 11. 50µl was discarded from col.11 (col. 12 has only VBD).

Mix plate I and II

1) 25 µl were transferred from plate II to plate I.
2) 50µl of complement in 1:40 dilution were added to all cups of plate I.
3) Plate I was placed in a refrigerator, covered with a second empty plate (cold fixation).
4) The following day, 50µl of 2% sheep red blood cells and amboceptor pre-mixture, equal volume, i.e. 25µl of sheep red blood cells and 25µl of a 1:100 working dilution of amboceptor, were added to all cups.
5) The plate was covered with a sealing tape, shaken well and put into a water bath at 37°C for 30 min.
6) The last cup with 50% (2+) sedimentation was read and recorded. The highest dilution of the antigen with 50% (2+) sedimentation was the limiting antigen concentration or the right corner value. The working antigen dilution was found to be 1:100.

The test proper, multiple sera technique (BgVV Service Laboratory, 1994):.

1) 25µl of VBD were added to all cups.
2) 25µl of control and test sera were added simultaneously to col. 1 and 4, 5 and 8, and 9 and 12.The sera were pre-diluted to 1:2.5 and inactivated at 58°C in a water bath for 30 min.
3) 25µl sera were serially transferred from col. 1 to 3, 5 to 7 and 9 to 11. 25µl are discarded from col. 3, 7, and 11. 25µl VBD are added to column 4, 8, and 12.
4) 25µl of antigen in a working dilution of 1:100 was added to column 1-3, 5-7, and 9-11.
5) 50µl of complement in working dilution was added to all cups.
6) The plate was placed at 4°C overnight covered with a 2nd empty plate (cold fixation).
7) The following day 50µl of equal volume pre-mixed 2% sheep red blood cells and amboceptor at a working dilution of 1:2000 were added. These were the same reagents used for complement evaluation.
8) The plate was sealed, shaken well, put into a water bath of 37°C for 30 min.

Interpretation:

Sera with a strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 and at least with 50% fixation of complement (2+) at a dilution of 1:10 and at a dilution of 1:20 were classified as positive (Hellmann *et al.*, 1984).

3.5. Data analysis

The total prevalence rate was calculated on the basis of RBPT+/CFT+ positive results; by dividing the number of RBPT+/CFT+ positive animals by the total number of animals tested. Herd prevalence was calculated for MRT results by dividing the number of pooled MRT positive herds by the number of MRT tested herds and on the basis of RBPT+/CFT+ results, by dividing the number of herds with at least one reactor in RBPT+/CFT+ by the number of all herds tested using RBPT+/CFT+. The within-herd prevalence was calculated by dividing the number of RBPT+/CFT+ reactors within a herd by the number of serum samples tested in that herd (Thrusfield, 1995). The apparent prevalence is the proportion p (T+), and the true prevalence is the proportion p (D+) (Martin, 1977; Zessin, 1996).

Calving intervals were calculated by adding the gestation length, 282 days on the average, and the interval between previous calving and last insemination, the days open. The age at first calving was defined as the date of the animal's first calving, and the number of services per conception as the number of services required per fertile conception (Mukasa-Mugrewa, 1989).

Test sensitivity is the proportion of diseased animals which are correctly identified by a test. Sensitivity was calculated using the conditional probability of p (T+/D+; given the animal is diseased the probability that the animal is test-positive) (Martin, 1977; Zessin, 1996). Specificity is the proportion of healthy animals which are correctly identified as healthy. Specificity was calculated using the conditional probability of p (T-/D-; given the animal is disease-free the probability that the animal is test-negative). The predictive value was calculated using the proportion p (D+/T+). The positive predictive value is the proportion of test positive animals which are diseased. The kappa statistic was used to test agreement between the serological tests. k measures if the results agree to an extent significantly in excess of "chance agreement"; k = % agreement beyond chance / maximum % improvement); (k = 0: no agreement, k = 1: perfect agreement, k = 0.4-0.5: moderate agreement, k>0.7: good agreement). In this study k was determined using the computer program Win Episcope Version 1.0.

The χ^2 test was used to test asociations between factors and seropositivity. The odds ratio (OR) is the ratio of the odds of disease occuring among animals exposed to a factor and the odds of disease occuring among animals not exposed to a factor. χ^2 and OR values were calculated using the computer program Epi Info Version 6.02. Descriptive statistics, t- test, and ANOVA were calculated using the Excel 5.0 program (Microsoft Corp.).

4. RESULTS

The cross-sectional investigation of bovine brucellosis was carried out between June and October 1997. The study was conducted on 42 dairy farms in four districts: Debre Zeit, Addis Ababa, Kaliti, and Sebeta. Bulk milk samples from all farms and serum samples from a total

of 950 non-vaccinated animals above 6 months of age were taken to establish *B. abortus* antibody prevalences.

4.1. The Milk Ring Test (MRT)

Bulk milk samples from each farm were collected in June, August, and October, respectively. Six (6) samples from 6 farms (14.3%) were positive during the first sampling. When the results of all sample dates were pooled, samples from 11 farms reacted positive (26.2%) (Table 5).

Table 5: Results of the bulk MRT from June to October 1997

Prod. System	District	No. farms	Pos. in MRT 1	2	3	Pos. farms in MRT pooled total	district	Pos. farms in RBPT+/CFT+ total	district	MRT pos.farms confirmed by RBPT+/CFT+	Avg.prop. of lactating cows
Intra-urban sec.towns	Debre Zeit	20	2	0	1	3 (7.1%)	15%	6 (14.3%)	30%	1	51/153 (33.3%)
Peri-urban	Kaliti	3	1	1	2	3 (7.1%)	100%	3 (7.1%)	100%	2	91/249 (36.5%)
"	Sebeta	3	1	2	2	3 (7.1%)	100%	3 (7.1%)	100%	3	171/467 (36.6%)
total peri-urban		6	2	3	4	6 (14.3%)	100%	6 (14.3%)	100%	5	262/716 (36.6%)
Intra-urban	Addis Ababa	17	2	1	0	2 (4.8%)	12.5%	2 (4.8%)	12.5%	2	88/245 (35.9%)
TOTAL		43	6	4	5	11 (26.2%)		14 (33.3%)		8	401/1114 (35.9)

4. 2. The Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT)

For the four districts 950 sera were screened by RBPT. In the RBPT 124 (13.1%) reacted positive and 169 (17.8%) reacted positive in the CFT. When those sera positive in the RBPT were further tested by CFT, 77 (8.11%) were confirmed positive (Table 6).

Table 6: Results of the RBPT and CFT serological tests

Prod System	District	No. serum samples	No. reactors RBPT	CFT	RBPT+/CFT+	Prev.rate (%) with 95% CI
Intra-urban sec.towns	Debre Zeit	124	7	24	7	5.6 (1.6-9.6)
Peri-urban	Kaliti	190	23	37	17	8.9 (6.8-10.9)
"	Sebeta	423	64	74	42	9.9 (7.1-12.7)
total peri-urban		613	87	111	59	9.6 (7.3-11.9)
Intra-urban	Addis Ababa	213	30	34	11	5.2 (2.2-8.9)
TOTAL		950	124	169	77	8.11 (6.4-9.8)

4.3. Test Agreement

The agreements of different test combinations were quantitatively assessed by calculating the *k* quotient (Table 7). The agreements between RBPT+/CFT+ (at least one reactor in a farm makes that farm positive) and MRT tests were 0.31, 0.43, and 0.58 (*k* quotients) for one-two-three-time MRT results. Moderate agreement (k=0.44) was found between RBPT and CFT in this study. Good agreement (k=0.74) was found between RBPT and RBPT+/CFT+ serial testing procedure.

Table 7: Agreement of MRT, RBPT, and CFT serological tests

Test combination			k quotient	Individual anim.	Herds
RBPT	-	CFT	0.44	*	
RBPT	-	RBPT+/CFT+	0.74	*	
MRT I	-	RBPT+/CFT+	0.31		*
MRT I & II	-	RBPT+/CFT+	0.43		*
MRT I, II, & III	-	RBPT+/CFT+	0.58		*

4.4. Sensitivity, Specificity, and Predictive Value

Three (3) of the farms were not confirmed for brucellosis by the RBPT+/CFT+ test results that were used as gold standard; this thus constitutes false-positives in the pooled MRT. Six (6) farms positive in the RBPT+/CFT+ confirmation were not detected by pooled MRT. These constitute false-negative findings (Table 8). The resulting sensitivity was 57.1% and specificity was 89.3% when the three MRT results were pooled. In individual animals, considering the RBPT+/CFT+ as gold standard, false-negative animals were not detected, but 47 false-positive animals were encountered in the RBPT. This resulted in a sensitivity of 100% and a specificity of 94.6%. Taking CFT as gold standard, the sensitivity and specificity of RBPT were 45.6% and 93.9%, respectively. The positive predictive values of pooled MRT and of RBPT, considering RBPT+/CFT+ as gold standard, were 72.7% and 62.1%.

Table 8: A 2 X 2 table for calculating sensitivities and specificities of MRT and RBPT

(a) pooled MRT and RBPT+/CFT+ (gold standard)

		RBPT+ / CFT+		
		+	-	TOTAL
MRT	+	8 (a)	3 (b)	11
	-	6 (c)	25 (d)	31
	TOTAL	14	28	42

(a) = true positives
(b) = false positives
(c) = false negatives
(d) = true negatives
SE = 57.1%
SP = 89.3%
PPV = 72.7%

(b) RBPT and RBPT+/CFT+ (gold standard)

		RBPT+ / CFT+		
		+	-	TOTAL
RBPT	+	77 (a)	47 (b)	124
	-	0 (c)	826 (d)	826
	TOTAL	77	873	950

SE = 100%
SP = 94.6%
PPV = 62.1%

(c) RBPT and CFT+ (gold standard)

		CFT+		
		+	-	TOTAL
RBPT	+	77(a)	47(b)	124
	-	92(c)	733(d)	825
	TOTAL	169	780	949

SE = 45.6%
SP = 93.9%
PPV = 62.1%

4.5. *Brucella abortus* Sero-prevalence Rates

4.5.1. Prevalence on herd level

The overall herd prevalence rate, based on MRT testing performed at three time points and after pooling was 26.2% (11/42). The rates were 7.1%, 14.3%, and 4.86% for intra-urban secondary town, peri-urban, and intra-urban production systems, respectively. Debre Zeit, Kaliti, Sebeta, and Addis Ababa shared herd prevalence rates of 7.1%, 7.1%, 7.1%, and 4.8%, respectively. When the production systems were compared, all (100%), 15%, and 12.5% of the herds infected were from peri-urban, intra-urban secondary town, and intra-urban production systems, respectively (Table 5).

When the farm was tested with the RBPT+/CFT+ procedure, the overall herd infection rate was 33.3% (14/42). Debre Zeit, Kaliti, Sebeta, and Addis Ababa had herd infection rates of 14.3%, 7.1%, 7.1%, and 4.8%, respectively. In respect to the production systems, all (100%), 30%, and 12.5% of the herds were infected in the peri-urban, intra-urban secondary town, and intra-urban systems, respectively.

The within-herd prevalences ranged from 0% to 16.7% based on the RBPT+/CFT+ test results. One or two reactors were recorded in 64.3% (9/14) of the farms.

4.5.2. Prevalence on individual animal level

The individual animal prevalence rate was determined by using the RBPT+/CFT+ serial serological test results. The overall individual antibody prevalence (seropositivity) was established at 8.11% (77/950) (Table 6). The infection rates were 0.11% (1/950) and 8% (76/950) for male and female sexes, respectively, in the total animals tested. This difference was not significant ($p = 0.71$).

Infection rates were higher in older age groups (Table 9). The prevalence in animals above 4 years was 4% while it was 1.3% in animals between 6 months and two years. Comparing the age groups, animals aged two to four years had the highest prevalence rate of 9.8%. Prevalence in animals in the above four years age group was 8.9% and in the age group six months to two years 4.5%. The infection rates were significantly different between the age group ≤2 years, and >2 years ($p = 0.04$).

Infection rates were also compared based on parity. Differences in prevalence rates between animals without parturition and at least one (≥1) parturition were significant ($p < 0.1$) (Table 9).

Sebeta and Kaliti shared prevalence rates of 9.9% and 8.9%. The rates for Debre Zeit and Addis Ababa were 5.6% and 5.2% respectively. For the peri-urban dairy production system, a prevalence rate of 9.6% was found. The rates for the intra-urban system was 5.2% and 5.6% for the intra-urban secondary town production system. However, these differences between districts and production systems were statistically not significant ($p = 0.56$ and $p = 0.54$, respectively).

When the RBPT+/CFT+ serial test results were considered as gold standard, the apparent prevalences and true prevalences were the same both in herds and in individual animals.

Table 9: Intrinsic and extrinsic risk factors for brucellosis prevalence rates

Risk factor		Prevalence rate (%)	CI (95%)	p-value	OR
Sex				0.71	
	male	0.11	0 -30.5		
	female	8.0	6.3-9.7		
age					
	total animal				
	0.6-≤2 yrs	1.3	0.5-2.1		
	2-≤4 yrs	2.4	1.3-3.5		
	>4 yrs	4.0	2.7-5.7		
	age group				
	0.6-≤2 yrs	4.5	1.8-7.2		
	2-≤4yrs	9.8	5.5-14.1		
	>4yrs	8.9	5-11.9		
	>2 yrs	9.2	6.72-11.6	0.04	2:14 (1.04-4.83)
Parity					
	no parturition	2	0.9-3.1		
	single parturition	1.7	0.71-2.67		
	more than one part.	4.2	2.65-5.74		
	at least (≥1) one part.	5.7	4.2-7.22	0.07 (90% CI)	1.89 (0.95-3.83)
District					
	Debre Zeit	5.6	1.6-9.6	0.56	
	Kaliti	8.9	6.8-10.9		
	Sebeta	9.9	7.1-12.7		
	Addis Ababa	5.2	2.2-8.9		
Production system					
	Intra-urban	5.2	2.2-8.1	0.54	
	Intra-urban sec.town	5.6	1.0-10.2		
	Peri-urban	9.6	7.3-11.9		
Herd size					
	≤50	3.3	0.9-5.7	0.01	2.99 (1.29-8.06)
	>100	7.8	6.05-9.6		

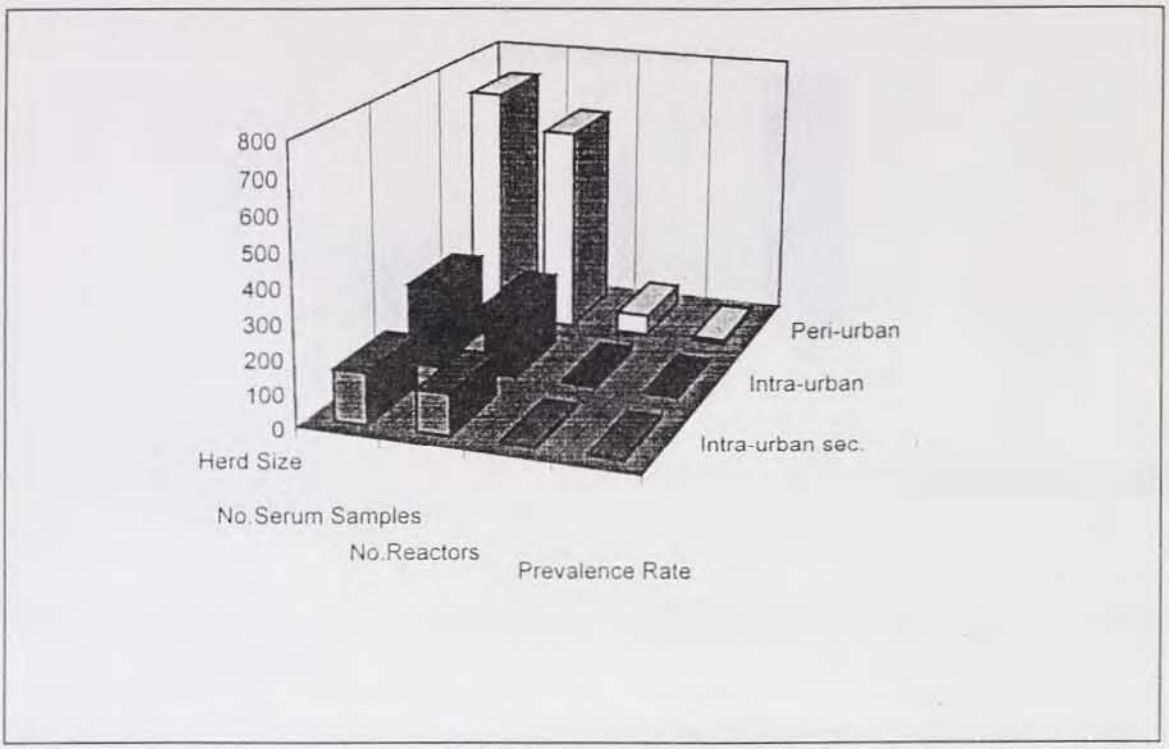


Figure 2: Description of herd sizes, no. serum samples, no. reactors, and prevalence rates in intra-urban, intra-urban secondary town and peri-urban dairy production systems

4.6. Herd size and brucellosis prevalence rate

A *Brucella* infection rate of 9.1% was found in farms that kept more than 100 animals and 3.3% in farms that kept 1 to 50 animals. This difference was statistically significant ($p = 0.01$). On the herd basis, all herds (100%) were infected in farms that kept more than 50 animals (Table 10). The herd rate in farms that kept less than 50 animals, in contrast, was 17.6%.

Table 10: Relationship of herd size and brucellosis infection rates (seropositivity) in individual animals and herds using RBPT+/CFT+ test results

Herd size Class	No.animals	No. farms	No.infected animals	% infected animals & confidence interval	% infected farms
1 - 50	215	34	7	3.3% (0.9-5.7)	6 (17.6%)
51 - 100	461	6	30	6.5% (4.2-8.8)	6 (100%)
>100	438	2	40	9.1% (6.4-11.8)	2 (100%)
TOTAL	1114	42	77	6.9% (5.4-8.4)	14 (33.3%)

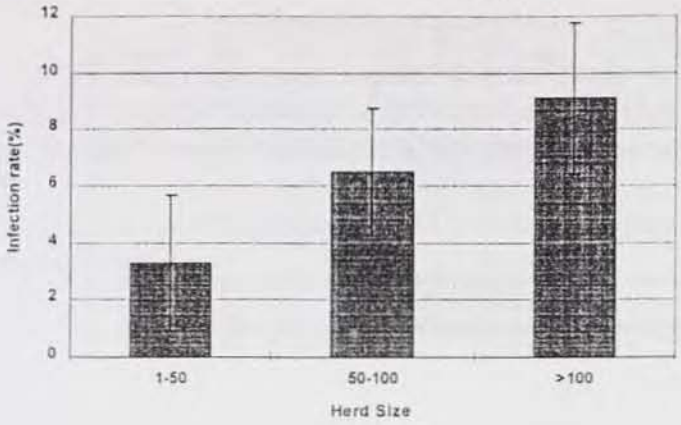


Figure 3: Relationship between herd size and brucellosis sero-prevalence rates (mean values and 95% confidence intervals)

4.7. Descriptive statistical results of cow reproductive parameters

Results of descriptive statistics of cow reproductive parameters are summarized in Table 11. Significant differences were observed in calving interval and ages at first calving between production systems ($p < 0.05$). These differences were apparent between intra-urban and peri-urban systems, but not between intra-urban and intra-urban secondary town systems ($p > 0.05$).

Table 11: Summary of descriptive statistics of cow reproductive parameters in intra-urban secondary town, intra-urban and peri-urban production systems separately and combined

DESCR.	INTRA	URBAN.	SEC.	INTRA	URBAN		PERI	URBAN		COMB.		
STAT.	CI*	AFC	NSC	CI	AFC	NSC	CI	AFC	NSC	CI	AFC	NSC
Mean	483.1	3.07	1.89	421.28	2.27	2.41	501.2	3.25	1.59	497	3.02	1.77
SD	111.12	0.62	1.29	89.16	0.42	1.69	111.3	0.67	0.87	112.2	0.71	1.14
SE	14.35	0.085	0.21	14.86	0.085	0.41	7.47	0.08	0.09	6.63	0.06	0.09
CV	0.23	0.20	0.68	0.21	0.19	0.70	0.22	0.21	0.55	0.23	0.24	0.64
Median	451.5	2.85	1	393.5	2	2	494	3	1	488	3	1
Mode	417	2.6	1	317	2	2	557	3	1	408	3	1
Range	307-752	2-4.3	1-6	301-762	1.5-3	1-8	325-1087	2-5	1-4	301-1087	1.5-5	1-8
Count	60	54	37	36	24	17	223	66	87	319	144	141
Confid	28.12	0.17	0.41	29.13	0.17	0.81	14.63	0.16	0.18	12.98	0.12	0.19

* CI Calving interval (days)
AFC Age at first calving (days)
NSC Number of services per conception

4.8. Reproductive and productive parameters and brucellosis status

Measures of effect (OR) were calculated for possible associations between reproductive and productive parameters and brucellosis status in individual animals (Epi info Version 6.02). No association was established between *Brucella* infection and these parameters (Table 12).

Table 12: Associations between productive and reproductive parameters and brucellosis infection status

Parameter	p-value
Abortions	0.94
Life births	0.69
Clinical signs	0.61
Lactation status	0.16
No. services per conception	
1 service	0.77
> 1 service	
Age at first calving	
< 3.5 yrs	0.91
≥ 3.5 yrs	
Calving interval	
≤ 540 days	0.31
> 540 "	

4.9. Results of the questionnaire survey

4.9.1. Management and husbandry activities and brucellosis status

Associations between farm management and husbandry activities and the brucellosis status were analyzed (odds ratio) using the Epi Info Version 6.02. Previous abortions and the use of natural service were associated with *Brucella* infection (p = 0.009) (Table 13).

Table 13: Associations of management and husbandry activities with brucellosis status

Farm management Activities	% of farms affected	p-value	OR	OR CI
Abortions	23/41 (56.1)	0.009	15.58	1.72-79.16
Use of natural mating	18/41 (43.9)	0.009	0.06	0-0.61
Use of AI	8/41 (19.5)	0.32		
Knowledge of brucellosis (no)	36/41 (87.8)	0.96		
Proportion of lactating cows	443/648 (68.4)			
Proportion of pregnant cows	173/648 (26.7)			
Presence of parturition pens	2/41 (4.9)	0.89		
Separation of cows during parturition	11/41 (26.8)	0.32		
Cleaning and disinfection	12/41 (29.3)	0.45		
Disposal of after birth (not disposed)	23/41 (56.1)	0.87		
Farm replacement strategy (outside source)	9/41 (21.9)	0.911		
Culling of infected animals	6/41 (14.6)	0.47		

4.9.2. Occurrence of brucellosis in man

Ninety-nine (99) milkers, farm workers and owners in the 41 farms were interviewed. Nine persons showed prolonged fever since starting keeping dairy cattle. In addition, other clinical symptoms of brucellosis in man were recorded (Table 14). Diagnoses made by physicians in these cases were malaria, kidney problems, gastritis, anaemia, hypertension, typhus, lameness, typhoid fever, bronchitis, and headache. No diagnosis of human brucellosis was ever made by physicians.

Table 14: Results of a questionnaire administered to investigate occurrence of brucellosis in man (n=99)

Questionnaire	No. of positive answers
Visited a health institution in the last 6 months	17
Prolonged fever since started keeping dairy cattle (intermittently for at least 6 months)	9
Clinical symptoms shown since started keeping dairy cattle	
insomnia	6
pain over the spine	4
vague generalized pain	5
pain over the joint	9
pain of testes	1
nervous disorders	1

5. DISCUSSION

The Milk Ring Test

The MRT was performed in all farms for three times in order to identify infected herds. Only 42.9% (6/14) of RBPT+/CFT+ farms were also detected by the MRT, taking results of the first sampling. In Uganda, only 25% of the CFT-positive herds were detected by the MRT (Oloffs, 1994). In this study, one milk sample from a pool of 8 lactating animals was taken. Obviously with increasing herd size the ability of the MRT to detect one infected animal in a herd decreases due to the dilution effect (Staak, 1996; Crawford *et al.*, 1990).

The RBPT and CFT Tests

The RBPT was performed on all sera collected from individual animals. Those reacting positive were further tested by the CFT as confirmatory test. Such serial testing procedure does maximize the specificity of the test system. The number of false-positives is reduced. Improvements in the specificities are particularly useful in control programmes when the strategy is removal of positive reactors. Although some false-negative animals are missed, healthy animals are less likely to be considered infected (Thrusfield, 1995; Zessin, 1996). The use of the serial testing procedure, first screening all animals by RBPT and then applying CFT as confirmatory test thus improves the efficiency of detecting brucellosis (Staak, 1990; Kagumba *et al.*, 1978). It is recommended that either the buffered plate antigen test or the indirect enzyme immuno-assay test be used as screening test and either the complement fixation test or the indirect enzyme immunoassay be used as confirmatory test in situations requiring high specificities (Dohoo *et al.*, 1986). However, RBPT as screening test is cheap and technically not complicated which may explain its still wide use. The CFT is regarded as a complicated and time consuming test, but it can be performed with little equipment, and can be adapted to tropical conditions (Staak *et al.*, 1995). It is for these reasons why the CFT at present is the preferred test for bovine brucellosis (OIE, 1992).

Test agreement, sensitivity, and specificity

The agreement between MRT and RBPT+/CFT+ results was improved when MRT sampling was repeated. The first MRT sample detected only 42.9% of RBPT+/CFT+ positive farms. By pooling three samples, MRT test results improved to 78.5% of RBPT+/CFT+ positive farms. It is recommended that the MRT to be effective should be taken at intervals of 14-16 weeks (Staak, 1996) or three times annually (Blood *et al.*, 1994). This is, because new animals are added or withdrawn and the number of lactating cows fluctuates from time to time.

The agreement between RBPT and RBPT+/CFT+ was good. This shows that the RBPT test is an effective testing technique for bovine brucellosis investigations. The good agreement between RBPT and CFT reported by Staak (1990), however, was not observed in this study. Reasons could be that the reagents, especially the complement quality could have deteriorated during storage under tropical environmental conditions.

An explanation for the false positive results in the MRT could be the effect of colostrum milk; also, a high percentage of mastitic cows were reported in the farms; it is reported that the mean [incidence] of subclinical mastitis was 37.6% and of clinical mastitis 1.2% (ILRI, 1996). The other explanation is when sampling is done in early and late lactation. The false-

negative results, in contrast, relate to the low proportion of lactating cows and the low within-herd prevalence. The sensitivity of the MRT test was low. In order to determine point prevalence rates, it is therefore preferable to screen the population, using blood samples. RBPT is a highly sensitive but less specific test system. The sensitivity of RBPT ranked second among six serological tests (Stemshorn *et al.*, 1985). Dohoo *et al.* (1986) reported a 97.9% sensitivity for it. A report on investigations on infected animals exists whereby the RBPT detected all culturally positive animals, whereas the SAT failed to detect nearly half of these (Staak, 1990). The false positive result in the RBPT could be due to cross reactions with other bacteria. Cross reactions of *Brucella* with *Yersinia enterocolitica*, *E. coli*, *Salmonella* spp., and *Pasteurella* spp. are reported (Bisping and Amstberg, 1988).

The positive predictive values of both pooled MRT and RBPT tests were low in this study. However, the PPV was better in the MRT than in the RBPT. In another investigation at a herd prevalence rate of 0.67%, the PPV of the MRT test was 8% and of the RBPT 18% (Crawford *et al.*, 1990).

Seroprevalence rates

In this baseline investigation, an overall herd prevalence rate of 33.3% was found. The importance of brucellosis in the Addis Ababa milk shed is clearly indicated.

Overall prevalence rates of 8.11% in individual animals and of 8.9% in cows alone were established. In Arsi region, a prevalence rate of 8.26% was detected in crosses using the RBPT (Bayleyegn, 1989). For Southern Ethiopia, using the same test, Endrias (1989) reported a prevalence rate of 15.8%. Tariku (1994) detected a prevalence rate of 22% using RBPT and SAT. In dairy cattle owned by Institute of Agricultural Research (IAR) a prevalence of 38.7% was found using the RBPT as screening and SAT as confirmatory tests (Muktar, 1993). Both latter studies, however, were carried in single herds and used SAT as confirmatory test. The relatively high prevalence rate in this study is explained by a number of factors. Except for one farm, vaccinated ten years before, vaccination has not been practiced so far in all farms. 43.9% of the farms also use natural mating. Only bulls in government institutions are tested for brucellosis, no test is done on farm bulls. Owners take cows on heat to places where bulls are rented for a single mating. These bulls serve a number of cows indiscriminately. The role of the bull in the transmission of brucellosis has been particularly studied. Manthei and Carter (1950) reviewed controlled and field studies on natural service and AI. They concluded that transmission to susceptible cows by natural service has not been demonstrated in controlled experiments. However, Seifert (1996) states that brucellosis is an important venereal disease. 87.7% of owners in this study do not have knowledge about brucellosis. In 36.6% of cases general farm hygiene is poor. For example, in 56.1% of farms the placenta is left on the ground or fed to dogs; cleaning of stables with antiseptics is practiced only in 4.9% of the farms. Separate parturition pens are not present in most of the farms (95.1%). Under intensive systems of cattle husbandry, the use of parturition pens has been shown to reduce the level of infection markedly (Crawford *et al.*, 1990). Dogs are found in 85.7% of the farms. A study in which nine dogs, orally infected with *B. abortus*, were housed with eleven pregnant heifers demonstrated that infected dogs can be a source of *Brucella* infection for cattle (Crawford *et al.*, 1990). Horses and mules could also be a source of infection to the smallholder farms. Poll-evil and fistulous wither in these species is endemic in the the study area.

For indigenous cattle in central Ethiopia, Tekeleye *et al.* (1989) reported a brucellosis prevalence rate of 4%, using the RBPT. In peri-urban private and state owned farms the rate

was 10.3%, while in the far extreme rural villages the value was 0.54% (Bakunzi *et al.*, 1993). Jiwa *et al.* (1996) reported higher prevalence rates in dairy (6.3%) and ranch (15.8%) animals than in animals kept under extensive grazing conditions (4.3%). In the dairy management system the prevalence rates for brucellosis were 11%, 5.6%, and 3.2% in pure exotic, crossbred, and dual purpose local (Mpwapwa) animals, respectively. This apparent difference in infection rates could have been due to the fact that under extensive grazing conditions pasture contamination in dry climatic and arid conditions is reduced and hence, exposure is limited. Additional studies on breed susceptibilities to brucellosis in different production systems are required for further rational explanations.

Female cattle were more infected when compared to male cattle. However, the difference was not statistically significant. This could be due to the small number of males tested (n = 10 vs female = 940). However, based on reactor rates, males are considered more resistant than female cattle (Nicoletti, 1980). Also sexually mature, pregnant cattle are more susceptible to infection with brucellosis than sexually immature cattle of either sex (Enright, 1990). When herd bulls stay permanently with cows, they appear to be less susceptible, and serologic data may underestimate *B. abortus* infection in males as infected bulls in tests were found to be nonreactors or only had low antibody titers (Crawford *et al.*, 1990).

The prevalence was low in young animals and higher in older cattle. An OR value of 2.14 in this study explains that older animals were twice as likely affected by brucellosis than younger animals. It is widely accepted that sexually immature cattle are quite resistant to exposure to *B. abortus* and that susceptibility increases with sexual development and pregnancy (Nicoletti, 1980; Crawford *et al.*, 1990). Goode *et al.* (1957) assert, however, that there are no significant differences in susceptibility of non-vaccinated cattle of different ages to virulent *B. abortus* when the animals are raised in a *Brucella* free environment. Still susceptibility generally appears to be more commonly associated with sexual maturity than with age (Blood *et al.*, 1994). Young sexually immature cattle generally do not become infected following exposure or recover quickly from infection. In this study, a higher infection rate was observed in the age group two to four years. This high rate constitutes a high risk for the incidence of brucellosis in the study farms in the future. The greater the number of infected cows that abort or calve, the greater is the exposure risk to other cattle in the herd (Blood *et al.*, 1994). An important conclusion from this observation is that infected cows need to be removed from the herd prior to parturition.

Significant differences were found in *Brucella* infection rates between animals with no birth and those with at least one parturition. Susceptibility to infection increases with gestation and with stages of lactation. The probability of isolation of *Brucella* organisms at parturition increased from 0.22 to 0.90 when fetal age at exposure of non-vaccinated heifers increased from 60 to 150 gestation days (Crawford *et al.*, 1987). This high susceptibility is related to the presence of a sugar alcohol known as erythritol in the placenta and in fetal fluids; the substance is produced by the fetus and is capable of stimulating the growth of *B. abortus* (Blood *et al.* 1994). Enright (1990) relates increased susceptibility also to the presence of hormones and secretory proteins in metabolically active chorionic trophoblasts.

Herd size and prevalence rate

Clear differences in infection rates were demonstrated when animals were stratified in respect to herd size. In individual animals, herd size was significantly associated with *Brucella* infection, with prevalence increasing with herd size. The OR value of 2.99 shows that farms

with more than 100 animals were almost three times more likely to be affected than small farms. In Rukungiri district in Uganda, the majority of CFT reactors were detected only in large and medium sized herds (Oloffs, 1996). Kerr (1968), too, had observed that infection with brucellosis was greater in herds with more than 25 cows than in those with fewer numbers. The trend towards larger herd size hereby not only results in greater probability of infection but also in a higher prevalence (Christie, 1969). Intensive dairy farms enhance the exposure potential, especially following an abortion. It is practically impossible to isolate individual cows at calving or detect imminent abortion. During abortion, a high number of pathogens are excreted. 10^{12}-10^{14} bacteria are excreted during this period (Sewell *et al.*, 1990). Increases in herd size are often also accompanied by an increase in cattle density, promoting transmission by contact and common feeding. The failure of many cows to show serologic evidence of infection until after parturition or abortion in these cases nearly assures perpetuation of the disease in spite of hygienic or other control measures (Nicoletti, 1980). Large herd sizes often are maintained furtheron by the introduction of replacement cattle from outside sources. 21.9% of the farms in this study did acquire replacement stock from such outside sources. The animals may have originated from multiple sources, increasing the probability of introducing infected cattle. Another explanation for the association between herd size and infection with *Brucella* relates to logistic and managerial problems that occur in controlling the disease as herd size increases. Control of brucellosis in larger herds often demands concerted managerial inputs which may be difficult to implement.

Reproductive and productive parameters and brucellosis status

Statistically significant associations with brucellosis were not established for any of the reproductive and productive parameters. The mean calving interval of 497 days in the farms studied was long when compared with 410.65 days in Boran-Holstein crosses in Ethiopia (Goshu, 1983). On the average animals had their first birth at 36.2 months. Their non-productive life was obviously longer when compared to Boran-Holstein crosses studied in Debre Zeit (Goshu, 1983) which were 29.68 months old at first calving. In most farms, anoestrus, lack of programmed heat and pregnancy detection, and inadequate services offered by AI personnel were observed. Shortage of feed due to high prices also were witnessed in most of these farms. Cow reproductive and productive parameters are strongly influenced by feeding and management, among other factors (Mukasa-Mugrewa, 1989). Brucellosis with prevalence rates of 8.11% in individual animals and 56.1% of farms experiencing previous abortions, could be a likely explanation for the situation on the study farms. Brucellosis causes abortions mostly during the first pregnancy and rarely later; this could contribute to the high age at first calving. The other sequels of brucellosis are retained placenta and metritis. In this study, all 22 cases of metritis occured in conjunction with abortion, retained placenta, or abortion with retained placenta. Inflammation of the uterus prevents conception, resulting in extended calving intervals. Hinojosa *et al.* (cited by Mukasa-Mugrewa, 1989) attributed a short calving interval in a well managed herd in Mexico due to the absence of brucellosis and a resulting reduced abortion rate.

Significant differences were observed for cow reproductive parameters (calving interval and age at first calving) between the different production systems. Differences, however, were not seen between the intra-urban secondary town and the intra-urban systems. The peri-urban farms usually keep more than 50 animals, while intra-urban farms usually keep 1 to 15 animals. Management and logistic problems are greater in larger farms than in small farms. This could be one explanation for the differences observed in calving intervals and ages at first calving.

Management and husbandry activities and brucellosis status in farms

A significant association between *Brucella* infection with abortion and natural mating was observed. Among farms 56.1% experienced abortions in the last three years. The fact that no associations between abortions and brucellosis infection could be established for individual animals but for herds can be explained. Data on herd abortions for the last three years could be analyzed whereas for individual animals records of only one year were available.

Abortion and retention of placenta are characteristics of *Brucella* infection (Sewell *et al.*, 1990). The association between natural mating and *Brucella* infection observed in this study is interesting. Conflicting views exist about the role of infected bulls. Blood *et al.* (1994) and Manthei *et al.* (1950) state that infected bulls do not usually transmit infection from infected to non-infected cows mechanically by natural mating even when the semen contains *Brucella* organisms. Seifert (1996), in contrast, asserts that transmission during mating is important and that brucellosis has to be considered as an important venereal disease.

Brucellosis in man

Prevalence rates for humans could not be established because taking of blood samples was not possible. However, the observations of leading symptoms in people closely associated with cows justify to suspect the presence of brucellosis in man. This suspicion is further strengthened by the fact that only 11.9% of farms sell pasteurized milk. 82.9% of the farms sell raw milk directly to individuals nearby soon after milking. Also, on 87.8% of farms no knowledge exists about brucellosis and hence personal caution is not practiced. A survey of brucellosis in man would be essential to determine the exact epidemiological picture, and human doctors should be made aware of the disease in order to consider it in the differential diagnoses.

6. CONCLUSION AND RECOMMENDATION

A cross-sectional investigation of bovine brucellosis in the Addis Ababa dairy shed showed a relatively high prevalence of infection in dairy cattle. The high prevalence in the younger age group shows brucellosis to be in the incubative stage in the herds, carrying the potential for an imminent explosion and spread in the future. Females, older animals and cows at reproductive status life were comparatively more affected. Higher brucellosis rates were observed in intensive farms when compared to smallholder farms. Abortions, delayed age at first calving, and long calving interval were concurrently observed. Herd management practices and hygiene on the farms were found poor.

Test agreement between MRT and RBPT+/CFT+ serial testing improved consistently when the number of MRT tests was increased. Hence, three or four MRT tests should be carried annually. The good agreement between the RBPT test and the recommended RBPT+/CFT+ serial testing procedure shows that RBPT can be used effectively in bovine brucellosis surveys. Brucellosis, besides being of economic importance, also is of public health significance. As urban and peri-urban farms are the sole sources of milk to the majority of inhabitants of Addis Ababa and closer secondary towns, particular emphasis should be given to the dairy enterprise in regards to the hygienic qualities of milk.

In these lines, the following recommendations are made for at least in the Addis Ababa milk shed:

(1) General surveys should be carried out in all dairy farms using the RBPT and possibly CFT.
(2) All dairy farmers should be instructed to implement recommendations of the veterinary authorities for the control and prevention of brucellosis.
(3) Slaughter of positive animals may not be economically feasible. Slaughter of positive animals though is recommended once the prevalence drops to 1-2%. To achieve this, vaccination of young animals at 3-6 months of age with a full dose of strain 19 and the use of strain 45/20 in adults is recommended.
(4) Extensive extension service must be launched to make the stock owners aware of the disease.
(5) In constructions of dairy farms, all hygienic measures should be adhered.
(6) The pasteurization of milk should be enforced.
(7) Cooperation between veterinarians and the medical personnel should be sought, strengthened and coordinated.
(8) Restriction of movement of infected animals should be enforced by law.
(9) Control of the importation of live animals and frozen semen, to limit introduction of brucellosis to the country, should be enforced by law.
(10) During internal animal movements from one locality to another, a certificate should be presented stating that animals are free from brucellosis.

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ANNEX 1: EQUIPMENTS AND REAGENTS FOR SEROLOGICAL TESTS

Milk ring test

Materials:

- stained MRT antigen. The antigen was obtained from BgVV (Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin), Berlin, Germany. It was standardized *B. abortus* antigen.
- pipettes

Rose Bengal Plate Test

- RBPT antigen, obtained from B_gVV. The antigen is standardized *B. abortus* antigen.
- Positive and negative control sera, obtained from B_gVV.
- A 10 - 100ml nicrotitre pipette
- White enamel or ceramic plate
- Wooden applicator stick.

Complement Fixation Test

i) equipments, glassware and plastic material,

- Water bath
- Centrifuge
- pH meter
- Magnetic Stirrer
- Reading mirror
- Test tubes, 1.2cm by 10cm.
- Pipettes, 1ml, 5ml, 10ml
- Cornwall 2ml
- U-type microtitre plates
- Eppendorf pipettes (10-100µl, 100-1000µl)
- Adjustable multichannel pipette
- Plastic tips (10-100µl, 100-1000µl)
- Sealing tape

ii) Diluents,

Veronal buffered diluent (VBD). Preparation of 200ml of 5 fold concentrated VBD.

NaCl	83.00g
Na 5,5,- diethy/barbiturate	10.10g

dissolved in about 800ml of hot distilled water. Added slowly 34.6ml HCl (1N), and afterwards 5ml of the following "VBD stock solution"

1M	M_gCl_2	+	0.3M $CaCl_2$
$M_gcl_2 . 6H_2O$			20.3g
$CaCl_2 . 2H_2O$			4.4g

add 100ml distilled water

Fill up to 200ml with distilled water. The pH of a 1.5 diluted stock VBD should be between 7.4 to 7.5

iii) Preservatives,

a) Alsever's Solution

Dextrose	18.66g
NaCl	4.19g
NA-Citrate	8.00g
distilled water add	1000ml
sterilized by steaming	

b) Richardson sol

Richardson sol A

Boric acic	1.86g
Borax	4.58
Sorbitol	22.94
Satur. NaCl add	200ml

Richardson sol B

Borax	1.14g
Na Azide	1.62g
Satur. NaCl add	200ml

Reagents,

a) Complement. Mixed serum from healthy male guinea-pigs. The serum was preserved by adding 1ml of Richardson sol.B to 8ml of serum and after mixing well 1ml of Richardson A was added and mixed. Preserved complement was stored at 4°C. Preserved complement is obtained from BgVV.

B) Amboceptor. Amboceptor is a serum containing lytic antibodies against sheep red blood cells, prepared in rabbits. The amboceptor was obtained from Behring-Werke, Marburg, Germany.

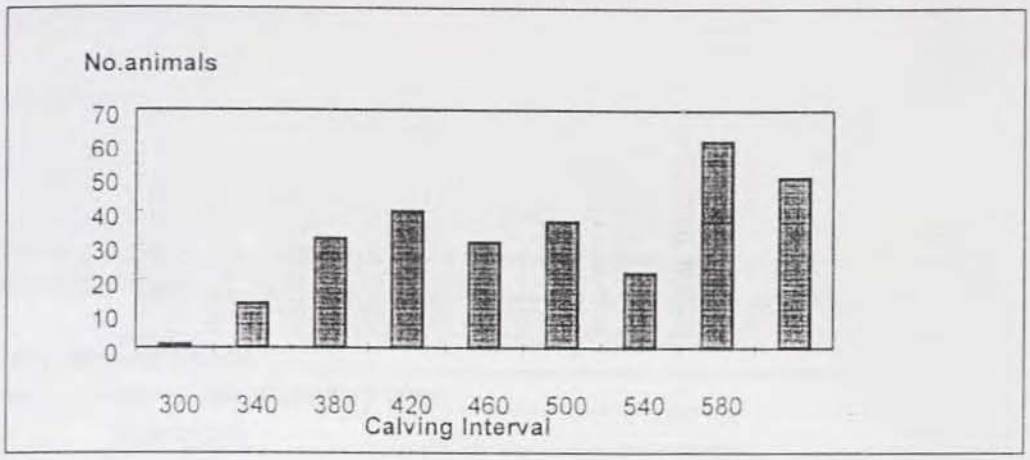
C) Sheep red blood cells. 75ml of free flowing blood was drawn from the jugular vein directly into a bottle containing 125ml Alsever's solution. A dash of crystalline penicillin was added. Stored at 4°C.

D) Antigen. Obtained from BgVV. The antigen is standardised *B. abortus* against the Second International Standard Anti - *B. abortus* Serum, so as to give 50% fixation at a dilution of 1/200 of this serum.

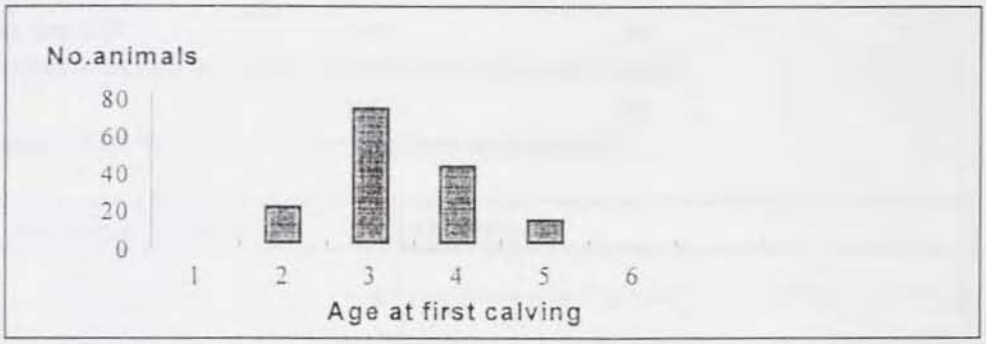
E) Control sera. Positive control sera originate from infected animals with sufficiently high titres. Negative control sera are derived from non-infected animals. In this study both positive an d negative control sera were obtained from BgVV, Berlin, Germany.

ANNEX 2 GRAPHICAL REPRESENTATION OF SOME COW REPRODUCTIVE PARAMETERS

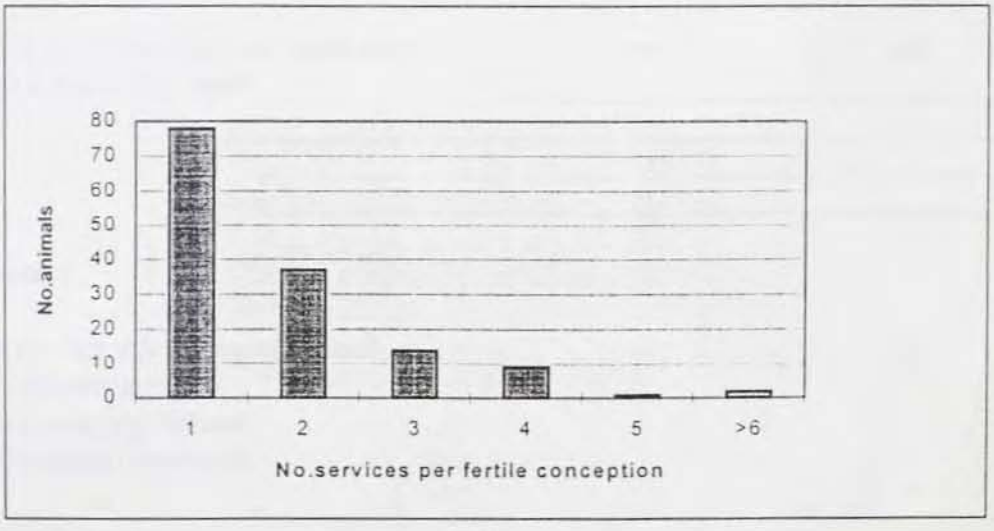
(A) Calving Interval



(B) Age at first calving



(C) Number of services per fertile conception



ANNEX 3 QUESTIONNAIRE FORMAT

CROSS-SECTIONAL AND LONGITUDINAL PROSPECTIVE STUDY OF BOVINE BRUCELLOSIS, CLINICAL AND SUBCLINICAL MASTITIS, IN INTRA-URBAN AND PERI-URBAN DAIRY PRODUCTION SYSTEMS IN AND AROUND ADDIS ABABA, ETHIOPIA.

Date_____

GENERAL INFORMATION

Farm Structure

Farm owner

_____sex_____age_____yrs

Occupation _____

Farm physical address_____

_____Tel._____

Year dairy farming started_____

Location intra-urban secondary town_____

intra-urban _____

peri-urban _____

Distance from town center_____km.

Farm size_____ha.

Is the farm fenced? yes no

Is there frequent contact between your animals with other herds?

yes no

Crop livestock farming: Types of crops grown and quantity.

Type of crop livestock farming	Quantity

Do you own land elsewhere e.g. rural area? yes no

What do you do on this land?_____

_____

_____

_____

Social Factors

What was the last school you attended?

(1) elementary

(2) junior high school

(3) college / university

Professional qualifications _____

How did you acquire skills to raise dairy cattle?

(1) agricultural training (level) _____

(2) from extension agents (which ones) _____

(3) from parents

(4) other _____

How did you start dairy buisness?

(1) inherited the enterprise

(2) bought the enterprise

(3) bought dairy animals

(4) upgrade the local breed

(5) other _____

What are your reasons for producing milk?

(1) to earn a living

(2) to supplement family food

(3) to supplement family income

(4) as a hobby

(5) other _____

Do you have another job?

yes no

specify _____

Herd Composition

Breed of cows _____

No. of milking cows _____

No. of first lactation heifers _____

No. of female calves _____

Bulls _____

Others _____

ON BRUCELLOSIS

What are your culling criteria?

(1) disease _____

(2) old age _____

(3) reproductive diseases _____

(4) poor production _____

(5) other _____

What type of insemination do you use for your animals?

(1) artificial insemination _____

(2) natural service _____

(3) both _____

Do you have knowledge about brucellosis?

yes no

Are there separate parturition (maternity) pens?

yes no

Do you separate cows during parturition?

yes no

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

_____

_____

_____

How do you dispose off the after birth?

_____

_____

Where do you get your replacement stock?

(1) buy in
(2) raise own replacement
(3) both

What do you do with known *Brucella* infected animals?

_____

_____

_____

Have you ever observed abortions in the farm?

yes no

How many abortions have you observed in the last three years?

_____

Did the farm been tested for brucellosis since inception/when?	yes	no
Did vaccinations for brucellosis been carried since inception?	yes	no
Are dogs kept in the farm?	yes	no

Farm inspection report

housing

Building material used is

(1) traditional
(2) modern
(3) mixture

Housing type

(1) pen
(2) barn
(3) stanchion
(4) freestall

Floor type

(1) earth
(2) concrete
(3) stone
(4) other

Does the building structure allow sunshine into the building?	yes	no
Is the roof structure rain proof	yes	no
Cow stalls are free of hazards	yes	no
Parturition pens are clean and dry and well bedded?	yes	no

Dry cow facilities are clean yes no
Cow stalls are of adequate size (220cm × 110cm)? yes no
The drainage system
(1) excellent
(2) satisfactory
(3) poor
Ventilation in the building
(1) excellent
(2) satisfactory
(3) poor
General farm hygiene and orderliness
(1) excellent
(2) satisfactory
(3) poor

cow cleanliness

Soiling of the udders
(1) not apparent
(2) moderate
(3) serious
Flies on the cows
(1) negligible
(2) moderate
(3) too much
Is hair clipped from the udder
yes no

Format to investigate occurrence of brucellosis in man

The number of milkers, other workers in the farm: _____
_____

Has any member of the family, milker , and worker visited a health institution in the last six months?
yes no
If yes for what health problem? _____
_____
_____

Has any member of the family/milker/worker shown symptoms of prolonged fever since starting keeping dairy cattle and for how long?
yes no
Has any member of the family/milker/worker shown the following symptoms since starting keeping dairy cattle?

headache	yes	no
insomnia	yes	no
pain over the spine	yes	no
vague generalized pain or aches	yes	no
pain over the joint	yes	no
pain on testes	yes	no
nervous disorders	yes	no

ANNEX 4 DESCRIPTION OF FARM CATTLE STRUCTURE IN THE STUDY SITES*

DISTRICT	FARM NO.	Herd size	Lact. Cows	Dry Cows	Preg. Cows	Tested	RBPT+/CFT+
DEBRE ZEIT	2	7	1	1	4	5	negative
"	3	7	3	2	3	6	positive
"	4	7	6	1	1	9	positive
"	5	5	1	0	1	3	negative
"	6	5	2	0	1	3	negative
"	9	8	3	0	1	4	negative
"	10	7	2	0	0	6	negative
"	11	9	4	1	0	12	positive
"	12	5	2	0	3	4	positive
"	13	9	3	1	4	7	negative
"	14	46	21	4	15	28	positive
"	15	6	2	0	1	5	negative
"	16	6	1	0	0	3	negative
"	19	4	3	0	3	4	negative
"	20	5	1	0	0	4	negative
"	21	4	3	0	0	7	positive
"	22	2	0	1	1	3	negative
"	23	2	0	1	1	2	negative
"	24	5	3	0	2	6	negative
"	25	4	2	0	1	3	negative
KALITI	1	99	38	8	32	75	positive
"	2	79	30	11	23	57	positive
"	3	71	23	9	4	58	positive
SEBETA	1	80	26	17	12	72	positive
"	2	61	20	7	16	62	positive
"	3	326	134	69	18	291	positive
ADDIS ABABA	1	112	58			87	positive
"	2	71	24	23	12	62	positive
"	3	5	1	1	1	5	negative
"	4	8	3	1	0	5	negative
"	5	5	1	1	1	3	negative
"	6	2	1	0	1	3	negative
"	7	4	0	0	2	5	negative
"	9	4	1	0	2	4	negative
"	10	3	2	0	1	3	negative
"	11	3	1	1	1	4	negative
"	12	1	0	1	1	4	negative
"	13	5	3	0	1	4	negative
"	14	2	1	0	0	1	negative
"	15	3	2	0	0	4	negative
"	16	11	7	0	0	9	negative
"	17	6	4	1	3	8	negative
TOTAL	42	1114	443	162	173	950	14

* herd parameters were taken in October

CURRICULUM VITAE

Name	Yilkal Asfaw
Sex	Male
Age	30
Marital status	Single
Place of birth	Feres Bet Michael, Gojjam, Ethiopia.

Language

1) Amharic, speaking and writing
2) English, speaking and writing
3) Deutsch, speaking and writing

Education

1) Elementary: Feres Bet Elementary and Junior Secondary School, Gojjam, Ethiopia.
2) High School: Damot Comprehensive Secondary School, Gojjam, Ethiopia.
3) Undergraduate study: Faculty of Science and Faculty of Veterinary Medicine of the Addis Ababa University.
4) Postgraduate study: Freie Üniversität Berlin and Addis Ababa University.

Academic Awards

1) Ethiopian School Leaving Certificate Examination
2) A Degree of Doctor of Veterinary Medicine (DVM)

Work Experience

1) Assistant lecturer and lecturer at the Faculty of Veterinary Medicine from 1989-1995. In this time I have handled General Medicine, Large Animal Medicine, and Veterinary Clinical Diagnosis as junior lecturer.
2) Meat Inspection at the abattoir of the Faculty of Veterinary Medicine.

Research Activities

1) Hydatidosis in cattle, sheep, and pigs around Dessie.
2) The efficacy of Kosso (*Hagenia abyssinica*) on *Taenia hydatigena*.
3) A monograph: Veterinary Therapeutic Guide
4) Major causes of carcass condemnation at the abattoir of the Faculty of Veterinary Medicine, Debre Zeit.
5) The epidemiology of bovine brucellosis in intra- and peri-urban dairy production systems in and around Addis Ababa.

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I the under signed, declare that the thesis is my original work and has not been presented for a degree in any University.

Name Yilkal Asfaw

Signature

Date of submission 7. 1. 1998

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

Prof. Zessin

Dr.Bayleyegn

1998
YIL/1415

The epidemiology of bovine brucellosis in intra- and peri-Urban dairy production systems in & Around Addis Ababa.

Yilkal Asfaw

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