ASSESSMENT OF MAJOR REPRODUCTIVE PROBLEMS OF DAIRY CATTLE IN SELECTED SITES OF CENTRAL ZONE OF TIGRAI REGION, NORTHERN ETHOPIA

By

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BIOGRAPHICAL SKETCH

Bahlibi was born in 1982 from his father Weldegebriall Sahlu and his mother Ametemichael Aregawi in Eastern zone, Tigray region, Northern Ethiopia in a district called Saesie Tsaeda Emba, a place named Edagahamus. He attended his primary school in Mealfiat and Welwalo starting 1995 and attended his high school in Edagahamus senior secondary school until 2003. After he completed his high school he joined Hawassa University in 2003 and attended Veterinary Medicine until 2008. After graduation he worked in Dalol Bureau of Agriculture, in Afar region as Animal health expert for seven month and also in Tigray Agricultural Research Institute, in position as associate animal health researcher up to date. He again joined Mekelle University College of Veterinary Medicine to study his M.Sc. in Veterinary Reproduction and Obstetrics from September 2013 to date.

DEDICATION

This thesis is dedicated to my wife, **Zebib Lema** and our children **Blen** and **Saron Bahlibi.**

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LIST OF ABBREVIATIONS

AHA	Animal Health Australia
AI	Artificial Insemination
c-ELISA	Competitive Enzyme Linked Immuno-Sorbent Assay
CFSH	Center for Food Security and Public Health
CFT	Complement Fixation Test
ELISA	Enzyme Linked Immuno-Sorbent Assay
FAO	Food and Agricultural Organization
FAWC	Farm Animal Welfare Council
IBM	Interim Brucellosis Manual
i-ELISA	Inhibition Enzyme Linked Immuno-Sorbent Assay
Ig	Immunoglobulin
ILCA	International Livestock Center for Africa
LIVES	Livestock and Irrigation Value Chain for Ehiopian Smallholders
MRAHDIL	Mekelle Regional Animal Health Diagnosis and Investigation Laboratory
MR	Milk Ring Test
MZN	Modified Ziehl- Neelsen
OIE	Office of International de Epizootics
PA’s	Peasant Associations
PGF2	Prostaglandin F2 alpha
RB	Repeat Breeding
RBPT	Rose Bengal Pate Test
RBS	Repeat Breeding Syndrome
RFM	Retained Fetal Membrane
SAT	Serum Agglutination Test
WHO	World Health Organization

ABSTRACT

A cross sectional study to identify the prevalence of major reproductive problems of dairy cattle and the possible risk factors in selected sites of Central zone of Tigrai Region from September 2014 to April 2015 was conducted. In this study a total of 120 respondents were interviewed and the finding revealed that the most frequently encountered reproductive health problems are repeat breeding, anoestrus, retained fetal membranes, abortion, uterine prolapsed, and testicular swelling as responded by 26.7%, 20.0%, 5.8%, 5.8%, 28.3%, and 26.7% of the interviewee. Similarly a record of 265 dairy cows was examined to determine the reproductive problems of dairy cattle and associated risk factors and the finding indicated that 19.3% of the cattle have reproductive problems. The major reproductive problems found were 9.1% repeat breeding, 4.2% anoestrus, 3.8% abortion, 1.1% RFM, 0.8% pyometra and 0.4% uterine prolapse. In addition, a total of 414 blood samples for sera were collected from both sexes and greater than 6 months age to determine sero-prevalence and associated risk factors of bovine brucellosis in the study area. The Rose Bengal Plate Test (RBPT) was used on sera as a screening test for brucellosis. The prevalence of bovine brucellosis found was 0.0%. Analysis of risk factors of major reproductive problems from the retrospective study showed that except in the case of sanitation and pregnancy status, the overall prevalence of reproductive problems were significantly ($p<0.05$) influenced by breed, location, lactation status, production system, age, parity, body condition score, herd type, herd size and housing system. Generally the current finding revealed that reproductive health problems commonly exist in the study area through their percentage and types vary from time to time; hence, regular reproductive health management and proper formulation of ration could be the possible solutions to alleviate the problems encountered in different production systems.

Key words: Abortion; breed; brucellosis; repeat breeding; anoestrus

1. INTRODUCTION

Cattle production is the main component of agricultural growth in many parts of sub-Saharan countries. The overall cost of keeping cattle is associated with the health care, nutrition and reproduction management, however, has not matched to their contribution to the livelihood and economy of the people in sub-Saharan countries. As in many other countries, livestock, particularly cattle play an important role in Ethiopia as being a source of milk, meat, hide, etc (Mekonnen *et al*., 1989). However, the productivity of indigenous cattle breeds is low due to many constraints including diseases and parasites, nutrition, poor management systems, poor reproductive performance and large socio-economic factors by decreasing reproductive efficiency, shortening the expected length of productive life and by lowering milk production. Reproductive problems are the most common which occur in lactating dairy cows and can dramatically affect reproductive potential of the dairy herd. Poor reproductive performance is a major cause of involuntary culling and therefore reduces the opportunity for voluntary culling and has a negative influence on the subsequent productivity of a dairy herd (Tegegne, 1989; ILCA, 1988, 1998; Hossein-Zadeh, 2013).

The reproductive problems result in heavy economic losses and have been public health concern in dairy farms. Reproductive problems are the main causes of poor productive performance in smallholder dairy farms (Roberts, 1986; Bekena *et al*., 1994, 1997; Arthur *et al*., 1996). Among the major reproductive problems that have a direct impact on reproductive performance of dairy cows, retained fetal membrane (RFM), bovine brucellosis, repeated breeding, abortion, anoestrus, dystocia, endometritis, prolapse(uterine and vaginal) and pyometra have been reported to be the most common economic problems (Hadush *et al.,* 2013; Dinka, 2013; Haile *et al*.,2014). These reproductive problems could also be classified as before gestation (anoestrous and repeat breeding), during gestation (abortion, vagina prolapsed and dystocia) and after gestation (RFM and uterine prolapsed).The impaired function of the reproductive system results failure of a cow to produce a calf yearly and regularly (Arthur *et al*., 1989; Hoojjar *et al*., 1999; Shiferaw *et al*., 2005; Lobago *et al*., 2006).

It has been reported that among infectious diseases, brucellosis is a common genital disease which induces abortion in animals and humans. Brucellosis is an infectious bacterial zoonotic disease affecting both humans and animals causing a serious economic loss in animal production and deterioration of public health (Arthur *et al*., 1996; Richey and Dix Harrell, 1997; Moti *et al*., 2012) by inducing abortion. It is a chronic infectious disease of cattle that causes birth of weak or dead calves, infertility and, as a consequence, reduced milk production (AHA, 2005). Studies conducted in Ethiopia (Shiferaw *et al*., 2005; Hadush *et al*., 2013; Dinka, 2013; Haile *et al*., 2014)revealed poor reproductive performance of dairy cows in many parts of the country.

1.1. Statement of the Problem

The livestock production system, especially the dairy production, is the most important issue in central zone of Tigrai region, where dairy cows and their products are the main sources of income and food. Central zone is a large, historical place in the country and majority of the people live concentrated. The zone is also a highly populated area in the region. The demand of dairy and dairy products is coming greater and greater. But production constraints, mainly reproductive health problems, make a bottle neck in its productivity. Therefore it is important to generate useful information on the major reproductive problems of dairy cattle in the study area. Moreover, no investigation has been done on the presence and risk factors of reproductive problems of dairy cattle in the present study area, which gave impetus to the initiation of this study. Thus, the objectives of this study were to determine the prevalence of major reproductive problems of dairy cattle and to identify the possible risk factors for the occurrence of the problems in selected sites of Central Zone of Tigrai Region, Northern Ethiopia.

2. LITERATURE REVIEW

2.1. Reproductive Problems of Dairy Cattle

Reproductive disorders are the most common problems affecting the production and productivity of dairy cows (Dinka, 2013). The presence of the reproductive problems result in considerable economic loss to the dairy industry due to lesser number of calves crop, slower uterine involution, prolonged inter-conception and calving interval, early depreciation of potentially used cows, decreased milk yield per lactation as well as overall lifetime production, and increased costs due to veterinary services and earlier culling of cows (Lobago *et al.,* 2006; FAWC, 2009).

Reproductive and production disorders of cross-bred dairy cattle significantly reduce their productivity because of reproductive disorders. These disorders are of great concern of dairy producers because of their effect on the future fertility of dairy cows (Khair *et al*., 2013). The major reproductive problems that have most important influence on reproductive performance of dairy cows are include abortion, dystocia, RFM, metritis, prolapse (uterine and vagina), mastitis, anoestrus, bovine brucellosis and repeated breeding. These reproductive problems could be categorized as prepartum and postpartum reproductive problems (Shiferaw *et al*., 2005; Lobago *et al*., 2006; Dinka, 2013; Benti and Zewdie, 2014).

2.1.1. Repeat breeding

Repeat breeding (RB) is defined as a cow that did not conceive after three or more consecutive inseminations with a fertile bull or fertile semen, despite, it comes normally in heat and shows clear estrous signs with no clinical detectable reproductive disorders or without anatomical abnormalities and infections by exhibiting a variety of reproductive disturbances (Bage *et al*., 1997; Perez-Marin and Espana, 2007; Yusuf *et al*., 2010). Repeat breeder syndrome (RBS) is a major economic loss in the dairy industry due to greater insemination costs and increased calving interval. Repeat breeder animals exhibit normal cycle every 18 to 24 days without any clinical abnormalities, but fail to conceive even after at least three successive artificial inseminations (Kumar *et al*., 2014).The reproductive objective of dairy farms is to produce one calf in a year when dairy cows become pregnant in a single insemination having parturition after 270 days and inseminated again for second pregnancy within two months. But, this is not always possible and cows must be re-inseminated many times in several consecutive estrus cycles (Opsomer *et al*., 1996). Gupta and Deopurkar (2005) reported incidence rate of repeat breeding (5-30%) in cows. Repeat breeding is commonly associated with improper management of reproduction than hormonal inherited problems in the dairy cows (Bage *et al*., 1997; Perez-Marin and Espana, 2007).

Nutritional insufficiency, poor management, natural servicing with sub-clinically infected bulls or heat stress could also be the potential causes of repeat breeding (Ahmed, 2009). Early embryonic deaths in cows may be associated with repeat breeding because of the embryonic mortality mostly occurs earlier in pregnancy. Maurer and Echternkamp (1985) reported that there is a higher prevalence of RBS in heifers (15.1%) than multiparous females (8.3%) in beef cattle. The effective treatment of repeat breeder cows could be uterine lavage plus PGF2α because of some advantages, such as no milk waste or side effects on the endometrium, and may have improved the conception rate (Ahmadi and Dehghan, 2007).

2.1.2. Abortion

Abortion is defined as the premature expulsion of fetus between 42 days (the estimated time of attachment and approximately 260 days of gestation (the age of the fetus in which fetus can survive outside of the uterus). Abortion is the most common problem of dairy cows which limit the cow's ability to produce a calf yearly and can largely affects the profit of the dairy farm (Peter, 2000). Sarder *et al*. (2010) also defines abortion as a condition in which fetus is delivered live or dead before reaching the stage of viability where the delivered fetus is visible by naked eyes. The diagnosis of abortions is a major challenge to the herd owner and veterinarian. There is sudden and dramatic increase of abortion in herds over a long period of time. For this reason, prompt and thorough action is required when abortions do occur. Breeding dates, parity, production information and health events (*e.g.,* disease or vaccination) can all help to identify factors which may be associated with the abortions (Hossein-Zadeh, 2013). Other herd level information such as ration changes, new additions, personnel changes, etc., should also be recorded.

Numerous bacterial, viral, protozoan and fungal pathogens have been associated with infertility and abortion in cattle. These pathogens can result in substantial economic losses, indicating the need for control measures to prevent infection or disease. Embryonic or fetal death may result in resorption, mummification, maceration, or abortion. Factors that impact the outcome of embryonic fetal death includes gestation, age, cause of death and source of progesterone for pregnancy maintenance. Aborted fetuses may be autolytic due to death within 24–48 h before abortion (Givens and Marley, 2008).

Table 1: Infectious Causes of Abortion in Cattle

Bacterial	**Fungal**	**Protozoan**	**Viral**
Campylobacter fetus	*Aspergillus fumigatus*	*Neospora caninum*	*Bovine herpesvirus1*
Histophilus somni	*Mucor spp*	*Tritrichomanas fetus*	*Bovine viral diarrhea virus*
Ureaplasma spp.	*Morteriella wolfii*	*Toxopllasma gondii*	*Bluetongue virus*
Brucella abortus		*Anaplasma marginale*	*Epizooticcbovine abortion*
Leptospira spp.			*Akabane virus*
Listeria monocytogenes			
Arcanobacterium pyogenes			
Chlamydophila spp			
Salmonella			
Coxiella burnetti			

Source: Givens and Marley, 2008.

The low reproductive efficiency in normal bovine populations is due to abortion. Spontaneous abortion of dairy cows is most common problem that contributes substantially to low herd viability and decreasing production potential by reducing the number of potential female herd replacements and lifetime milk production, and by increasing costs associated with breeding and premature culling (Thurmond *et al.*, 2005). Fetal mortality in pregnant cows between 35 and 45 days of gestation ranges from 8 to 10% (Forar *et al.*, 1996), with abortion often exceeding 14% in some herds (Thurmond *et al.*, 1990; Hossein-Zadeh *et al.*, 2008). The primary focus of abortion is prevention of infectious diseases because of the infectious agents probably cause less than half of the fetal deaths. The cost of abortion depends mainly on the time of gestation, milk production, days in milk, the time of insemination after parturition, the cost of nutrition, sperm costs, insemination time, and labor costs (Rafati *et al.*, 2010). Early pregnancy abortions could result in increased days open. The loss of potential replacement heifers and early culling of productive cows is mainly due to late term abortions. Moreover, extended calving intervals may result in a loss of 2-5% of the herd's potential calf production.

Infectious agents (bacteria, viruses, protozoa, and fungi), toxic agents, heat stress, genetic abnormalities (Hovingh, 2009), twin pregnancies (Nielen *et al.*, 1989) and mastitis (Santos *et al.*, 2003) are some of the causes of abortion. Jamaluddin *et al.* (1996) reported infectious agents (37.1%), noninfectious agents (5.5%) and undetermined causes (57.3%) from 595 abortion submissions in California. The three most commonly diagnosed infectious agents were bacterial (24%), fungal (7%), and viral (6%) as reported by Khodakaram- Tafti and Ikede (2005) in Canada. Control of these infectious factors is achieved by appropriate vaccination programs.

The complex vertebral malformation (CVM) gene (Agerholm *et al.*, 2001) is one of the genetic abnormalities that cause abortion in Holsteins which results in malformations from middle to late gestation period. Non-infectious factors such as genetic and non-genetic disorders have been reported in some investigations. The most important non-genetic factors are heat stress, production stress, seasonal effect and seasonal changes (Labernia *et al.*, 1996; Markusfeld- Nir, 1997; Hansen, 2002; López-Gatious *et al.*, 2002; Sani and Amanloo, 2007). The most important genetic disorders include chromosomal and single gene disorders and these disorders resulted in high abortion rate in cows and increased calf sterility (Geoffrey *et al.*, 1992). Cow parity, sire effect, age at conception and abortion history could be some of the non-infectious maternal and paternal factors that cause fetal death (Thurmond *et al.*, 1990, 2005; Markusfeld-Nir, 1997; Hanson *et al.*, 2003; Lee and Hwa Kim, 2007).

2.1.3. Dystocia

Dystocia is defined as an abnormal and difficult birth in which the first or specially the second stage of parturition was markedly prolonged and subsequently found impossible for the dam to deliver without artificial aid (Benti and Zewdie, 2014). Dystocia is most commonly known as difficult calving and defined shortly as prolonged or difficult parturition is a problem where most of the dairy producers encountered (Mee, 2004, 2008). Improper cervical dilation, failure of uterine expulsive forces (uterine inertia) and neoplasms of vagina, vulva and uterus could be the maternal causes of dystocia in cows (Purohit *et al.*, 2011). Assistance at calving (including lower degrees of difficulties) is much more prevalent, varying from 10% to over half of the calving than severe or considerable difficulty in calving vary from just below 2% to over 22% (Mee, 2008).

The fetal oversize and fetal abnormalities are major causes of dystocia. Fetal oversize is common in heifers, cows of beef cattle breeds, prolonged gestations, increased calf birth weight, male calves and perinatal fetal death with resultant emphysema. Monsters, fetal diseases and fetal mal-dispositions are fetal abnormalities resulting in difficulty to deliver such fetuses because of their altered shape. Hydrocephalus, ascites, anasarca and hydrothorax are the most common diseases of fetus resulting in dystocia. The most common cause of dystocia in cattle seems to be fetal maldispositions, of which limb flexion and head deviation appear to be the most frequent (Purohit *et al.*, 2012).

The most common causes of dystocia in dairy cattle results from maternal and foetal factors. Foeto-pelvic incompatibility (FPI) is the most common reason for dystocia which results from a physical incompatibility between the pelvic size of the mother and the size of the calf at birth (Meijering, 1984; Mee, 2008). This is highly influenced by the weight and morphology of the dam and the calf, respectively. The pelvic area available at birth is affected by the size of pelvis and fatness of the dam which might partially obstruct the birth canal. The calf's physical factors such as a calf of a big size or mal-presentation contribute to a size of mismatch between the calf and the dam. These morphological factors also depends on different variables including age, breed and parity of the dam, twinning, the sex and weight of the calf, the sire and breed of the calf as well as the nutrition of the dam during gestation (Meijering, 1984; Hickson *et al.*, 2006; Mee, 2008; Zaborski *et al.*, 2009; Hossein-Zadeh, 2010). Lack of uterine contractions (weak labour), incomplete dilation of the cervix and vagina due to stenosis (narrowing and stiffening of the tissue) and uterine torsion are other causes of dystocia exhibiting repelling force to expel a calf during delivery. Hormonal imbalances such as reduction in plasmatic oestradiol concentration, high levels of oestradiol-17ß at parturition or high ratios of cortisol to progesterone are risk factors for the lack of uterine contractions. The hormonal imbalances in the uterus reduce the expression of oxytocin receptors in the uterus by changing the preparation of the soft tissues resulting in weak uterine contractions and weak dilatation of soft tissues (Sorge *et al.*, 2008).

2.1.4. Pyometra

Pyometra is defined as an accumulation of pus in the uterus resulting in infertility and postpartum anoestrus (Ael-G and Fahmy, 2011).In cows; the presence of persistent corpus luteum could be associated with pyometra. In cattle, infectious causes of pyometra includes *Campylobacter spp*, *Staphylococcus spp* and *Streptococcus spp* bacteria, as well as protozoa including *Tritrichomonas spp* and *Brucella spp* (Foldi *et al.*, 2006).

The common clinical signs are decreased milk yield, dullness or other signs of toxemia, including fever and purulent vaginal discharge. Laboratory tests showing a leucocytosis may help to establish a diagnosis (Sheldon *et al*., 2006). Administration of PGF2α or its analogs at normal luteolytic doses and Lavage of the uterus using large volumes of fluid are the appropriate treatments, but the condition frequently recurs, and permanent cure in these cases requires hysterectomy.

2.1.5. Retained placenta

Retention of placenta is the inability of fetal membrane to be expelled within 8 hrs after parturition and ranging of the retention from 8 to 48 hours post partum (Beagley *et al*., 2010). The normal physiological stages of birth during parturition include dilatation of parturient canal, delivery of the fetus and expulsion of the fetal membranes. In normal condition, fetal membranes are usually expelled within two to eight hours of parturition. Any retention of fetal membranes beyond 12 hours could be considered as pathological (Wetherill, 1965). The incidence of retained placenta varies from 4-18% of calving (Paisley *et al*., 1986; Eiler, 1997; Noakes *et al*., 2001). The uterus normally contracts approximately fourteen times an hour immediately following parturition but the frequency gradually diminishes to once every hour at 42 hours. Delayed involution of the uterus is usually associated with retention of membranes. Retained placenta had a significant negative effect on milk yield for several weeks after calving (Rajala and Gröhn, 1998; Lucey *et al*., 1986). The interval from calving to first service and conception were higher in the retained placenta and increases the risk of fatty liver syndrome and ketosis (Han and Kim, 2005).

Retained placenta delays the postpartum resumption of cyclic ovarian function and prolongs the interval from calving to first ovulation (Opsomer *et al*., 2000). Early or induced parturition, dystocia, hormonal imbalances, and immune-suppression are risk factors in interrupting the normal process resulting in retention of the placenta. Systemic administration of antibiotics can be beneficial in treating metritis and collagenase injection enhances placenta release during fetal retention (Beagley *et al*., 2010).

2.1.6. Uterine prolapse

Uterine prolapse is a non-hereditary abnormal complication of the uterus usually expressed as expulsion of the uterus through the vulva to the outside of the body which is occurring immediately after parturition and occasionally up to several hours afterwards (Roberts, 1971; Cuneo *et al*., 1993). Gustafsson *et al.* (2004) also defines uterine prolapse as a protrusion of the uterus from the vulva with the mucosal surface exposed. Uterine prolapsed occurs in the third stage oof labour when fetus is expelled and fetal cotyledons separated from the maternal caruncles (Noakes *et al*., 2001). Uterine prolapse is a common complication of the third stage of labour in the cow during parturition (Joseph *et al*., 2001). Uterine prolapse is generally a complete inversion of the gravid cornua in ruminants (Arthur *et al*., 1996).

Uterine prolapse is a one the most common obstetrical problem, affecting productive and reproductive performance of cattle by reducing postpartum return to estrus, conception rate and calving interval in dairy cattle (Kumar and Yasotha, 2015*)*. Uterine prolapse is one of the most important complications associated with calving in dairy cows. Uterine prolapsed is usually associated with hypocalcaemia or milk fever, poor uterine tone, increased straining, weight of the retained fetal membrane, tympany and excessive estrogen content in the feed (Jackson, 2004; Hanie, 2006; Kumar and Yasotha,2015). Cows recovered from uterine prolapse can become pregnant with a post operative fertility rate of 40-60% (Tyagi and Singh, 2002) when treatment is successful. The successful treatment in uterine prolapsed cases depends on type of case, duration of case, degree of damage and contamination. Administration of injectable broad spectrum antibiotics(Ceftiofur sodium 2mg/kg) for three to five days after replacement of the prolapsed uterus can prevent secondary bacterial infection (Hosie, 1993; Plunkett, 2000; Borobia-Belsue, 2006) so that prolapsed animals could become properly recovered from the problem and can conceive again without problems.

2.1.7. Anoestrus

Anoestrus is defined as failure of cows to exhibit overt estrus but is more commonly a problem with estrus detection (Johnson, 2008). The anoestrus is usually associated with the presence of inactive ovaries even in the presence of follicular development where none of the growing follicles become mature enough to ovulate (Montiel and Ahuja, 2005). Anoestrus is categorized in to four clinical forms: silent heat; cystic ovarian disease; ovarian afunction and corpus luteum pseudo-graviditatis (Mwaanga and Janowski, 2000; Zdunczyk *et al*., 2002). Anestrus is a result of managerial, physiological, pathological and nutritional factors. These factors include age, breed, pre- and postpartum nutrition, body condition at calving, milk yield, suckling, calving season, presence or absence of the bull, delayed uterine involution, dystocia and general health status influence duration of postpartum anoestrus (Short *et al*., 1990; Yavas and Walton, 2000; Webb *et al*., 2004; Hess *et al*., 2005; Montiel and Ahuja, 2005; Peter *et al*., 2009). The incidence of post partum anoestrus (10-40%) varies from herd to herd as reported by many researchers (Martinez and Thibier, 1984; Mwanga and Janowski, 2000; Zdunczyk *et al*., 2002). Treatment of anoestrous depends on cause, diagnostic facility, availability and efficiency of drugs, response of the animal to the drug, dose of administration and health status of the animal.

2.1.8. Status of some reproductive disorders of dairy cows in Ethiopia

Reproductive efficiency is one of the most important factors impacting the profitability of the cow-calf operation and is largely dependent on maintaining a short breeding and calving season and increasing calf crop in the dairy farms (Ahmadzadeh *et al*., 2011). The high economic loss to the dairy industry occurs due to slower uterine involution during parturition, reduced reproductive rate, prolonged inter-conception and calving interval, negative effect on fertility, increased cost of medication, drop in milk production, reduced calf crop and early depreciation of potentially useful cows (Gebremariam,1996; Lobago *et al*., 2006). Studies on major reproductive problems of cattle in different parts of the country has shown the presence of the reproductive problems (Shiferaw *et al.*, 2005; Haftu and Gashaw, 2009; Bitew and Shiv, 2011; Dinka, 2013). The study conducted by Haftu and Gashaw (2009) on major clinical reproductive health problems of 217 dairy cows in and around Bako of West Ethiopia showed that 30.4% of the total dairy cows affected with one or more clinical reproductive problems. A study of the major reproductive health disorders of dairy cows in ILCA and Almaz dairy farms in Ada'a district, Debre Zeit town in East Shoa showed that 37.1% of them had at least one of the reproductive disorders (Esheti and Moges, 2014). Other study conducted in Addis Ababa Milk shed on major reproductive disorders in cross breed dairy cows under small holding indicated that an overall observed prevalence of 67.8% of reproductive health problems (Haile *et al.*, 2010).

A study conducted by Dinka (2013) showed that 18.3% of dairy cattle have been affected by either one or more reproductive disorders based on questionnaire interviews in and around Assella in Central Ethiopia. A retrospective study by Hadush *et al.* (2013) revealed that 44.3% of the cows were found with major prepartum and postpartum reproductive problem from 711 cows in three selected farms in Debre Zeit town. Another study conducted by questionnaire and observational survey in urban and per urban area of Hossana indicated that 43.07% of prevalence on major reproductive health problems of dairy cattle (Haile *et al.*, 2014). A study in and around Bedelle showed a prevalence of 26.5% of reproductive problems in South west Ethiopia (Bitew and Shiv ,2011) and 8.7% and 18.3% abortion and retained fatal membrane respectively in selected sites of Arsi zone (Degefa *et al.*, 2011). A prevalence of 40.3% was reported at Kombolcha town in Noth east Ethiopia by Dawit and Ahmed (2013). A study conducted by Gizaw *et al.* (2007) and Benti and Zewdie (2014) also reported 37.76% and 47.7% in Nazareth town of central Ethiopia and Borena zone in Southern Ethiopia, respectively. A study conducted by Simenew *et al.* (2011) on major gross reproductive tract abnormalities in female cattle slaughtered at Sululta Slaughterhouse reported 1.6% prevalence rate of pyometra. Reproductive problems are most common in smallholder dairy cows (Gizaw *et al.*, 2007). In order to reduce these problems formulation of strategic control measures, including health education about the disease transmission, treatment and control has to be introduced to reduce reproductive wastage and their risks factors (Dinka, 2013).

2.1.9. Bovine brucellosis

2.1.9.1. Definition

Brucellosis is a bacterial disease of animals and humans caused by the genus *Brucella*. *Brucella abortus* is a contagious disease of cattle, but occasionally sheep, swine, dogs and horses may also be affected. In horses, *Brucella abortus* together with *Actinomyces bovis* is commonly present in poll evil and fistulous withers (Roberts, 1971; Radostits *et al*., 2000).

2.1.9.2. Epidemiology

In cattle and other Bovidae, *Brucella* is usually transmitted from animal to animal by contact following an abortion, pasture or animal barn contamination, ingestion, inhalation, conjunctival inoculation, skin contamination udder inoculation from infected milking cups and the use of pooled colostrums for feeding newborn calves. Sexual transmission and artificial insemination can transmit the disease and semen must only be collected from animals known to be free of brucella infection (FAO, OIE and WHO, 2006).

2.1.9.3. Etiology

Brucellosis in cattle is primarily caused by the bacterium *Brucella abortus,* which is one of six species of the genus *Brucella*. Nine biotypes have been identified, all of which are intracellular, parasitising, gram-negative and facultative intracellular coccobacillus or short rods. *Brucella* has a wide host range but cattle are the preferred host of *B. abortus* (AHA, 2005). Six named species occur in animals: *B. abortus*, *B. melitensis*, *B. suis*, *B.*

ovis, *B. canis* and *B. neotomae*. One or more unnamed species of *Brucella* have been found in marine mammals. Formal names proposed for marine mammal isolates are *B. maris* for all strains, or *B. pinnipediae* for strains from pinnipeds (seals, sea lions and walruses) and *B. cetaceae* for isolates from cetaceans (whales, porpoises and dolphins). Some species of *Brucella* contain biovars. Five biovars have been reported for *B. suis,* three for *B. melitensis*, and up to nine for *B. abortus*. Each *Brucella* species is associated most often with certain hosts. *B. abortus* usually causes brucellosis in cattle, bison and buffalo (CFSPH and OIE, 2009).

2.1.9.4. Transmission

The risk associated with exposure of susceptible animals to the disease following parturition or abortion of infected cattle depends on three factors: the number of organisms excreted, the survival of these organisms under the existing environmental condition and the probability of susceptible animals being exposed to enough organisms to establish infection. B. *abortus* achieves its greatest concentration in the contents of the pregnant uterus, the fetus and the fetal membranes after birth (Radostits *et al*., 2000).

In animals, *B. abortus* is usually transmitted from infected animals to healthy animals by contact with the placenta, fetus, fetal fluids and vaginal discharges. Infected animals after abortion or full-term parturition could be infectious for the other healthy animals. *B. abortus* may also be present in the milk, urine, semen, feces and hygroma fluids. Shedding in milk may be prolonged or lifelong, and can be intermittent. Many infected cattle can become chronic carriers. Infection with B. *abortus* can also be transmitted by ingestion or through mucous membranes or through broken skin. Mammary gland is usually colonized during the course of an infection and may be infected by direct contact because of subsequent shedding of the organisms in the milk (CFSPH, 2009).Humans are infected from drinking raw or un-pasteurized infected milk, from exposure to infected discharges or tissues (Roberts, 1971). *B. abortus* can be spread on through feed, water and by contaminated semen during artificial insemination when semen is deposited in the uterus but not in the mid cervix. In conditions of high humidity, low temperatures, and no sunlight, the *Brucella* organisms can survive in water, aborted fetuses, manure, wool, hay, equipment and clothes for several months. *Brucella* species can resist drying and survive in dust and soil if there is an organic material in the environment. Survival rate of *Brucella* organism is longer in low temperatures especially in deep freezing (CFSPH, 2009).

2.1.9.5. Immunity

2.1.9.5.1. Humoral immune response

Naturally infected animals and those vaccinated as adults with strain 19 remain positive to theserum and other agglutination tests for long periods. The serum of infected cattle contains highlevels of IgG1, IgG2, IgM, and IgA isotypes of antibody (Radostits *et al.*, 2000). Similar isotypes at different relative concentrations occur in milk, although most of the IgA is present in secretory form. The first isotype produced after an initial heavy infection or strain 19 immunization is IgM and is soon followed by IgG antibody. IgG1 immunoglobulin is the most abundant in serum and exceeds the concentration of IgG2. 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 immunization with a standard dose of strain 19 during calf hood, IgG antibody concentrations usually decline to diagnostically insignificant levels over 3-6 months. Residual antibody if present, is usually predominantly of the IgM class. Following exposure to virulent Br. *abortus*, antibody may appear in 4-10 weeks or longer, depending on the size and route of entry of the inoculums and the stage of pregnancy of the animal. Antibodies of IgG, IgM, IgG1 and IgG2 isotypes can all react in the tube agglutination test, but those of the IgM class are by far the most efficient (WHO, 1986; WHO, 1997; Tolosa, 2004).

2.1.9.5.2. Cellular immune response

Brucella species are facultative intracellular pathogens. They are readily phagocytised by macrophages and polymorphonuclear leukocytes and, in the case of virulent strains, are capable of surviving within these 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 phagocytic cells. Macrophage activation occurs when T-lymphocytes of the appropriate subset are stimulated to release lymphokines (interleukins) (WHO, 1986; WHO, 1997). 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. The role of cytotoxic cells, including cytotoxic T-lymphocytes, natural killer (NK), and killer (K) cells, in the cell-mediated immune response to Brucella has not been elucidated. Further studies are also needed to determine the basic processes underlying the development of protective immunity to Brucella in the natural host species (WHO, 1986; WHO, 1997, Tolosa, 2004).

2.1.9.6. Occurrence and prevalence of infection

Brucellosis has a worldwide distribution and poses a major threat to sub-Saharan countries including Ethiopia (FAO, 2009). It is one of the economically important disease in livestock and people in this region. Brucellosis has a considerable impact on animal and human health, as well as wide socio-economic impacts, especially in countries in which rural income relies largely on livestock breeding and dairy products.

Table 2: Distribution of Bovine Brucellosis in Some African Countries

Country	Host	Prevalence (%)	No. tested	Tests used	Reference
Kenya	Cattle	4.9	-	ELISA	Kang'ethe *et al*., 2000
	Cattle	3.9	-	MRT	Kang'ethe *et al*., 2000
Zambia	Cattle	20.7	395	c-ELISA	Muma *et al*., 2013
Sudan	Cattle	20	250	SAT	Senein and Abdelgadir, 2012
	Cattle	2	250	c-ELISA	Senein and Abdelgadir, 2012
	Cattle	8.4	250	RBPT	Senein and Abdelgadir, 2012
Somaliland	Cattle	1.96	153	RBPT	Ahmed, 2009
Nigeria	Cattle	5.45	220	RBPT	Bwala *et al*., 2015
Tanzania	Cattle	5.3	655	RBPT	Swai and Schoonman, 2010
Eritrea	Cattle	7.1	130	CFT	Omer *et al*., 2002
Uganda	Cattle	5	423	c-ELISA	Makita *et al*., 2011
Gambia	Cattle	1.1	465	RBPT	Unger *et al*., 2003
	Cattle	1.1	465	CFT	Unger *et al*., 2003
Senegal	Cattle	0.63	479	RBPT	Unger *et al*., 2003
	Cattle	0.63	479	CFT	Unger *et al*., 2003
Guinea	Cattle	7.8	3861	RBPT	Unger *et al*., 2003
	Cattle	5.54	3861	CFT	Unger *et al*., 2003
Guinea Bissau	Cattle	19.23	733	RBPT	Unger *et al*., 2003
	Cattle	15.14	733	CFT	Unger *et al*., 2003
Ghana	Cattle	2.93	444	RBPT	Folitse, 2014
Cameroon	Cattle	4.88	840	RBPT	Shey-Njila, 2005
	Cattle	9.64	840	iELISA	Shey –Njila, 2005

2.1.9.7. Clinical signs

The primary clinical sign of brucellosis is late-term (5–7 months) abortions in cow's and inflammation of the testis (orchitis) and lameness due to bursitis in bull. Sexually immature may remain sub-clinically infected until maturity and pregnancy without showing any sign of the disease (AHA, 2005). Brucellosis should be suspected in flocks and herds when abortions and stillbirths occur in the late term pregnancies without concurrent illness (Radostits *et al*., 2008; CFSPH and OIE, 2009). In male, localization in the testis, epididymis and accessory sex organs is common, and bacteria may be shed in the semen. This may result in acute orchitis and epididymitis and later in infertility. Arthritis is also observed occasionally in both sexes. Animals generally abort once, although reinvasion of the uterus occurs in subsequent pregnancies and *Brucella* organisms are shed with the membranes and fluids. In cattle, the consequences of *B. abortus* include abortions, stillbirths, retention of placenta and weak calves. Retention of placenta and metritis are common sequels to abortion (Walker, 1999). Females usually abort only once, presumably due to acquired immunity. In general, abortion with retention of the placenta and the resultant metritis may cause prolonged calving interval and permanent infertility.

The important signs of brucellosis especially to bulls are Epididymitis, seminal vesiculitis (Weidmann, 1991), orchitis and testicular abscesses. Infertility occurs in males and females due to orchitis /epididymitis or metritis respectively. Hygromas on the leg joints of brucella infected animal is a typical sign of the disease which is resulted due to chronic infection with Br. *abortus* (Walker, 1999). Arthritis can develop after long-term infections. Systemic signs do not usually occur in uncomplicated infections, and deaths are common in the fetus or newborn (CFSPH, 2009).

2.1.9.8. Pathogenesis

Following exposure, *Brucella* penetrates intact mucosal surfaces, and survives and multiplies in cells of the reticuloendothelial system, such as the bone marrow, lymph nodes, liver, spleen, and also kidney (Isselbacher *et al.*, 1980; Walker, 1999). Multiplication of the organisms here may last for several months, resolve itself, or be recurrent for at least two years in 5-10% of animals. Recurrence occurs particularly at the time of parturition. During the bacteraemia, organisms are carried intracellularly in neutrophils and macrophages or free in the plasma and localize in various organs, especially the gravid uterus, udder, and suprammamary lymph nodes. Localization may also occur in other lymph nodes and the spleen, testes, and male accessory sex glands. Occasionally bacterial localization occurs in synovial structures causing a purulent tendovaginitis, arthritis, or bursitis (Bishop *et al.*, 1994). The preferential localization to the reproductive tract of the pregnant animals is due to the presence of unknown factors in the gravid uterus. These are collectively referred to as allantoic fluid factors that would stimulate the growth of *Brucella*. Erythritol, a four-carbon alcohol, is considered to be one of these factors (Walker, 1999) which are elevated in the placenta and fetal fluid from about the fifth month of gestation (Bishop *et al.*, 1994). The preferential replication of Br. *abortus* in the extraplacentomal site within trophoblasts of the chorioallantoic membrane results in rupture of the cells and ulceration of the fetal membrane. The damage to placental tissue together with fetal infection and fetal stress will induce maternal hormonal changes. As a result, abortion occurs principally in the last three months of pregnancy, the incubation period being inversely proportional to the stage of development of the fetus at the time of infection (Radostits *et al.*, 2000; Tolosa, 2004).

2.1.9.9. Diagnosis

The isolation and identification of *Brucella* offers a definitive diagnosis of brucellosis. It is useful for epidemiological purposes and to monitor the progress of a vaccination programmes in animals (FAO, OIE and WHO, 2006). The method of diagnosis includes the following:

2.1.9.9.1. Direct diagnosis

- **Microscopic staining**

The disease can be confirmed by demonstration of the bacteria in smears. The smears made from vaginal discharges, placenta, colostrum, foetal stomach fluid or of the aborting cow's lochia, and the abomasum of the aborted fetus using the modified Ziehl-Neelsen stain (MZN) (Kusiluka *et al*., 1996; AHA, 2005; FAO, OIE and WHO, 2006). Impression smears may be taken from freshly cut and blotted tissue surfaces, e.g. cotyledons, by firmly pressing the slide surface against the tissue. Allow to air dry and heat fix. Smears may be made of foetal stomach fluid, cotyledons or lochia and stained with the modified Ziehl-Nielsen stain or Stamp stain. In MZN-stained smears the bacteria appear as red intracellular coccobacilli whereas most other bacteria stain blue.

- **Bacteriological culture**

All *Brucella* strains are relatively slow growing, and because the specimens from which isolations best attempted are frequently heavily contaminated, the use of a selective medium, e.g. Farrell's medium is advocated. Incubation normally continues for 72 hours, but a negative diagnosis can only be made after week long incubation. Specimens which may be used for *B. abortus* isolation include: foetal stomach fluid, spleen, liver, placenta, lochia, milk (especially colostrum or milk within a week of calving), semen and lymph nodes (supramammary (chronic and latent infections) and retropharyngeal (early infections) are preferred, but iliac, prescapular and parotid may be used). If serological reactions are thought to be caused by S19 vaccine strain then it is important to collect prescapular lymph nodes as well. All *B. abortus* isolates should be forwarded to laboratories capable of biotyping (IBM, 2013). Farrels' medium and Albimi Brucella medium are selective enriched media for isolation of Brucella species (WHO, 2006; CFSPH, 2009; OIE, 2009).

2.1.9.9.2. Indirect diagnosis

In the absence of culture facilities, the diagnosis of brucellosis traditionally relies on serological testing with a variety of agglutination tests such as the Rose Bengal plate test, the serum agglutination test, and the antiglobulin (Ruiz-Mesa *et al*., 2005). Detection of antibodies (and at a lesser degree the measure of the cell mediated immunity) against relevant *Brucella* epitopes is the more practical approach (MacMillan, 1990). Serology can be used for a presumptive diagnosis of brucellosis, or to screen flocks. Indirect or competitive enzyme-linked immunosorbent assays (ELISAs) are also used (CFSPH and OIE, 2009).

- **Rose bengal plate test**

This very sensitive test is used to screen serum samples. It does not differentiate between field and S19 vaccine strain reactions, but is quick, inexpensive and easy to perform. False negative reactions are rare but may sometimes be due to excessive heating in storage or in transit. RBPT has a sensitivity of 98.3% and specificity of 68.8% (Morgan *et al*., 1969; Dohoo *et al*., 1996). Positive reactions should be investigated using suitable confirmatory and/or complementary strategies (including the performance of other tests and an epidemiological investigation) (IBM, 2013).

- **Complement fixation test**

The CFT is the most widely used test for the serological confirmation of brucellosis in animals. The CFT is both sensitive and specific, in the hands of experienced users, and is used as a definitive (confirmatory) blood serum test. In most cases, the CFT is used on RBPT positive sera, but like the RBPT, it is also affected to a large extent by the misuse of strain 19vaccine, particularly when recent or repetitive vaccinations have been used in sexually mature heifers and cows. It is almost impossible to prescribe strict cut-off readings that indicate infection particularly when S19 vaccination reactions play a role due to its misuse. The CFT is a relatively complex test. The reagents include *B. abortus* CFT antigen, complement, amboceptor (haemolysin), ovine erythrocytes and test serum with Veronal buffer as the diluents (WHO, 2006; IBM, 2013).

2.1.9.10. Control and eradication

The treatment of brucellosis in the cow has generally been unsuccessful because of the intracellular sequestration of the organisms in lymph nodes, the mammary gland, and reproductive organs and the bacteria are facultative intracellular which survive and multiply within the cells (Radostits *et al*., 2000; Tolosa, 2004). Bovine brucellosis is usually introduced into a herd in an infected animal, but it can also enter in semen from infected bulls and on fomites. In endemic areas, vaccinated calves or non-pregnant heifers are the best herd additions in uninfected herds. Any pregnant or fresh cows should come from brucellosis-free areas or herds, and should be sero-negative. Herd additions should be isolated for approximately a month and retested for *B. abortus* before they are added to the herd. Selective breeding for disease-resistant genotypes may also be feasible as a control strategy in water buffalo. *B. abortus* can be eradicated from a herd by test and removal procedures, or by depopulation. Eradication can be accomplished by quarantine of infected herds, vaccination, test-and-slaughter techniques, various forms of surveillance and trace backs. *Brucella* species are readily killed by most commonly available disinfectants including hypochlorite solutions, 70% ethanol, isopropanol, iodophores, phenolic disinfectants, formaldehyde, glutaraldehyde and xylene; however, organic matter and low temperatures decrease the efficacy of disinfectants. Two *B. abortus* vaccines, Strain 19 and RB51, can be used to control this disease in endemic areas, or used as part of an eradication program. Routine vaccination is often done in calves to minimize the production of persistent antibodies that can interfere with serological tests (CFSPH, 2009).

2.1.9.11. Public health significance

B. abortus is pathogenic for humans. Occupational exposure is seen in laboratory workers, farmers, veterinarians and others who contact infected animals or tissues. Brucellosis is one of the most easily acquired laboratory infections. People who do not work with animals or tissues usually become infected by ingesting unpasteurized dairy products. The Strain 19 *B. abortus* vaccine is also pathogenic for humans and must be handled with caution to avoid accidental injection or contamination of mucous membranes or abraded skin. Adverse events are also reported with the RB51 vaccine, although it appears to be safer than Strain 19(CFSPH, 2009).

2.1.9.12. Status of bovine brucellosis in Ethiopia

Extensive system in Ethiopia covers 95% of the cattle farming. In the last 4 decades, several serological surveys have showed that bovine brucellosis is an endemic and widespread disease in the country. These studies showed that high incidence of brucellosis in pastoral and mixed livestock production systems where people live very closely with livestock and thus, are at higher risk of acquiring the disease (Berhe *et al.*, 2007). The evidences of *Brucella* infections in Ethiopian cattle have been serologically evaluated in different parts of the country by different authors (Berhe *et al.*, 2007; Tolosa *et al.*, 2008; Asmare *et al.*, 2010; Haileselassie *et al.*, 2010,2011; Adugna *et al.*, 2013).According to some reports, *Brucella* seroprevalence is higher in intensive farming system than within extensive cattle rearing systems. In Borena Zone of Oromia Region, the highest seroprevalence (50%) was documented using ELISA in Didituyura Ranch (Alem and Solomon, 2002). Tolosa *et al.* (2008) reported overall individual animal prevalence and herd prevalence of 0.77 and 2.9%, respectively in Jimma Zone. Reports from North West, Tigray region (Haileselassie *et al.*, 2010) and Southern Sidama Zone (Asmare *et al.*, 2010), recorded an overall prevalence of 1.2 and 1.66% following screening 848 and 1627 cattle from extensive system, respectively. Another study conducted on cattle brucellosis in traditional husbandry practice from 1623 cattle sera in southern and eastern Ethiopia showed that 3.5% of the animals and 26.1% of the herds were tested positive (Megersa *et al*., 2011).

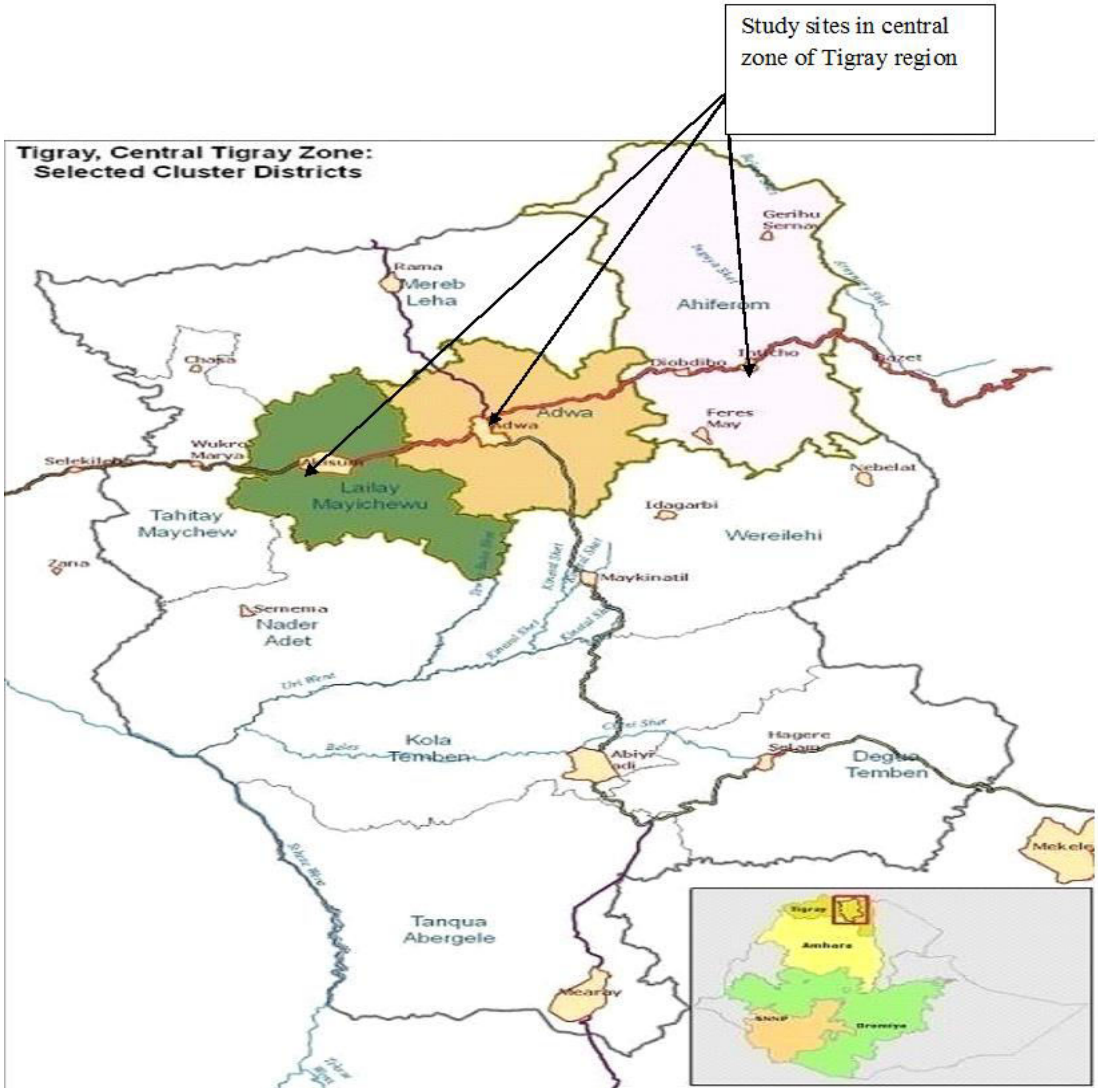
Table 3: Prevalence of Bovine Brucellosis in Ethiopia

Breed	**Location in Ethiopia**	**No. of animals tested**	**Prevalence (%)**	**Tests**	**Reference**
Local	South east	180	1.4	RBPT	Donde, 2013
Mixed	West	1813	0.61	CFT	Tolosa, 2004
Mixed	West	1813	0.94	RBPT	Tolosa, 2004
Mixed	Central	1136	11	CFT	Kebede *et al*., 2008
Mixed	Central	1136	12.5	RBPT	Kebede *et al*., 2008
Local	West	1152	1	CFT	Adugna *et al*., 2013
Local	West	1152	1.2	RBPT	Adugna *et al*., 2013
Local	North	1968	4.9	CFT	Haileselassie *et al*., 2010
Mixed	Assela	304	14.14	RBPT	Deselegn and Gangwar, 2011
Mixed	Central	1238	4.9	RBPT	Jergefa *et al*., 2008
Mixed	Central	1238	2.9	CFT	Jergefa *et al*., 2008
Cross	Ambo	169	0.2	RBPT	Bashitu *et al*., 2015
Cross	Ambo	169	0	CFT	Bashitu *et al*., 2015
Cross	Derebrhan	246	0.7	RBPT	Bashitu *et al*., 2015
Cross	Derebrhan	246	0.2	CFT	Bashitu *et al*., 2015
Local	South east	862	1.4	RBPT	Gumi *et al*., 2013
Local	South east	862	1.4	CFT	Gumi *et al*., 2013
local	Southern	1627	1.66	CFT	Asmare *et al*., 2010
Cross and exotic	Southern	811	2.46	CFT	Asmare *et al*., 2007
Local	East Showa	1106	11.2	RBPT	Dinka and Chala, 2009
Local	Eastern	435	1.84	RBPT	Degefu *et al*., 2011
Local	Eastern	435	1.38	CFT	Degefu *et al*., 2011
Mixed	East Wollega	406	2.96	RBPT	Yohannes *et al*., 2012
Mixed	East Wollega	406	1.97	CFT	Yohannes *et al*., 2012
Local	Arsi zone	370	0.05	RBPT	Degefa *et al*., 2011
Local	Arsi zone	370	0.05	CFT	Degefa *et al*., 2011
Mixed	Debrezeit	300	2	CFT	Alemu *et al*., 2014
Mixed	Debrezeit	300	3	RBPT	Alemu *et al*., 2014

3. MATERIALS AND METHODS

3.1. Description of Study Area

The study was carried out in central zone of Tigrai region, Northern Ethiopia of LIVES project districts (Laelay Maichew, Adwa and Ahferom). The Central Tigrai Zone is one of the six zones in Tigrai National Regional State and is 1024 km far away from Addis Ababa. The central zone of Tigai covers about 9,741 km^2 with a total population of 1,132,229.the zone consists of about 859,066 cattle, 949,130 shoats, 98,910 honey bee colonies and 1,312,844 poultry. Most parts of the selected districts are classified as *Dry Weina Dega* followed by *Dega* in the high lands of Ahferom and Adwa, and *Kolla* in the northern part of Ahferom district. The zone approximately extends between $13^o15'$ and $14^o39'$ North latitude, and 38^o 34' and $39^o25'$ East longitude. The altitude of the zone mainly falls within the category of 1332 to 2921 m.a.s.l. The larger part of the zone receives mean annual rainfall ranging from 400 to 800mm. The mean monthly maximum and minimum temperatures of the zone are 30^oC and 10^oC, respectively. Central Tigrai zone is bounded by Eritrea in the north, East Tigrai zone in the East and South east, North West Tigrai zone in the West and Amhara National Regional State in the South. The zone with its capital in the ancient city of Aksum encompasses ten districts. The zone has the largest human population in the region. The farming system of the study area is largely characterized by mixed crop-livestock production system, which is in turn grossly divided into the lowland and highland parts.



Source: LIVES, 2013.

Fig 1: Map of the Study Area

3.2. Study Design

For the purpose of the study cross sectional study using different approaches namely questionnaire survey to assess the perception of farmers on major reproductive problems of dairy cattle, retrospective data to determine the prevalence and associated risk factors of major reproductive disorders of dairy cattle and serum sample collection from dairy cattle to assess the prevalence of brucellosis and its risk factors were deployed. During the assessments data on reproductive history of the study cows were collected from the farm records or owners. In the retrospective study, information on reproductive disorders as well as management system and particulars related to individual cow such as parity, breed, age, body condition, herd size, herd type, mating system used, lactation status, pregnancy status, sanitation and history of reproductive problem in 265 cattle were documented. The risk factors such parity, breed, age, body condition, herd size, herd type, mating system used, lactation status, pregnancy status ,sanitation and history of reproductive problem was evaluated as to associate with one of the reproductive health problems of dairy cows namely brucellosis.

3.3. Study Population

The study population consisted of cattle that were managed under the extensive, semi intensive and intensive production systems in each district. The cattle under study comprise of the local and cross breeds with no history of vaccination against brucellosis. In both sexes all animals greater than six month age were included in the cross sectional study.

3.4. Sample Size Determination

For sample size determination the 95% of level of confidence and absolute precision of the 5% was used. Since the previous prevalence report of 4.9% prevalence of bovine *brucella* infection in Western Tigray region was reported by Haileselassie *et al.* (2010), the current sample size calculation used the reported prevalence for the sample size determination. Hence using the Formula stated by Thrustfield (2007) the calculated sample for the current study becomes 72 heads of cattle; however a total of 414 serum samples from both sexes having different ages were sampled to increase the precision of the result. It was computed with the expected precision at 5 and at 95% confidence interval. The sample size was 72 (Thursfield, 2007); however a total of 414 serum samples of both sexes and different ages were sampled to increase the precision of the result.

$$N = \frac{1.96^2 (P)\,(1-P)}{d^2}$$

Where

N= Total calculated sample size

P= expected prevalence

d= absolute precision

3.5. Sampling Technique

Multistage sampling method was used in the study. Districts, peasant associations, flocks and animal were the primary, secondary, third and fourth units respectively. At each stage, sampling units were selected randomly (Dohoo *et al.*, 2003). From each district, two peasant associations (PA's) were taken using purposive sampling. A total of 200 herds with an average herd size of 5 (ranging from 1 to18), were randomly sampled. Simple random sampling was used to sample individual cattle from selected herds of the PA's. A total of 414 animals were sampled, 149 from Ahferom, 128 from Adwa and 137 from Laelay Maichew districts. Male animals above six months of age and females of age 2year and above kept for breeding purpose was selected from the sampled herds.

3.6. Study Animals and Their Management

A total of 414 cattle under different management system were included in this study during the period September 2014 to April 2015. Classification of management systems was done based on the criteria adopted by Richard (1993). Accordingly, semi-intensive system included all animals that were kept in door and fed and watered in their house/shade by cut and carry system while intensive system covers all animals which were kept in closed housing system and feed concentrated as well as mixed feed. Extensive management system included all animals that were kept out-door during the day time and allowed to graze on a communal or private owned pasture land. Of the 414 cattle, 218 were local /zebu breeds whereas the remaining 196 were cross breeds.

3.7. Body Condition Scoring

Body condition of the study animals was scored based on the criteria set by Richard (1993), which ranged from 0 to 5. Body condition score 0 stands for cows with the poorest body condition while score 5 for cows with the best condition.

3.8. Study Protocol

3.8.1. Collection of blood samples

Blood samples were collected from jugular vein of each selected animal using plain vacutainer tube by needle. Identification of each animal was labeled on corresponding vacutainer tubes and kept overnight at room temperature to allow clotting. At the next morning sera were collected from the clot (unrestricted blood centrifuge) by siphoning in to the sterile cryotube (2ml), to which animals /identification was coincided and Serum samples was kept at -20°C(OIE, 2009) at Axum Agricultural Research Center. The collected serum in sterile tubes was transported to Mekelle Regional Animal Health

Diagnosis and Investigation Laboratory(MRAHDIL) using ice box where stored at -20°C until tested.

3.8.2. Secondary data

Secondary data of 265 cattle were collected from the record books and owners of the farms in the last two years; from March, 2013 to March, 2015 to identify the major reproductive problems and assess associated risk factors of the dairy cows in the study area. Heifer that has age of 2year and above was included in the study.

3.8.3. Questionnaire survey

A total of 120 owners and/or attendants of cattle were randomly selected and interviewed using structured questionnaire. The questionnaire survey on the households/cattle owners or attendants was conducted to determine the prevalence of reproductive problems and their linkage to brucellosis sero-positivity. The questionnaire was designed to collect information on factors that are believed to be a risk factor for *Brucella* infection and for the other reproductive problems. In addition the clinical indicators such as history of abortion, testicular swelling and other reproductive problems were interviewed and recorded. Based on their cattle production availability, two sites (PA's) was purposively selected from each of three districts of LIVES project sites for questionnaire administration. The questionnaire was administered using face to face interview of respondents.

3.8.4. Serology test

At MRAHDIL, Mekelle, Ethiopia, the RBPT was employed as a screening test on the serum samples for the presence of *brucella* agglutinins and the degree of positivity was recorded using agglutinations observed during the test of sera using RBPT. During the test animals were considered as positive if there is agglutination reaction of the serum sample with the antigen but if there is no agglutination reaction the animals were considered as test negative.

3.8.4.1. Rose Bengal Plate Test

The protocol of RBPT was used as screening test to test the presence of *Brucella* antibody in the sampled sera at MRAHDIL. The antigen was obtained from Institut Pourquier, rue de la Galera 34097 Montpellier, France. The test was performed according to manufacturer's protocol. Before performing test, antigen and sera was brought to room temperature (+4c^0) for half an hour, since active materials straight from the refrigerator react poorly. One drop (0.03 ml) of serum was taken on a glass slide by micropipette. The antigen bottle was shaken well to ensure homogenous suspension and then one drop (0.03 ml) of Rose Bengal antigen will be added. The antigen and serum was mixed thoroughly with using an applicator stick and then the slide was rocked by hand for four minutes. The tests were read immediately after four minutes by examining for agglutination in a good light. Magnifying glass was used to read micro agglutination when suspected. Those samples with no agglutination was recorded as negative while those with +, ++ and +++ was recorded as positive (Appendix IV).

3.9. Data Analysis

The collected data were recorded in Microsoft excel spread sheet and coded properly. The coded data were transferred on to SPSS version 20. For the data analysis descriptive statistics were deployed and to test the association between the risk factors with the occurrence of the disease Chi-square statistics was used. To test the association between the disease and the possible risk factors, the point brucellosis prevalence rate was calculated by dividing the number of RBPT positive animals by the total number of animals tested. Similarly, prevalence of the other reproductive problems was calculated by dividing cows with history of positivity to reproductive problem cases to the total cows inspected. The questionnaire survey on farmer's perception was calculated by descriptive analysis (Thrustfield, 2007).

4. RESULTS

4.1. Questionnaire Survey

The present result indicated that respondents having of different age groups with the maximum age of 70 and minimum age of 28 with average mean age of 50.0$\pm$ 0.9 years were included. Out of the respondents 86.7% of them were males and 13.3% females. Out of the total households interviewed, 98.3% are cattle owners, 56.7% sheep owners, 36.7% goat owners and 77.5% poultry owners. With regard to educational status, among the respondents 44.2% had not received education, while 42.5% were attended from grade 1 to grade 7. More specifically, 10.8% and 2.5% of the respondents had attended from grade 8-12 and greater than grade12 respectively. The respondents reported 54.2% communal type and 45.7% small scale commercial type of farming system were used in the selected sites of the study areas (Table 4).

Table 4: Socio-Economic Characteristics of Respondents on Educational Status, Sex and Farming type

	Total sample (n=120)
Parameter	Number of respondents (%)
Educational level	
Illiterate	53(44.2)
Grade1-7	51(42.5)
Grade8-12	13(10.8)
> Grade 12	3(2.5)
Sex	
Male	104(86.7)
Female	16(13.3)
Farming type	
Communal	65(54.2)
Small scale commercial	55(45.7)

n= Number of observations

According to the survey result 63.3%, 5% and 31.7% were in rural, urban and peri-urban household classification. Majority of the sample respondents practice intensive production system (56.7%). Others, 29.2% and 14.2% of the respondents practice semi intensive and extensive production system, respectively. Most of the respondents (46.7%) reported herd trend increment (Table5).

Table 5: Response of the Interviewed Respondents on Household Classification, Production System and Herd Trend

	Total sample (n=120)
Parameter	Number of respondents (%)
House hold classification	
Rural	76(63.3)
Urban	6(5.0)
Peri-urban	38(31.7)
Production system	
Intensive	68(56.7)
Semi-intensive	35(29.2)
Extensive	17(14.2)
Herd trend	
Increasing	56(46.7)
Decreasing	21(17.5)
Stable	33(27.5)
Unknown	10(8.3)

n= Number of observations

Most of the respondents (53.3%) breed their animals using artificial insemination (AI) while 17.5%,13.3%,11.7% and 4.2% were used uncontrolled mating, hand mating, group mating and both hand mating and AI , respectively. Majority of the interviewed respondents (79.2%) were mentioned their access to government veterinary clinics where as 9.2%, 10.8% and 0.8% had access to private veterinary shop, extension service and no service, respectively(Table 6).

Table 6: The Response of Respondents on Mating System and Access to Veterinary Service

	Total sample (n=120)
Parameter	Number of respondents (%)
Mating system	
Group mating	14(11.7)
Hand mating	16(13.3)
Uncontrolled mating	21(17.5)
Artificial insemination	64(53.3)
Hand mating and Artificial insemination	5(4.2)
Access to veterinary service	
Government veterinary clinics	95(79.2)
Private veterinary shop	11(9.2)
Extension service	13(10.8)
No service	1(0.8)

n= Number of observations

Respondents were also interviewed to describe the occurrence of reproductive problems and indicated 26.7% repeat breeding, 20% anoestrus, 5.8% retained fetal membranes, 5.8% uterine prolapsed, 28.3% abortion and 26.7% testicular swelling. Only a few of the farmers (2.5%) move their animals to other areas in search of feed during summer. Most of the farmers (96.7%) had no knowledge of isolating aborted animal from others. Some of the respondents indicated the consumption of raw milk (13.3%) and raw meat (18.3%). The farmers have habit of assisting cows during parturition (41.7%) but assisting with bare hand (84.2%) is practiced in the community. The questionnaire survey showed that most of the respondents had only little knowledge about the transmission of brucellosis when assisting parturition and the farmer's perception on the cause of the disease is about 29.2%. Most of the respondents (55.8%) have awareness on the transmission of brucellosis by drinking raw milk to humans (Table 7).

Table 7: Occurrence of Reproductive Problems and Associated Factors Based on Interview of Respondents

Variables	Number of respondent (n=120)	
	Yes (%)	No (%)
Repeat breeding	32(26.7)	88(73.3)
Anoestrus	24(20.0)	96(80.0)
Retained fetal membranes	7(5.8)	113(94.2)
Uterine prolapsed	7(5.8)	113(94.2)
Abortion	34(28.3)	86(71.7)
Retention of placenta after birth	43(35.8)	77(64.2)
Assisting cows during parturition	50(41.7)	70(58.3)
Use of protective gloves when assisting with parturition	19(15.8)	101(84.2)
Isolation of aborted animal from others	4(3.3)	116(96.7)
Use of separated grazing and watering from other flocks	58(48.3)	62(51.7)
Use of separated house	61(50.8)	59(49.2)
Migration of animals	3(2.5)	117(97.5)
Disease transmitted by drinking raw milk to human	67(55.8)	53(44.2)
Raw milk consumption	16(13.3)	104(86.7)
Treated milk consumption	92(76.7)	28(23.3)
Testicular swelling	32(26.7)	88(73.3)
Raw meat consumption	22(18.3)	98(81.7)
Perception on cause of abortion	35(29.2)	85(70.8)

n= Number of observations

4.2. Frequency of Studied Dairy Cattle

In the retrospective study, a total of 265 dairy cows were examined for the presence of reproductive health problems based on cow's history obtained from the owner and record books during the last two years. The dairy cows under this study were local and crossbred kept under different management system. Accordingly, 70, 23 and 173 dairy cows were kept under extensive, semi intensive and intensive production system respectively. The number of animals sampled across the three locations based on parity level is described as in Table 8.

Table 8: Frequency of Studied Dairy Cattle by Location and Parity

Locations	Parity levels among female animals (%)								
	Parity1	Parity2	Parity3	Parity4	Parity5	Parity6	Parity7	Parity8	Heifer
Ahferom	12(28.6)	13(32.5)	8(33.3)	15(44.1)	5(45.5)	3(33.3)	2(100.0)	_	47(46.5)
Adwa	10(23.8)	11(27.5)	5(20.8)	10(29.4)	2(18.2)	3(33.3)	_	_	26(25.7)
L/Maichew	20(47.6)	16(40.0)	11(45.8)	9(26.5)	4(36.4)	3(33.3)	_	2(100.0)	28(27.7)
Total	42	40	24	34	11	9	2	2	101

4.3. Prevalence of Major Reproductive Problems

A retrospective study, from a total of 265 cattle were assessed and recorded from all the three production systems during the last two years from March 2013 to March 2015. Results revealed 51(19.3%) were found to be affected with the major reproductive problems. The results of the study shown in Table 9 indicated that repeat breeding (9.1%) anoestrus (4.2%) and abortion (3.8%) were found to be the major reproductive problems. The other reproductive problems found with lower frequency were RFM (1.1%), pyometra (0.8%) and uterine prolapse (0.4%) in selected sites of Central zone of Tigrai region.

Table 9: Overall Prevalence of Major Reproductive Problems of Dairy Cattle in the Study Area

		Total sample (n=265)
History of reproductive problem	Number of animals affected	Overall prevalence (%)
Repeat breeder	24	9.1
Anoestrus	11	4.2
Abortion	10	3.8
Retained fetal membrane	3	1.1
Pyometra	2	0.8
Uterine prolapsed	1	0.4
Total	51	19.3

n= Number of observations

4.4. Factors Associated with Reproductive Problems of Cattle

In these study risk factors such as breed, location, lactation status, pregnancy status, system of production, age, parity, body condition score, mating system, herd size, herd type, housing system and sanitation were assessed and their association with reproductive problems were presented on the tables below.

Reproductive problems showed a statistically significant association ($p<0.05$) with respect to breed. Higher prevalence of reproductive health related problems were found in local cattle than the cross breed. The present study revealed that there is highly significant association ($p<0.05$) of reproductive problems with location and lactation status where highest prevalence of reproductive problems with location was found in Adwa followed by Laelay Maichew, while the lowest in Ahferom. Significantly higher prevalence of reproductive problems was observed in non lactating cows than lactating cows.

Pregnancy status had no statistically significant association ($p>0.05$) on reproductive problems. Higher prevalence was recorded in non pregnant cows than pregnant dairy cows (Table 10).

Table 10: Prevalence and Association of Reproductive Problems Based on Breed, Location, Lactation Status and Pregnancy Status

Risk factors	Total cows examined	Total cows affected	Percentage	X^2 value	p-value
Breed					
Local	94	20	21.3	15.512	0.017
Cross	171	31	18.1		
Total	265	51	19.3		
Location					
Ahferom	105	7	6.7	64.246	0.000
Adwa	67	21	31.3		
Laelay Maichew	93	23	24.7		
Total	265	51	19.3		
Lactation status					
Lactating	61	11	18.0	17.583	0.007
Non-lactating	204	40	19.6		
Total	265	51	19.3		
Pregnancy status					
Pregnant	47	8	17.0	6.260	0.395
Non-pregnant	218	43	19.7		
Total	265	51	19.3		

There was no statistically significant association ($p>0.05$) of production system with the prevalence of reproductive problems. Highest prevalence was found in extensive production system (22.9%), followed by intensive production system (18.6%) and lowest in semi intensive system (13.0%) of production.

The influence of age on the prevalence of major reproductive problems was assessed and the result showed that there was significant association ($p<0.05$) with respect to age. The prevalence was significantly highest in cows with greater than 6 year's age group (29.7%) followed by 3 to 6 years age group (23.2) while the lowest in less than three years age (6.7%) as indicated (Table 11) .

Table 11: Prevalence of Reproductive Problems of Cattle Associated with Production System and Age

Risk factors	Total cows examined	Total cows affected	Percentage	X^2	p-value
Production system					
Extensive	70	16	22.9		
Semi-intensive	23	3	13.0	20.719	0.055
Intensive	172	32	18.6		
Total	265	51	19.3		
Age					
<3years	89	6	6.7		
3-6years	112	26	23.2	26.75	0.008
>6years	64	19	29.7		
Total	265	51	19.3		

Parity number had highly significant effect ($p<0.01$) on the prevalence of major reproductive problems and the effect progressively increased its prevalence from heifer (6.9%) to the sixth parity (55.6%). The significantly higher prevalence of major reproductive problems was found in the sixth parity. The prevalence rate of major reproductive problems increased from heifers to older cows with respect to parity number (Table 12).

Table 12: Prevalence and Association of Reproductive Problems with Parity

Risk factor	Total cows examined	Total cows affected	Percentage	X^2 value	p-value
Parity					
Heifer	101	7	6.9		
1	42	9	21.4		
2	40	7	17.5	97.008	0.000
3	24	8	33.3		
4	34	9	24.5		
5	11	6	54.6		
6	9	5	55.7		
7	2	0	0.0		
8	2	0	0.0		
Total	265	51	19.3		

Reproductive problems were assessed with respect to body condition score of dairy cattle and there was highly significant association ($p<0.05$) between the prevalence rate of reproductive problems and body condition of the cows. The prevalence was higher in body conditioned score 1or lean (25.0%) followed by body condition score 5 or fat body conditioned animals (Table 13).

Mating system has a significant ($p<0.05$) effect on the prevalence rate of major reproductive problems, where the prevalence rate of the reproductive problem is significantly higher in natural mating (24.2%) when compared with that of artificial insemination (Table 13).

Table 13: Prevalence and Association of Reproductive Problems with Body Condition Score and Mating System

Risk factors	Total cows examined	Total cows affected	Percentage	X^2 value	p-value
Body condition score					
1	8	2	25.0		
2	29	5	17.2	46.927	0.003
3	141	30	21.3		
4	74	11	14.9		
5	13	3	23.1		
Total	265	51	19.3		
Mating system					
AI	186	32	17.2		
Natural mating	66	16	24.2	21.139	0.048
Natural mating + AI	13	3	23.1		
Total	265	51	19.3		

Reproductive problems were assessed in relation to herd type of the study animals and their association was found to be statistically significant ($p<0.05$). The mixed herd type (23.1%) revealed significantly higher prevalence of reproductive problems than single herd type (18.0%). Similarly, herd size had a significant influence ($p<0.05$) on the overall prevalence of reproductive problems where herd size with 6 to 10 animals were having significantly higher reproductive problems than others. Housing system showed highly significant effect ($p<0.05$) on the occurrence of reproductive problems. A significantly higher prevalence of reproductive problems was also observed in semi open housing system (25.0%). On the other hand, sanitation of the farms did not show a significant difference ($p>0.05$) on the occurrence of reproductive problems of the animals (Table14).

Table 14: Prevalence of Reproductive Problems Associated with Herd Type, Herd Size, Sanitation and Housing System

Risk factors	Total cows examined	Total cows affected	Percentage	X^2 value	p-value
Herd type					
Single	200	36	18.0	14.234	0.027
Mixed	65	15	23.1		
Total	265	51	19.3		
Herd size					
1-5 animals	187	35	18.7		
6-10 animals	54	16	29.6	21.579	0.043
>10 animals	24	0	0.0		
Total	265	51	19.3		
Housing system					
Open	83	20	24.1		
Semi-open	56	14	25.0	38.51	0.000
Closed	126	17	13.5		
Total	265	51	19.3		
Sanitation					
Good	139	29	20.9	7.467	0.280
Poor	126	22	17.5		
Total	265	51	19.3		

4.5. Sero-prevalence of Bovine Brucellosis

In this study, Dairy cattle of above 6 months age were considered for the purpose of the study and, 218 (52.7%) were local breed and 196 (47.3%) cross breeds of indigenous zebu and Holstein Friesian dairy cattle. A total of 414 animals, 149(36.0%) male animals and 265(64.0%) females were sampled. The overall prevalence rate of brucellosis in the current finding was 0.0% using the RBPT test. The present study attempted to compare the sero-positivity between the breeds of animals. Thus, the prevalence of local breeds and cross breed animals was compared in Table 15. The sero prevalence of local as well as crossbred cattle was found to be 0.0% in all of the three districts/locations. The prevalence of brucellosis of both the age category and sex of the study animals was 0.0%. Generally, the prevalence and frequency distribution of risk factors were summarized in the following tables (Table15, Table16 and Table17).

Table 15: Seroprevalence of Brucellosis Associated with Location, Breed, Age and Sex using RBPT

Risk factors	Total examined (%)	Number (%) positive
Location		
Ahferom	149(36)	0(0)
Adwa	128(30.9)	0(0)
Laelay Maichew	137(33.1)	0(0)
Total	414(100)	0(0)
Breed		
Local	218(52.7)	0(0)
Cross	196(47.3)	0(0)
Total	414(100)	0(0)
Age		
<3 years	136(32.9)	0(0)
3-6 years	154(37.2)	0(0)
>6 years	124(30)	0(0)
Total	414(100)	0(0)
Sex		
Male	149(36)	0(0)
Female	265(64)	0(0)
Total	414(100)	0(0)

Among 414 dairy cattle selected for study, 12(2.9%) of the animals show history of abortion, 3(0.7%) with history of retained fetal membrane and 2(0.5%) were with a history of testicular swelling. In the current study the occurrence of abortion, RFM and testicular swelling did not indicate *brucella* positivity. The Sero-prevalence of brucellosis with respect to parity was 0.0%. The study does not show the risk of *brucella* infection as the increases its number of parturition. Most of the sampled animals were from intensive and extensive production systems (Table 16).

Table 16: Seroprevalence of Brucellosis Associated with Parity and Other Management Factors based on RBPT

Risk factors	Total examined (%)	Number (%)positive
Parity		
0	102(38.5)	0(0)
1-3	105(39.6)	0(0)
4-5	45(17)	0(0)
6-8	13(4.9)	0(0)
Total	265(100)	0(0)
Production system		
Intensive	207(50)	0(0)
Semi intensive	39(9.4)	0(0)
Extensive	168(40)	0(0)
Total	414(100)	0(0)
Housing system		
Open	168(40.6)	0(0)
Semi open	91(22)	0(0)
Closed	155(37.4)	0(0)
Total	414(100)	0(0)
History of reproductive problem		
Normal(with no problem)	397(95.9)	0(0)
Retained fetal membrane	3(0.7)	0(0)
Abortion	12(2.9)	0(0)
Testicular swelling	2(0.5)	0(0)
Total	414(100)	0(0)

The study did not indicate the sero positivity to brucellosis across body condition score, herd type, herd size as well as pregnancy and lactation status. The Seroprevalence across these factors is found 0.0%.

Table 17: Seroprevalence of Brucellosis Associated with BCS, Herd Type, Herd Size, Pregnancy Status and Lactation Status using RBPT

Variables	Total examined (%)	Number (%)positive
Body condition score		
1	11(2.7)	0(0)
2	57(13.7)	0(0)
3	233(56.3)	0(0)
4	100(24.1)	0(0)
5	13(3.1)	0(0)
Total	414(100)	0(0)
Herd type		
Single	256(61.8)	0(0)
Mixed	158(38.2)	0(0)
Total	414(100)	0(0)
Herd size		
1-5 animals	308(74.4)	0(0)
6-10 animals	81(19.6)	0(0)
>10 animals	25(6)	0(0)
Total	414(100)	0(0)
Pregnancy status		
Pregnant	47(17.7)	0(0)
Non-pregnant	218(82.3)	0(0)
Total	265(100)	0(0)
Lactation status		
Lactating	61(23)	0(0)
Non-lactating	204(77)	0(0)
Total	265(100)	0(0)

5. DISCUSSION

The educational status attained by majority of the respondents was low which falls between illiterate and grade7. In agreement to this study, Asaminew and Eyassu (2009) reported low educational level of households in Bahir Dar Zuria and Mecha Woredas, North western Ethiopia. This low level of educational status may lead to reduced production of dairy farms because of low use of dairy innovations such as cultivation of improved forages, breeding techniques and use of modern dairy farming in the study area.

The present findings using the questionnaire survey in this study revealed the occurrence of the major reproductive problems were mainly repeat breeding, anoestrus, abortion and RFM. The lack of knowledge on breeding and disease transmission, faulty insemination or heat detection, shortage of feed, problems in semen handling and insemination techniques, lack of awareness on isolation of aborted animal from healthy animals and inability of the cattle owners to use protective gloves when assisting parturition could possibly be associated with the high prevalence rate of reproductive problems.

Majority of the sample respondents practice intensive production system (56.7%). Others, 29.2% and 14.2% of the respondents practice semi intensive and extensive production system, respectively. Most of the respondents revealed that their cattle were managed under intensive and semi intensive management system. This could reduce the transmission of diseases and improves animal management. Most of the respondents (53.3%) breed their animals using AI. It was found that the use of artificial insemination is high and this may possibly prevent the transmission of sexually transmitted diseases especially brucellosis among the flocks in the area.

The retrospective study revealed that a total of 265 animals were examined during the study period of which 51 animals showed to be affected by the reproductive problems with an overall prevalence rate of 19.3%. The prevalence of major reproductive problems reported in the current study was lower than the values reported by Gizaw *et al*. (2007) 37.76% in Nazareth town of central Ethiopia, Esheti and Moges (2014) 37.1% in ILCA and Almaz dairy farms in Ada'a district of Debre Zeit town in East Shoa and Haile *et al*. (2014) 43.07% in urban and peri-urban areas of Hosanna in Southern Ethiopia and fairly agrees with the report of Dinka (2013) who reported prevalence rate of 18.3% in and around Assella in Central Ethiopia. The difference in the results could be related to production system, sample size, study methodology, breed of animals and environmental conditions that might be appeared in the different study areas.

The prevalence rate of repeat breeding (9.1%) found in this study is fairly agrees with the values reported by Gizaw *et al*. (2007) Haftu and Gashaw (2009) and Hadush *et al*. (2013) who reported 8.91, 8.72%, and 10.6%, respectively. When compared with the findings of Haile *et al*. (2010), Bitew and Shiv (2011), Gashaw *et al*. (2011), Dawit and Ahmed (2013) and Mesafint and Guesh (2014) who reported prevalence's of 6.2%, 3%, 1.3%, 3.87% and 7.29%, a higher prevalence rate of repeat breeding was obtained in the current study, but it is lower than when compared with the findings of Dinka (2013), Esheti and Moges (2014) and Haile *et al*. (2014), who reported prevalence of 26.8%, 15.9% and 13.8%, respectively. A number of factors can contribute for the cause of repeat breeding such as climatic condition of the area, sub fertile bulls, endocrine imbalance, reproductive tract infections, communal use of bull for natural service and other managerial factors (Arthur *et al*, 1989). Hence, the variation between the values of the current study and previous reports could be due to the above factors.

The prevalence rate of abortion recorded in the present study was (3.8%) which is fairly consistent with the finding of Gizaw *et al*. (2007) who reported 2.23% and Haile *et al*. (2014) who reported 2.56%. On the other hand, Bitew and Shiv (2011), Degefa *et al*. (2011), Dinka (2013) and, Benti and Zewdie (2014) reported 13.9%, 8.7%, 14.6% and12.2%, respectively which are higher than the current finding, but compared with the finding of Gashaw *et al*. (2011) who reported prevalence rate of 1% the present finding is higher. The difference in prevalence of abortion may be due to variation in practice of

AI, genetic, nutritional status, infection, level of toxicities and husbandry management system in different areas. When the incidence of abortion is 2% or more it should be diagnosed soon to know its cause and viewed seriously (Roberts, 1986).

The prevalence rate of RFM found in this finding is 1.1% which is a little bit higher compared with the report of Esheti and Moges (2014) having prevalence of 0.8%, but it is much lower than Gizaw *et al*. (2007) who reported prevalence of 12.91%, Haile *et al*. (2010) who reported prevalence of 17%, Gashaw *et al*. (2011) who indicated prevalence of 19.2% and Degefa *et al*. (2011) who reported 18.3% prevalence. The variation in the prevalence of RFM may be attributed to the difference in nutritional status and management factors. Uterine paresis, abortion, stress, late or premature birth, dystocia, twinning, infections, seasonal and hormonal disorders, immune-suppression vitamin and mineral deficiencies have been identified as causes of RFM (Joosten, 1987; Akar and Yeldiz, 2005; Lotthammer, 2005; Beagley *et al*., 2010).

The prevalence rate of anoestrus found in the current study (4.2%) is higher than the previous reports by Haftu and Gashaw (2009), Bitew and Shiv (2011) and Gashaw *et al*. (2011) who reported an overall prevalence rate of 2.29%, 1.7% and 0.3%, respectively. Previous reports of the prevalence of anoestrus of 10.1% by Haile *et al*. (2010), 10.26% by Haile *et al*. (2014) and 10.3% by Benti and Zewdie (2014) are higher than the current finding. The difference observed in the prevalence rate of anoestrus could be due to difference in heat detection practice and management system particularly nutritional variation in animals.

The prevalence rate of uterine prolapse in this study (0.4) is consistent with the previous reports by Haile *et al*. (2014) who reported 0.76%, Bitew and Shiv (2011) who reported 0.65%, Dawit and Ahmed (2013) who reported 0.43% and Gashaw *et al*. (2011) who reported 0.5%. However, this study is relatively lower than the study of Benti and Zewdie, who reported the prevalence rate of 2.7% in indigenous Borena breed cows in

Borena zone in Southern Ethiopia. This could be fairly related to the incidence of dystocia cases and associated factors.

The results of current study showed that prevalence of pyometra was 0.8%. These results are lower than the finding of Simenew *et al*. (2011) who reported the prevalence rate of 1.6% at Sululta Slaughterhouse in Ethiopia. The differences could be attributed to microbial infections, management and animal age.

Analysis of the prevalence of major reproductive problems revealed that breed had statistically significant association and a higher (21.2%) prevalence rate of major reproductive problem was obtained in local breed cows than the cross breeds (18.1%) in contrary to the previous reports (Bitew and Shiv,2011; Dawit and Ahmed,2013). The higher reproductive problems in local breed cows is possibly due to the cross breeds included in the study are having at most 50% Holstein Friesian blood type and this could help them to adapt the tropical weather conditions and have better management than the local breeds.

The variation of the reproductive health problems of dairy cattle in different districts showed that showed that high significant association ($p<0.05$) among the different districts were observed with highest (31.3%) prevalence rate of major reproductive problem in Adwa district followed by Laelay Maichew district (24.7%) and lowest in Ahferom district (6.6%) in contrary with the finding of Benti and Zewdie (2014). This variation could be due to difference in agro ecology, animal husbandry management and extension services.

Lactation status revealed a highly significant association ($p<0.05$) on the occurrence rate of major reproductive problems. This revealed that non lactating cows could be more susceptible to the reproductive problems than lactating cows. This might be due to better management of lactating animals and they may attribute to develop good body defense than dry cows. Even though a slightly higher prevalence rate was observed in non pregnant cows as compared to the pregnant dairy cows, there was no statistically significant association between pregnancy status and reproductive health problems.

The study indicated an increased reproductive problem in animals managed extensively than those managed under intensive and semi intensive management practice, but there is no significant association between the production system and reproductive problems. The current finding is in line with the report of Haftu and Gashaw (2009). This could be due to lack of records, faulty heat detection, poor nutrition and management in the extensive production system.

A statistically significant effect was found between reproductive problems and age. A higher prevalence rate of reproductive problems was observed in these cattle its age greater than 6 years age (29.7%) followed by 3 to 6 years of age when compared to the less than3 years of age cows in contrary to the report of Esheti and Moges (2014). This result is in accordance with the incidence of reproductive problems reported with respect to age by Benti and Zewdie (2014).

Parity number showed highly significant association with the occurrence of reproductive health problems in the sixth parity (55.6%) than heifers. This work is similar to the previous findings by Dinka (2013), Hadush *et al.* (2013), and Haile *et al.* (2014). The effect of parity number on the occurrence of reproductive problems is probably due to repeated exposure of the genital tract to environmental factors which result in increased uterine infections. Longer recovery time from pregnancy, lactation stress and the low feed intake capacity of the older cows could also be other reasons for this variation.

A highly significant association was indicated between body condition score and reproductive problems in poor body condition cattle (25.0%) being more susceptible, followed by fattened and medium body condition. This finding is in agreement with reports of Haftu and Gashaw (2009) and Benti and Zewdie (2014). On the other hand the current finding contradicts with the report of Gashaw *et al.* (2011).This difference may be due to animals with poor body condition have poor body defense mechanism, high infection rate during parturition because of weak expulsive force to deliver the fetus and fetal membranes leading to secondary bacterial complications. Cows with fattened body conditions were more susceptible to infections and metabolic problems which expose them to have difficult calving, retention of placenta and uterine infections. In conclusion, very poor body condition might be an indication of improper animal management, diseases and nutritional imbalances (Benti and Zewdie, 2014).

Mating system significantly ($p<0.05$) influences the occurrence of overall reproductive health problems. Animals that use natural mating system were more susceptible to reproductive health problems at 24.2% than using AI (17.2%). This could be due to easy access of reproductive diseases to the genital tract is common in natural mating than AI and management problems as a whole.

A statistically significant effect was observed between herd type and reproductive problems with mixed herd type to be more prevalent in reproductive problems than single type. This is probably being due to high transmission of diseases and lower management practices as a whole in mixed herd type. A significant association of herd size was obtained in herd size of 6 to 10 heads of cattle with 29.6% prevalence when compared with herd size of 1 to 5 and greater than 10 heads of cattle. This finding is inconsistent with the report of Haile *et al*. (2010).

The management aspects evaluated by housing system was found to have highly significant influence on the overall reproductive problems in the study area. This result is similar to the previous finding of Haile *et al*. (2010). Even though a slightly higher prevalence rate was found in good sanitation, there was no significant association between sanitation and reproductive health problems. This fairly agrees with the finding of Hadush *et al*. (2013). On the other side, the present result is on the contrary with the report of Haile *et al*. (2010). The difference in the management aspects could be due to much broader classification and have masked widely reported factors such as nutritional differences within the broad categories.

The present study on the prevalence of rate of bovine brucellosis in the selected sites of Central zone of Tigrai region was 0.0%. The result indicates that, bovine brucellosis is not prevalent in Laelay Maichew, Adwa and Ahferom districts of Central zone in Tigrai Region. This very low prevalence was in agreement with previous and recent reports done using RBPT by: Tolosa (2004) which reported prevalence rate of 0.94% in selected sites of Jimma zone, Asmare *et al*. (2010) reported 0.0% in Arroresa district of Sidama zone in Southern Region, Asmare *et al*. (2013) reported 0.0% in Mekelle and Gondar of Northern Ethiopia and in Nazeret of Central Ethiopia, Degefa *et al*. (2015) which reported 0.05% in Arsi zone and Bishatu *et al*. (2015) who reported 0.2% in Ambo and 0.7% in Debrebrhan. On the contrary, many other previous bovine brucellosis studies in different parts of the country reported a high prevalence rate using RBPT, 14.14% in Assela by Deselegn and Gangwar (2011), Yohannes *et al*. (2012) in East Wollega and 3% by Alemu *et al*.(2014) in Debrezeit. The absence of the disease in the current study area could be due to too little contact between different herds, increased awareness of farmers on use of zero grazing and AI, presence of trained animal health professionals as well as AI technicians in the areas of study, their good access to veterinary and extension services and due to the strict control of the government during introduction of animals where quarantine and testing of the animals imported from various areas where practiced.

6. CONCLUSION AND RECOMMENDATIONS

The current study revealed the high prevalence rate of reproductive health problems in selected sites of Central zone of Tigrai Region. Repeat breeder, anoestrus and abortion were the most important reproductive problems The possible risk factors associated with the incidence of reproductive problem in the study area includes breed, location, lactation status, production system, age, parity, body condition score, herd type., herd size and housing system. On the other hand, no prevalence of brucellosis was found in the study area. This also reflects the involvement of other possible causes of abortion and retained fetal membranes that pose reproductive wastage in the study area. This may be a clue for the presence of other causes of reproductive diseases. Based on the current finding the following points are recommended:

- ✓ Further investigation should be performed to isolate and characterize the causes of the reproductive problems and associated risk factors in the study area and in the country;

- ✓ Regular reproductive health management and proper formulation of ration could be the possible solutions to reduce the problems encountered in different production systems ;

- ✓ Strategic control measures of reproductive diseases had to be formulated based on early control and prevention of the possible causes;

- ✓ Dairy farmers, development agents and veterinarians should get training on dairy cattle management, breeding system, record keeping, and reproductive health management.

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8. APPENDICES

Appendix I: Data Recording Format for Blood Sampled Cattle

Name of Farmer/Dairy Farm__________Wereda_______Tabia________Kushet _____

S.No	Code Number	Animal ID	Breed	Age	Sex	Parity	BCS	Herd Size	Herd Type	Mating System	Production System	Housing System	Sanitation	Status of the Cow	History of Testicular swelling, Abortion, RFM	RBPT Result(+ or _)
1																
2																
3																
4																
6																
7																
8																
9																
10																
11																
12																
13																
14																
15																

Appendix II: Retrospective Data Collection Sheet for Some Reproductive Problems of Dairy Cattle

Name of Farmer/Dairy Farm_______Wereda_________Tabia__________Kushet ________

S.No	Number of cows assessed	Animal ID	Breed	Age	Parity	BCS	Mating system	Production system	Sanitation	Lactation Status	Pregnancy status	Reproductive problems found
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												

Appendix III: Questionnaire Survey for the Assessment Major Reproductive Problems and Associated Risk Factors of Dairy Cows

1. Personal Address

Name of Respondent________________________________Age__________sex_______

Educational level_________ Zone________District______Peasant Association________

2. Farm type

- Communal ☐
- Small-scale Commercial ☐
- Large-scale Commercial ☐

3. Agro ecology

- Highland ☐
- Midland ☐
- Lowland ☐

4. Household Classification

- Rural ☐
- Urban ☐
- Peri-urban ☐

5. Number of People Residing in Household

Males ☐ Females ☐ Children <15yrs ☐

6. Educational Level: Illiterate ☐ Grade1-7 ☐ Grade7-12 ☐ >12 Grade ☐

7. Livestock Activity

7.1. Is Livestock the major activity on your farm? Yes ☐ No ☐

7.2. Livestock Kept by type and Age

Livestock Type	Breed type		Total	Rank the most important species
	Local	Cross		
Cattle				
Sheep				
Goat				
Poultry				
Others				

8. Major Farming activity and rank (*tick in the first column and rank in the second column*)

1. Livestock Production		
2. Crop Production		
3. Mixed type Production		

9. System of Production

Intensive	
Semi intensive	
Extensive	

10. Purpose of Keeping Cattle (*tick in the first column and rank in the second column*)

1. Milk			7. Hide		
2. Meat			8. Cash from Sells		
3. Work (draft)			9. Dowry		
4. Stud breeding			10. Ceremonies		
5. Manure			11. Investment		
6. Bleed			12. Cultural		

13. Others (Specify)________________________

11. Members of Household responsible for cattle activities

	Adults		Boys	Girls	Labor
	Males	Females	(<15yrs)	(<15yrs)	
1. Purchase cattle					
2. Selling and slaughtering cattle					
3. Herding					
4. Breeding decisions					
5. Feeding					
6. Milking					
7. Making dairy					
8. Selling dairy products					
9. Animal health					
10. Others (specify)					

12. Grazing (Feeding)

	Dry season	Wet season
1. Herded		
2. Paddock		
3. Tethered (outdoor)		
4. Stall		
5. Yard		
6. Free grazing		
7. Others (specify) ___________		

13. Housing

	Dry season	Wet season
1. Kraal		
2. Stall (shed		
3. Yard		
4. None		
5. Others (specify) ___________		

14. Are calves housed together with adults?

Yes ☐ No ☐

15. Materials used for housing

1. Untreated Wood (bush) ☐ 3. Iron sheets ☐ 5. Mad ☐

2. Treated Wood ☐ 4. Bricks ☐ 6. Wire ☐

16. Form of housing

1. Roof ☐ 3. Floor a. Concrete ☐ b. Wooden ☐

2. Solid Well ☐ c. Earth ☐

17. Supplementation regime

Dry season   Wet season

1. Roughage (crop residue)
2. Minerals (salts)Vitamins
3. Bought in Feed (Concentrates
4. None
5. Others (specify) ______

18. How cattle were watered?

Dry season   Wet season

1. Animals go to water
2. Water is fetched (provided)
3. Both

19. Source of water

Dry season   Wet season

1. Bore hole
2. Dam(pond
3. River
4. Water well
5. Spring
6. Municipal (piped
7. Others (specify) ________________

20. Distance to farthest water point

Dry season   Wet season

1. At household
2. <1Km
3. 1-5Km
4. 6-10Km
5. >10Km

21. Frequency of watering

	Dry season	Wet season
1. Freely available		
2. Once a day		
3. Twice a day		
4. Every other day		
5. Once in 3 day		
6. Other (specify)__________________		

22. Water quality

	Dry season	Wet season
1. Good (clear)		
2. Muddy		
3. Salty		
4. Smelly		

23. Trend within herd

1. Increasing
2. Decreasing
3. Stable
4. Unknown

24. Mating system

1. Uncontrolled
2. Hand mating
3. Group mating
4. A.I
5. Other (specify) ________________

25. Access to veterinary services

1. Government vet
2. Private vet
3. Veterinary
4. Extension service

5. None

6. Other (specify) ________________

26. Have you ever seen reproductive problem in your farm?

Yes No

27. If you answer yes in question 26, list the local name or symptom of disease (Rank the most common first)

1.____

2.____

3.____

4. ____

5.____

28. Are animals free fed when sick? Yes No

29. If yes is the answer in question 28, are treatments or vaccinations (preventive treatments) given?

Yes No

30. If yes list:

Local name (symptom of disease) Done routinely Done when need arises

1. ________________________________

2. ________________________________

3. ________________________________

4.________________________________

5. ________________________________

31. Did you see any abortion in flock of cattle? Yes No

32. How many animals did you see with abortion at any time with in 5 years? _________

33. At what stage pregnancy do you face abortion? __________________

34. In which stage of parity abortion is observed? __________________

35. Have you seen any retention of placenta after birth? Yes No

36. What do think the cause of abortion (just to know farmers' perception?)

____

____

37. What is the local name for disease that causes abortion? If any____________________

38. Is there any vaccination given for?______________________

39. Do you assist the dairy cows during parturition?

Yes ☐ No ☐

39.1.If yes do you use protective gloves(masks) when assisting with the parturition (abortion of animals) hand ling placentas and aborted fetuses? Yes ☐ No ☐

40. Where do you dispense placentas, dead fetus and aborted fetuses?

1. Water canals ☐ 3.Burying ☐
2. Throwing on field ☐
4. Other (Specify)_______________________ ☐

41. Where do you dispose animal carcasses?

1.____________________ 2 .________________ 3.________________

42. What do you do when your animal is infected with Brucella?

1. Separate the infected animal ☐ 5.Sell to butcher ☐
2. Hot sell to neighbor ☐ 6.Call the local veterinarian ☐
3. Sell to neighbor ☐ 7.Buy a vaccine or treatments ☐
4. Sell to market ☐

43. Do you separate aborted animal from to other?

Yes ☐ No ☐

44. What do you do the milk produced from your farm?

1. Sell raw milk ☐ 5. Other (Specify)_________________________ ☐
2. Processed cheese ☐
3. Processed cream ☐
4. Processed butter ☐

45. Do you boiled raw milk? Yes ☐ No ☐

45.1. If yes 1. Before consumption ☐ 2. Boiled before processing ☐

46. Do you keep your animal separated from other flocks during grazing and watering?

Yes ☐ No ☐

47. Do you use separate housing for your cattle? Yes ☐ No ☐

48. Do you use mixed grazing and watering with other animals? Yes ☐ No ☐

49. Do you migrate your animals to other area? Yes ☐ No ☐

49.1 To where ______________________

49.2 In which season ______________

50. Do you milk your cattle? Yes ☐ No ☐

51. Who is responsible in milking? Woman ☐ Man ☐ Child? ☐

52. Do you consume milk? Yes ☐ No ☐

53. How do consume milk? Raw ☐ Treated ☐ Other_________ ☐

54. Do you know any disease transmitted to human by drinking raw milk? Yes ☐ No ☐

55. Have you seen any lameness in your cattle? A. Yes B. No

56. Did you see any testicular swelling? A. Yes B. No

57. Do you consume raw meat of cattle? A. Yes B. No

58. Do you heard of the disease brucellosis? A. Yes B. No

59. If your question in 35 is yes to which animals does this affects?

Cattle	☐	Poultry	☐	Sheep	☐
Donkey	☐	Goat	☐	Others	☐

60. Do you believe that brucellosis can be transmitted to humans?

Yes ☐ No ☐

60.1. If yes are you sure ? Yes ☐ No ☐

*If yes, what do you believe the means of transmission?

1. Physical contact with animals
2. Through contact with Fetuses' or total membranes
3. Through drinking contaminated raw milk
4. Through contact with untested humans

61. What are the symptoms of this disease? (mention)

1. No symptoms
2. Abortion
3. Drop in milk production
4. Fever
5. Loss of appetite
6. Loss of body weight
7. Other (specify)________________

Appendix IV: Rose Bengal Plate Test

Principle, Material and equipment and procedure of RBPT

The principle Test

The RBPT is rapid agglutination test effective in diagnosis of brucellosis when used as a screening test. The Antigen for RBPT is a dense suspense of inactivated *brucella* organism stained with Rose bengal adjusted at PH 3.65

Material and equipment

- Applicator
- Micropipette
- Micropipette tips
- Plates

Reagent:

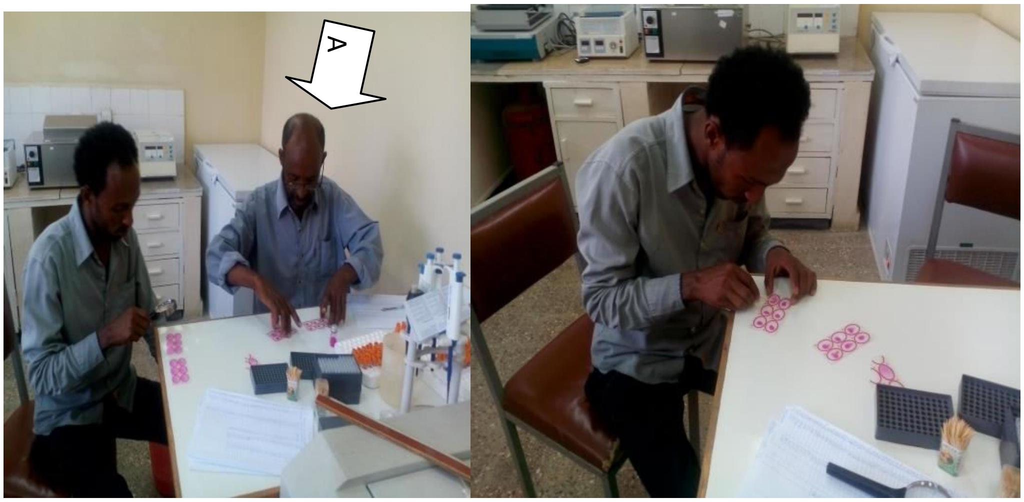
Rose bengal stained antigen

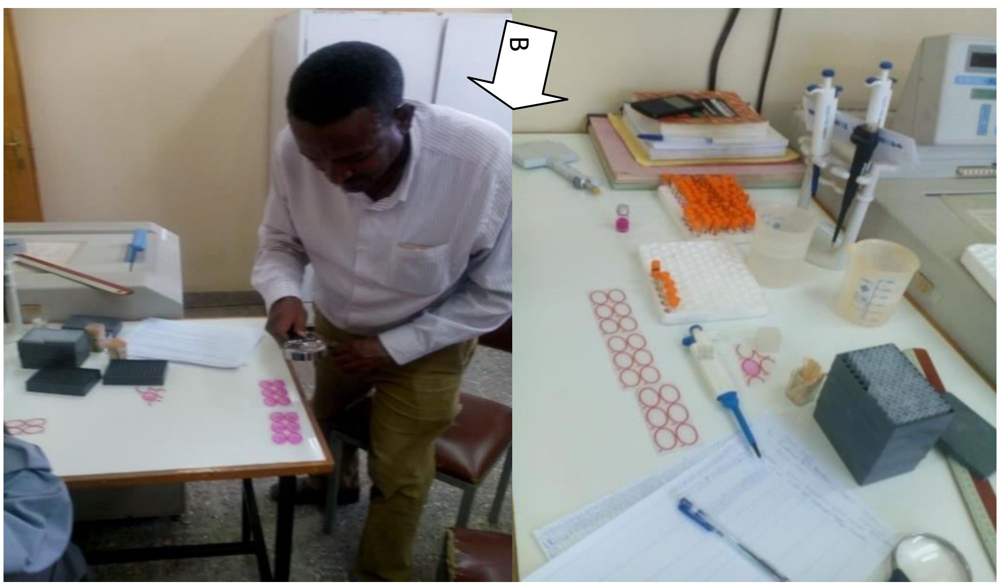
Positive and negative sera

Procedure

For the RBPT, the procedure described by OIE (2009) was followed. Both serum and rose bengal antigen was removed from the refrigerator and left at room temperature for at least 30 min before the test was performed *Brucella abortus* antigen Strain 99 was used manufactured by Institut Pourquier, rue de la Galera 34097 Montpellier, France and positive as well as negative control sera from Mekelle Regional Animal Health Diagnosis and Investigation Laboratory, Mekelle, Ethiopia, were used. Briefly, 30 µl of sera samples were dispensed on to the plate, and 30 µl of RBPT antigen was dropped alongside the sera. Using an applicator stick, the antigen and the sera were mixed and examined for agglutination. Positive and negative controls were employed for interpretation of the results. Results of RBPT were interpreted as 0, +, ++, and +++ as has been described by OIE (2009) with 0=no agglutination; +=barely visible agglutination (seen using magnifying glasses); ++=fine agglutination; and +++=coarse agglutination. Those samples with no agglutination (0) were recorded as negative while those with +, ++, and +++ were recorded as positive.

Appendix V: Some Figures during the Study Period





Some Photos in Mekelle Regional Animal Health Diagnosis and Investigation Laboratory with Dr. Teklay Nigusse(A) and My colleague Tadesse Gugssa (B).

Uterine prolapse Case in Sefho (Laelay Maichew District)

Statutory Declaration

I declare that this thesis presents the work carried out by myself and does not incorporate without the acknowledgement of any material previously submitted for a degree or diploma in any university; and to the best of my understanding, it does not contain any materials previously published or written by another person except where due reference is made in the text; all substantive contributions by others to the work presented including jointly authored publications, is clearly acknowledged.

Name of the Candidate: Bahlibi Weldegebriall Signature: ________________

Place: Mekelle University, Mekelle, Ethiopia

Date of Submission: June, 2015