ADDIS ABABA UNIVERSITY, COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE, DEPARTEMENT OF CLINICAL STUDIES

EPIDEMIOLOGY OF CANINE AND SWINE BRUCELLOSIS IN SELECTED AREAS OF EAST SHOA ZONE, OROMIA REGIONAL STATE, ETHIOPIA

MVSc Thesis

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EPIDEMIOLOGY OF CANINE AND SWINE BRUCELLOSIS IN SELECTED AREAS OF EAST SHOA ZONE, OROMIA REGIONONAL STATE, ETHIOPIA

A Thesis Submitted to Addis Ababa University College of Veterinary medicine and Agriculture in partial fulfillment of the requirements for the degree of Master of

Science in Veterinary Epidemiology

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As MSc research advisors, we hereby certify that we have read and evaluated this Thesis prepared under our guidance by **Aregawi Girmay** entitled: "**Epidemiology of canine and swine brucellosis in selected areas of East Shoa Zone, Oromia Regional State, Ethiopia",** we recommend that it can be submitted as fulfilling the MSc thesis requirement.

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DEDICATION

This thesis manuscript is dedicated to my Father, Mr. Girmay Gebreegziabher, who was always immersed in hope about my success though death comes a bit before a month.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my original work and that all source of material used for this have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirement for a postgraduate (MVSc) degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/ College Library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institute anywhere for the award of any academic degree, diploma, or certificate.

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AAUCVMA	Addis Ababa Universty college of veterinary medicine and agriculture
AD	Anno domini
ATVET	Agricultural and technical vocational education college
BC	Before Christ
BPAT	Buffered plate agglutination test
CFT	Complement fixation test
CI	Confidence interval
CIEP	Counter immuno electrophoresis
CSA	Central Stastical Agency
DCs	Dendritic cells
DNA	Deoxy ribose nucleic acid
ELISA	Enzyme linked immuno sorrbent assay
FPA	Fluorescence polarization assay
Ig	Immunoglobin
LPS	Lipo polysaccharide
mAb	monoclonial antibody
ME	Mercapto ethanol
NMSA	National Meteorological Service Agency
NVI	National Veterinary Institute
OIE	Office of International des Epizootics
OR	Odds ratio
PCR	Polymerase chain reaction
Po	per-os
PRRs	Pattern recognition receptors
RBPT	Rose Bengal Plate test
RSAT	Rapid slide agglutination test
SAT	Serum agglutination test
SLPS	Smooth lipopolysaccharide
SRBC	Sheep red blood cells
TAT	Test-tube agglutination test
WHO	World Health Organization

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ABSTRACT

A cross sectional study was carried out from November, 2017 to May 2018 in Batu town, Alage ATVET College, and Naka village using serological tests, to determine the seroprevalence of canine and swine brucellosis. Accordingly, a total of 389 owned dogs (207 from Batu, 107 from Alage, and 75 from Naka) and 196 pigs (167 from Alage and 29 from Batu swine farms) were included in the study. Rose Bengal Plate Test (RBPT) prepared from the smooth strain *B. abortus* antigen was used as a screening test; whereas Complement Fixation Test (CFT) was the confirmatory test for both swine and canine brucellosis. Furthermore, all sera samples from dogs had also screened by RBPT containing a rough strain *B. canis* antigen; and those positive samples had considered as positive for *B. canis* infection. The result of the present study indicated that, among the 389 sampled owned dogs, 21 (5.4%; CI: 3.35, 7.96) were positive for anti *B. abortus* antibodies using RBPT; and 19 of them (4.88%; CI: 2.7, 7.0) were confirmed by CFT. Moreover, 34 (8.74%; CI: 5.92, 11.56) owned dogs were positive for anti *B. canis* antibodies. Among 196 serum samples from pigs, 10(5.1%; CI: 0.95, 6.19) were found to be positive by RBPT and 7(3.57%; CI: 1.99, 8.21) of them were further confirmed by CFT. The final simplified multivariable logistic regression analysis of the risk factors revealed that sex, living condition, and history of obstetrical problem were significantly associated with the overall canine brucellosis due to both strains of *Brucella* species ($p< 0.05$). Moreover, location difference and age had also significantly associated with canine brucellosis due to smooth and rough strains, respectively ($p< 0.05$). On the other hand, age and history of obstetrical problems were the major risk factors for overall swine brucellosis seropositivity ($P< 0.05$). Thus, the present study suggests that canine and swine brucellosis are prevalent in the study areas. The seropositivity in both animals could give an insight that brucellosis could pose a public health hazards. The awareness of the people toward the disease was also the gap in the study area. Hence, this warrants public education among the community; and further extensive epidemiological and molecular investigation is recommended.

Key words: *Epidemiology, Canine, Swine, Brucellosis, Alage, Batu, Naka*

1. INTRODUCTION

Nowadays, our world has been threatened by numerous emerging and re-emerging pathogenic diseases; these diseases are seriously affecting the wellbeing of human, animal health, and animal production. Most of them are zoonotic diseases and have great veterinary and public health impact, particularly in developing countries where people are having daily frequent contact with livestock and animal products. Brucellosis is one of them. It is an ancient and one of the world's most widespread zoonotic diseases accounting for the annual occurrence of more than 500,000 human cases; affecting both public health and animal production which is caused by a Gram-negative, facultative, and intracellular bacteria of the genus *Brucella* (Pappas *et al*., 2006). Domesticated animals such as cattle, buffalo, sheep, goats, swine and dogs are the hosts of *Brucella*; and the species of *Brucella* that cause disease in domesticated livestock are: *B. abortus* (cattle and buffalo), *B. ovis* (sheep), *B. melitensis* (sheep and goats), *B. suis* (pigs) and *B. canis* (dogs). It is primarily a disease of the reproductive tract of animals mainly characterized by:- late stage of abortion, infertility, retention of placenta, reduced milk yield , orchitis, and epididymitis in animals; and undulant fever, head ache, sweating, and other complications in human (Radostits *et al.*, 2007).

Brucellosis is endemic in the Middle East, Mediterranean countries, Asia, Africa and Central and South America; and consistently ranked among the most economically important zoonoses globally, as it results in reduced productivity, abortions, weak offspring and major impediments for trade and export of livestock. It poses a barrier to trade of animals and animal products between countries and causes considerable economic losses which can lead for international trade ban (Girmay *et al.*, 2013; Yasmin and Lone, 2015).

In many high-income countries, it has been successfully controlled both in livestock populations and man. However, in low-income countries like Ethiopia, it is endemic with large disease and livelihood burdens in animals and people and very weak effective control (McDermott *et al.*, 2013). This might be due to mismanagement on animal quarantine, trans-boundary animal movement, weak eradication and

vaccination program, and lack of awareness of the disease among pastoralist, farmers and general public.

Currently in Ethiopia, because of its significance to the livestock health, public health and the economy of the country; it is of high national priority. Since the first report of brucellosis in the 1970s in the country, the disease has been noted as one of the important livestock diseases and has been reported from different localities, commonly targeted on bovine, occasionally on sheep and goats, and rarely on camels (Asfaw *et al.,* 2016).

Many Ethiopians keep dogs as a domestic pet both in rural and urban communities. However, the practice of providing due care to these human companions is very rare. Owners feed them family food leftover and taking pets to veterinary clinics for medical treatments is considered something of a luxury. In urban areas, it is common to find a large number of stray dogs roaming freely in the streets scavenging for their survival. If irresponsible owners continue to allow indiscriminate growth of the pet dog population, they will be added to the stray dog population. In this event, brucellosis infectivity rate in the stray dog population continues and infected dogs will increasingly contaminate the environment with aborted fetal tissue, vaginal discharges, faeces, ejaculate and urine. The role of infected dogs in spreading of *B. abortus, B. suis*, and *B. melitensis* to neighboring herds, flocks and humans had reported by Baek *et al.* (2003) and Vieira *et al.* (2015).

However, despite of these risk factors which serve as sources of infection to other domestic animals and human, to the best of knowledge, canine brucellosis had not studied in the country. Even in Africa, except in Nigeria (Cadmus *et al.* 2011), Zimbabwe (Chinyoka *et al.,* 2014), and South Africa (Gous *et al.* 2005), there is dearth of information on canine brucellosis. Furthermore, though the population of pigs has shown increment, the occurrence and epidemiology of brucellosis in these animals is poorly understood. Unlike other livestock distribution, swine farms are predominantly found in the central part of the country and the importance of the disease in these animals has not been addressed to date. Report had indicated that only one study by Kebeta *et al.,* (2015) had done before, to determine the seroprevalence of swine brucellosis in central part of Ethiopia, using RBPT. Hence, as

human and animal interactions occur in various ways, an investigation of the disease in these neglected companion animals and pigs will narrow the currently knowledge gap; and have a great contribution to over all public health, animal health and livestock profitability. This epidemiological investigation was therefore, designed with the aim:

- ✓ To estimate the sero-prevalence of brucellosis in dogs and pigs in selected areas of East Shoa Zone, Oromia Regional State, Ethiopia
- ✓ To evaluate the degree of association of potential risk factors and sero-prevalence of canine and swine brucellosis.
- ✓ To assess the awareness and practices of dog owners and swine farm employees towards the disease.

2. LITERATURE REVIEW

2.1. Historical perspectives of brucella

The paleo-pathological evidence from the partial skeleton of the late Pliocene *Australopithecus africanus* suggests that brucellosis occasionally affected our direct ancestors 2.3–2.5 million years ago (Danastasio *et al.,* 2011). The pathological, molecular (DNA analysis) and electron microscopy findings from the human skeletal remains, and remains of buried cheese also suggested the presence of brucellosis long time ago: 3000-1200 BC in Bahrain and Persian Gulf; and 2100-1550 BC in Palestine and Jordan. Recent examination of the ancient Egyptian bones, dating to around 750 BC, showed evidence of sacroiliitis and other osteoarticular lesions and common complications of brucellosis (Seleem *et al.,* 2010). A type of fever characterized by fairly regular remissions or intermissions has been also recognized along the Mediterranean littoral since the time of Hippocrates in 450 BC (Mantur *et al.,* 2007. It had also suggested its presence in Roman town 'Pompeii' and 'Herculaneum' (79 A.D.); and in Butrint and Albania from 1260- 1020 AD (Anisur Rahman, 2014).

Much later in the 19th century, the disease was found to affect British armed forces and the local population of Malta (Wyatt, 2005; Wyatt, 2009). Brucellosis has many synonyms derived from the geographical regions in which disease occurs e.g., Mediterranean fever , Malta fever , Gibraltar fever , Cyprus fever ; from the remittent character of the fever e.g., undulant fever; or from its resemblance to malaria and typhoid e.g., typhomalarial fever and intermittent typhoid (Wyatt, 2005; Wyatt , 2011). Its cause was obscure until 1887 when Sir David Bruce, a Scottish physician reported numerous small coccal organisms in stained sections of spleen, from a fatally infected soldier and isolated the organism in culture from spleen tissue of four other British soldiers stationed at Malta. This organism, which he designated *Micrococcus melitensis*, produced a remittent fever in inoculated monkeys. The organism then derived its species name from 'Melita' (honey), the Roman name for the Isle of Malta (Mantur *et al*., 2007).

Thereafter, Surgeon Cap-tain M.Louis Hughes and Captain James Crawford Kennedy discovered significant details on the zoonotic transmission of brucellosis, including venereal transmission in both humans and animals (Wyatt, 2009). Wright and Smith in 1897 detected antibodies to *M. melitensis* in human and animal sera through agglutination test, which unraveled the zoonotic potential of the disease. Later, Zammit a young Maltese physician working with "Mediterranean Fever Commission" in 1905 confirmed it by isolating the organism from the milk and urine of goats. Thus he concluded that the goat was the reservoir of *M. melitensis* and the consumption of the raw milk and cheese infects man. The discovery that apparently healthy goats could be carriers of the disease has been termed one of the greatest advances ever made in the study of epidemiology (Mantur *et al*., 2007).

In the same year that Hughes monograph appeared, Bang in Denmark isolated a gram-negative rod from cattle, which had aborted. The third member of the group, which also is bacillary in shape was recovered from the foetus of aborted swine, by Traum in 1914 in the USA and implicated as an agent of brucellosis in man by Huddleson in 1943 (Mantur *et al.,* 2007; Seleem *et al.,* 2010). In 1918, Alice Evans an American bacteriologist published reports which contained convincing evidence that *M. melitensis* from goats and a Gram-negative rod from cows could not be differentiated morphologically or by their cultural and biochemical reactions but there were antigenic differences which could be shown by agglutination absorption test. She also showed in 1920 that *M. melitensis* was also a bacillus. She showed that *M. melitensis*, isolates of cows and pigs belonged to one genus. Meyer and Shaw further confirmed Evan's observations and suggested the generic name *"Brucella"* in honour of Sir David Bruce. The possible pathogenicity of *B. abortus* to man was then suggested by Evan in 1918 and confirmed by others (Mantur *et al*., 2007; Mantur and Amarnath, 2008; Moreno, 2014).

In 1956, Buddle and Boyce discovered *B. ovi*s, the cause of epididymitis in rams. In 1957, Stoenner and Lackman isolated *B. neotomae* from desert wood rat in Utah in USA. In 1968, Carmicheal and Bruner discovered *B. canis* as the cause of an epidemic of abortions in beagles. Human infections due to *B. canis* have been reported. Thereafter, two new *Brucella* species (*B. pinnipediae* and *B.cetaceae)* have been isolated from marine hosts within the past few years (Mantur *et al*., 2007).

2.2. Descriptive features of brucella

2.2.1. Biology of brucella

Brucellae belong to α–2 subdivision of proteobacteria and interestingly (being animal pathogens) have close relationships with soil organisms (e.g. *Ochrobactrum* species), with plant symbionts (e.g. *Rhizobium* species) and with phytopathogens (e.g. *Agrobacterium* species) (Scholz *et al*., 2008). All of these bacteria inhabit eukaryotic cells, and comparative genomic studies indicated that they have evolved from a common ancestor. *Brucellae* are Gram-negative, measuring 0.6- 1.5µm length to 0.5-0.7µm width in size, partially acid fast, aerobic, non-spore forming, non-motile, and facultative intracellular coccobacilli or short rods (Bargen *et al.,* 2012). They are oxidase, catalase, and urease positive. The genome contains two circular chromosomes except *B. suis* biovar 3, which has a single chromosome. *Brucellae* have two types of smooth lipopolysaccharide (SLPS) surface antigens, designated 'A' and 'M'; 'A' antigen predominates in *B. abortus* and *B. suis*, while 'M' is the major antigen in *B. melitensis*. Numerous outer and inner membrane, cytoplasmic, and periplasmic proteins have also been characterized (Mantur and Amarnath, 2008).

2.2.2. Survival out of host

Brucella may remain viable within the environment for a period of time. Its viability outside the mammalian host is enhanced by cool temperatures and moisture and decreased by high temperatures, dryness and direct exposure to sunlight. For example, *B. abortus* survives a couple of hours under direct sunlight but up to 185 days in the cold and shade. It also survives in aborted fetuses, manure and water for periods of up to 150 to 240 days (Saegerman *et al.,* 2010). *B. suis* survives so well in raw meat; e.g. 128 days in sausage meat, means that prepared pork products are always a source of infection. *B. suis* is more resistant to adverse environmental conditions than *B. abortus*, although its longevity outside the body has not been fully examined. It is known to survive in feces, urine, and water for 4-6 weeks. The organism can survive on grass for variable periods depending on environmental conditions. In temperate climates, infectivity may persist for 100 days in winter and 30 days in summer (Straw *et al*., 2006; Saegerman *et al*., 2010).

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. Disinfectants reported to destroy *Brucella* on contaminated surfaces include 2.5% sodium hypochlorite, 2-3% caustic soda, 20% freshly slaked lime suspension, or 2% formaldehyde solution (all tested for one hour). Ethanol, isopropanol, iodophores, substituted phenols or diluted hypochlorite solutions can be used on contaminated skin. Autoclaving (moist heat of 121°C for at least 15 minutes) can be used to destroy *Brucella* species on contaminated equipment. These organisms can also be inactivated by dry heat (160-170°C for at least 1 hour). Boiling for 10 minutes is usually effective for liquids. Xylene (1ml/L) and calcium cyanamide (20 kg/m^3) are reported to decontaminate liquid manure after 2 to 4 weeks. *Brucella* species can also be inactivated by gamma irradiation (e.g. in colostrum) and pasteurization. Their persistence in unpasteurized cheese is influenced by the type of fermentation and ripening time. The fermentation time necessary to ensure safety in ripened and fermented cheeses in unknown, but is estimated to be approximately three months. *Brucella* is reported to persist for weeks in ice cream and months in butter. This organism survives for very short periods in meat, unless it is frozen; in frozen meat, survival times of years have been reported (IOWA State Universty, 2007).

2.3. Brucellosis in dogs and pigs

2.3.1. Etiology

Swine brucellosis is caused by *B. suis* and there are five biovars of *B. suis* with biovars 1, 2 and 3 infect primarily domestic and feral pigs, as well as wild boar (Aparicio, 2013). Despite the description of cases of brucellosis in dogs caused by four of the six species of the genus *Brucella*, three of these (*Brucella melitensis*, *Brucella suis*, *Brucella abortus*), produce occasional infections in individual animals, while *B. canis* is of epidemiological importance (Wanke, 2004). Generally, *Brucella* species that can infect pigs and dogs are summarized below (Table-1).

Table-1:- *Brucella* species affecting pigs and dogs (WHO, 2006).

Hosts	*B. abortus*	*B. melitensis*	*B. suis*	*B. canis*	*B.ovis*
Pigs	+	+	+	-	-
Dogs	+	+	+	+	-

2.3.2. Distribution

The epidemiology of brucellosis is complex and it changes from time to time. Wide host range and resistance of *Brucellae* to environment and host immune system facilitate its survival in the populations. Worldwide, it remains a major source of disease in humans and domesticated animals (Mantur *et al.,* 2007; Yasmin and Lone, 2015).

Swine brucellosis is found worldwide in most areas where pigs are kept. It is infrequent and occurs sporadically in Europe, Asia, and Oceania (Meng *et al.,* 2009). Many predominantly Muslim countries and Israel are regarded free of *B. suis* infection due to religious beliefs that limited swine production. Generally, although the disease is of widespread in occurrence globally, the prevalence is low with the exception of South America and South-East Asia, where the prevalence is higher. In Latin America, swine brucellosis is enzootic and the region is thought to have the highest prevalence in the world (Woldemeskel, 2013). Available epidemiological evidence shows that *B. suis* biovar 2 is the most common agent, but biovars 1 and 3 can also occur. In Africa, the disease is believed to occur sporadically; and a number of sub-Saharan African countries officially reported porcine brucellosis to the OIE (Godfroid *et al*., 2011).

Canine brucellosis had reported in the Southern states of the United States, Central and South America (Mexico, Brazil, Argentina and Chile), Europe (Germany, Spain, Italy, Czechoslovakia, Poland and France), Asia (India, Philippines, Korea, Japan, China, Turkey, Malaysia, and Taiwan), and in Africa (Wanke, 2004).

2.3.3. Transmission

B. canis can be a major problem in dog breeding kennels and transmission is mainly during breeding. The most common mechanism dog to dog transmission is by mouth and nose contact with vaginal discharges from an infected bitch. This can occur while the bitch is during estrus, after an abortion, or during whelping. It should be remembered that dogs can acquire infection with *B. abortus*, *B. melitensis* or *B. suis* from aborted ruminants or swine, usually by contact or ingesting fetal or placental material or with food or environment contaminated by abortion excreta. In aborted materials, bacteria can be found in concentrations up to 10^{10} per mililitre. Although both sexes excrete bacteria in urine, the concentrations in male urine are higher, reaching 10^{3}–10^{6} bacteria/ml of urine. For this reason, urine from a male is more dangerous as a source of infection. Excretion of bacteria through urine starts at 4–8 weeks after infection. Bacterial concentration in milk is high and low concentrations of bacteria have also been isolated from saliva, nasal and ocular secretions, and from feces (Wanke, 2004). In addition, cages and equipment in contact with infected dogs have been reported as sources of infection. Sexual transmission is also an important means of spread and males can excrete the organisms in large numbers in their semen. Puppies can become infected in utero from their mother during pregnancy, and as neonates through milk or contact with infected surfaces (Greene and Carmichael, 2006; Iowa State Universty, 2007; Megid *et al.*, 2010; Stella and Croney, 2015).

The transmissions for swine brucellosis are similar to those identified for other types of *Brucella* infection, being essentially the oral, nasopharyngeal, conjunctival and vaginal mucosa routes (Kebeta *et al.*, 2015). It is transmitted by direct contact with recently aborted sows, by ingestion of contaminated food or exposure to a contaminated environment. However, sexual transmission is particularly important and brucellosis may be introduced on to farms through the communal use of boars (WHO, 2006; Iowa State Universty, 2007; Megid *et al*., 2010; Kebeta *et al*., 2015).

2.3.4. Pathogenesis

i. Entry into the host

The ability of the pathogen to survive and replicate within different host cells explains its pathogenicity. Pathogenesis depends upon various factors such as the species, size of the inoculum, modes of transmission and the immune status of the host (Ray *et al*., 2009; Alavi and Motlagh, 2012; Muflihanah *et al*., 2013; Acharya *et al*., 2016). The most common portals of entry for *Brucella* in dogs and pigs are mucous membranes of the respiratory (aerosol) and digestive tracts. Besides, the conjunctiva and membranes covering the sexual organs are important. Following penetration of mucosal epithelium, bacteria are transported, either free or within phagocytic cells, to regional lymph nodes, which become enlarged due to lymphatic and reticulo-endothelial hyperplasia and inflammation. These changes may require several weeks to develop and may persist for months. If bacterias do not become localized and are not killed in regional lymph nodes, they will spread to other organs via lymph and blood (Lapaque *et al.,* 2005; Salcedo *et al.,* 2008).

Brucellae gain access to the uterus via a hematogenous route, and the bacteria initially localize within erythro-phagocytic trophoblasts of the placentome. Adjacent chorio-allantoic trophoblasts become infected and support massive growth of the bacteria. These cells eventually rupture and ulcerate the chorio-allantoic membrane. Bacteria and inflammatory cells both are present within the lumen of the uterus. Bacteria spread via a hematogenous route to the fetus and to the placentome. Fetuses may also ingest amniotic fluid containing brucellae. It is important to note that the endometrium is not infected with brucellae; and other than a diffuse submucosal inflammatory reaction, it remains largely intact. The host mechanisms responsible for increased susceptibility to infection as pregnancy advances are not known, but it may be related to the differential susceptibility of placental trophoblasts during the middle and late stages of pregnancy. The probability of isolation of *B. abortus* at parturition increased from 0.22 to 0.9, as fetal age at the time of challenge of non-vaccinated heifers increases from 60 to 150 days of gestation (Gyles *et al*., 2004; de Figueiredo *et al*., 2015).

The ability to utilize erythritol has been proposed as a virulence factor for brucellae, and genes for erythritol metabolism have been identified in a pathogenic *B. abortus* strain. Erythritol, a substance produced by the fetus and capable of stimulating the growth of brucella, occurs naturally in greatest concentration in the placental and fetal fluids and is responsible for localization of the infection in these tissues. The presence of elevated amounts of erythritol in uterine tissues of swine, suggests an important role for the ability to utilize erythritol in the tissue tropism of certain *Brucella* species. Moreover, extracts of fetal fluids, placenta, and chorion have been shown to stimulate growth of *B. abortus, B. melitensis,* and *B. suis* (Gyles *et al.,* 2004; Radostits *et al.,* 2007).

ii. Survival inside host cell

Brucellae lack classic virulence factors like toxins, fimbriae and capsules which raises the possibility that they might have unique and subtle mechanisms to penetrate host cells, elude host defenses, alter intracellular trafficking to avoid degradation and killing in lysosomes and modulate the intracellular environment to allow long-term intracellular survival and replication. The *Brucella* O-polysaccharide appears to be a key molecule for cellular entry, to prevent complement-mediated bacterial lysis and to prevent apoptosis (i.e. programmed cell death) of the macrophages within which they reside allowing them to extend their longevity (Lapaque *et al.,* 2005).

Brucella has developed mechanisms to avoid innate immunity by minimizing stimulation of pattern recognition receptors (PRRs) of the host. The *Brucella* cell envelope has high hydrophobicity and its LPS has a non-canonical structure that elicits a reduced and delayed inflammatory response compared with other Gram-negative bacteria. The O side chain on the LPS can form complexes with the major histocompatibility complex class II molecules that interfere with the ability of macrophages to present exogenous proteins. The rough strains (i.e., strains with lipopolysaccharide lacking the O-side chain) are less virulent because of their inability to overcome the host defense system. However, under in vitro conditions, up to 90% of virulent *Brucella* and 99% of nonvirulent *Brucella* may be killed following intracellular entry (Anisur Rahman, 2014).

Brucellae display strong tissue tropism and replicate within vacuoles of macrophages, dendritic cells (DCs), and placental trophoblasts. However, the pathogen has the ability to replicate in a wide variety of mammalian cell types, including microglia, fibroblasts, epithelial cells, and endothelial cells. The intracellular lifestyle of *Brucella* limits exposure to the host innate and adaptive immune responses, sequesters the organism from the effects of some antibiotics, and drives the unique features of pathology in infected hosts, which is typically divided into three distinct phases: the incubation phase before clinical symptoms are evident, the acute phase during which time the pathogen invades and disseminates in host tissue, and the chronic phase that can eventually result in severe organ damage (de Figueiredo *et al.*, 2015). *Brucella* species prevent apoptosis within the macrophage and their long-term survival in the reticulo-endothelial system of spleen, liver, and bone marrow will sustain chronic infection. During gestation, they replicate in large numbers in placental trophoblasts. The integrity of the placenta may be disrupted and abortion induced. The pregnant uterus is an immunological privileged site, which prevents the rejection of the fetus by modulating local immune responses which in turn may allow the bacteria to replicate extensively (Godfroid *et al*., 2011).

2.3.5. Clinical signs

i. In dogs

Clinical signs of brucellosis in dogs can range from weight loss and lethargy, to late-term abortions in females, epididymitis and prostatitis in males, and infertility, lymphadentitis, ocular problems, and diskospondylitis (a destructive, inflammatory process of the intervertebral disks) in both sexes (Radostits *et al*., 2007; Krueger and Lucero, 2014; Stella and Croney, 2015) (Fig-1)**.** The classic symptom of canine brucellosis in the bitch is a late-term abortion (45–55 days' gestation), resulting in the birth of stillborn puppies that are often autolysed, having subcutaneous edema, congestion and hemorrhage of the subcutaneous abdominal region. The bitch will continue to excrete vulvar discharge with high numbers of bacteria for several weeks after the abortion or parturition. If the puppies survive, they may be weak and die within a few hours or weeks of birth. Some apparently normal puppies will survive but show clinical signs or test positive for the disease as they age, sometimes waiting

until puberty. Females may also exhibit embryo resorption or conception failure (Hollett, 2006; Makloski, 2011).

Males may have more obvious signs of *B canis*. During the acute stages of the disease, many male dogs may have epididymitis, which results in swelling of the epididymis and leads to pain and discomfort in the scrotum. This may lead to licking of the scrotum, then scrotal edema, dermatitis, and scrotal asymmetry in unilateral cases. Chronically, the epididymis will decrease in size, as will the testes. Orchitis is an infrequent clinical sign but will result in testicular necrosis. Testicular damage initiates the development of anti-sperm antibodies that may be found in the blood and prostatic fluid at about 11 to 14 weeks post infection. Auto-agglutination of the sperm can then be visualized starting at approximately 18 weeks post infection. *B canis* also localizes in the prostate of the male, which may lead to classic clinical signs of prostatitis including enlarged and painful prostate and difficulty urinating and defecating (Hollett, 2006; Brennan *et al*., 2008).

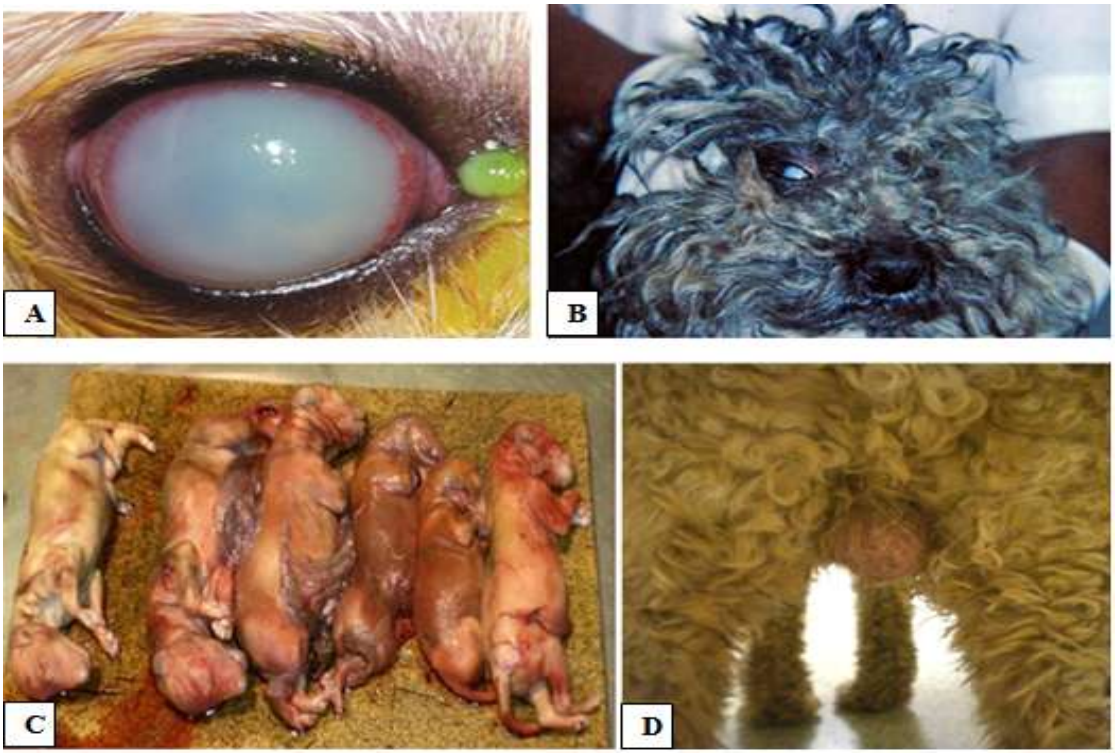

Fig 1: Clinical signs of Canine brucellosis (‘A’ Diifuse corneal edema, ‘B’ Uveitis, ‘C’ late term abortion of puppies, ‘D’ Scrotal asymmetry due to epidydimitis).
Source: ‘A’ (Ledbetter *et al.,* 2009); ‘B’, ‘C’, and ‘D’ (Megid *et al.,* 2010)

ii. In pigs

Brucellosis in pigs is a chronic disease most often characterized by infertility and abortion in sows and by orchitis in boars. The disease has drastically affected pig production through abortion, birth of weak/unthrifty piglets, infertility and orchitis in the boar (Fig-2) and these constitute serious economic losses. Extra-genital lesions such as lymphadenitis, subcutaneous abscesses, arthritis, and spondylitis are also common. There is generally a relatively long incubation period before clinical signs appear. These are not usually visible in young animals, and their occurrence will depend mainly on the age, sex and physiological state of animals at the time they are infected. As an example, animals infected during critical periods of the pregnancy (the first third to half of pregnancy) will abort approximately 30 to 45 days after infection. However, animals infected at full term do not abort. Similarly animals that are infected before the pregnancy period do not abort during their next pregnancies. Infected pigs excrete *Brucellas* in urine, sperm, vaginal discharge, milk, and also by placenta, lochial secretion, aborted fetuses and the content of subcutaneous brucellous abscesses (Iowa State Universty, 2007; Megid *et al*., 2010; Kebeta *et al*., 2015).

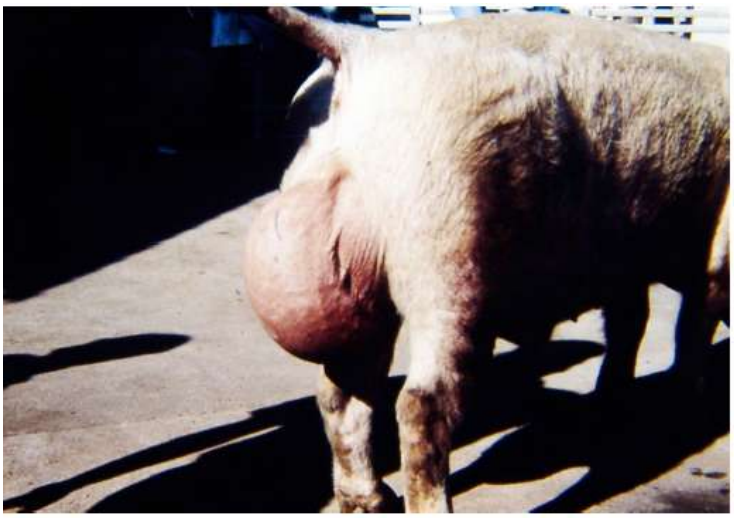

Fig 2: Testicular enlargement due to *B. suis* infection.

Source: Megid *et al.* (2010)

2.3.6. Diagnosis

i. In dogs

Clinical examinations and clinical findings are inadequate for diagnosis of canine brucellosis. The most common historical finding is infertility, but veterinarians must remember that many patients are adopted from shelters or purchased from breeding kennels as a family pet and may be spayed or neutered, so there is no known history of infertility in these cases. A thorough physical exam is necessary to gain basic information on vision, weight, locomotion, discharge, or any palpable swellings. Isolation of the organism and serological tests are the only reliable ways to confirm a presumptive diagnosis. Although isolation of the causative organism from fluids or tissues of suspected hosts is indisputable for definitive diagnosis of brucellosis, it is hazardous, time consuming, expensive, and not amenable to mass testing. Therefore, diagnosis is made by serological testing, usually of blood sample and some of the tests are described below (Nielsen *et al*., 2005; Lucero *et al*., 2009).

Serology

Serologic testing in the dog can be very challenging but can be helpful in screening for the disease. *B canis* has a rough, not smooth, plasma membrane as *B abortus*, *B melitensis,* and *B suis* possess. The surface antigens of this bacterium make serologic tests highly sensitive, but the specificity is low, making the occurrence of false-positive results very high. Given this information, it has come as a surprise that a significant amount of false-negative results have also been encountered. This may be due to the limitations of the serologic and microbiologic tests, but it may also be due to recent or chronic infection (Hollett, 2006).

Rapid slide agglutination test (RSAT) is a rapid commercially available countertop diagnostic test that can be used in-house for a quick diagnosis or screening. Results can be available within 2 minutes. The RSAT may cross-react with antibodies from *Bordetella, Pseudomonas, Moraxella*-type organisms, and other gram-negative bacteria. To decrease some of this cross reaction, 2-mercaptoethanol (2-ME) drops are added to increase the specificity of the RSAT; this is often referred to as the 2-ME

RSAT. The tube agglutination test (TAT) detects antibodies in the serum and can be quantitative; samples with titers less than 1:200 should be retested in 2 weeks. The Agar gel immuno diffusion test is used to confirm positive results from the RSAT, 2-ME RSAT, and TAT. There are 2 types of Agar gel immunodiffusion tests the first is the cell wall antigen test and the second is the more specific cytoplasmic protein antigen test. Both of these tests are more specific than the RSAT, 2-ME RSAT, and TAT and should be used to confirm any positive results before taking action (Makloski, 2011).

There are 2 other types of serology tests that have been used: the indirect fluorescent antibody (IFA) test and enzyme-linked immunosorbent assay (ELISA). The IFA sensitivity is uncertain so some infected dogs may go undetected with this test. In research, the ELISA is more specific than the IFA and can detect positive dogs within 30 days of infection (Hollett, 2006).

B. abortus, B. melitensis, and *B. suis* infection in dogs can be diagnosed using the serological procedures used for cattle, except for ELISA, which has not been widely assessed in dogs. For *B. canis* infection the most reliable procedure is isolation of the organisms. As persistent bacteraemia is common, blood culture is a useful procedure. Serological tests are less satisfactory. They must use antigens prepared from *B. canis* or *B. ovis* strains as the surface antigens of smooth *Brucella* species do not cross-react with these. ELISA is probably the most useful procedure but is not widely available (WHO, 2006).

Molecular

The use of real-time polymerase chain reaction (PCR) will detect the DNA of the *B canis* organism, whether it is alive or dead (Keid *et al.,* 2007). This is an area where bacterial cultures are limited. Only live organisms may grow and replicate on culture media. If there are not enough live organisms, then the bacterial culture may be considered negative, but the patient may be harboring the organism. PCR diagnostic testing is a new tool that may be used to diagnose *B canis*. Semen, vaginal swabs, uterine swabs, and urine are appropriate samples to submit for PCR. Whole blood can

also be submitted, but due to the limited time of bacteremia, this may not be an adequate sample (Keid *et al.,* 2010).

ii. In pigs

Brucella suis is readily isolated from live pigs by culture of birth products, and from carcasses by culture of lymph nodes and organs. Selective media are available for culture of contaminated samples. None of the serological tests used for the diagnosis of porcine brucellosis are reliable for diagnosis in individual pigs. The major antigen involved in the serological tests currently available is the smooth lipopolysaccharide (SLPS). The OPS moiety of this molecule contains epitopes that cross-react with those existing in the corresponding LPS from *Yersinia enterocolitica* serotype O:9 (16) (Jungersen *et al.*, 2006). Therefore, available serological tests are unable to distinguish between antibodies raised to these two infections. The sensitivities and specificities of the Rose Bengal test (RBT), the indirect and competitive Enzyme-linked immunosorbent assay (I- and C-ELISAs), and the Fluorescent polarisation assay (FPA) are similar. The RBT is useful for screening large numbers of sera, whereas ELISA offers the highest sensitivity and specificity of all currently available serological tests (WHO, 2006).

Swine serum may sometimes also contain nonspecific antibody, thought to be of the IgM isotype, further reducing the specificity of conventional tests, especially the serum agglutination test (SAT). Hence SAT is not recommended. For international and other trade, e.g. purchasing boars, the disease status of the herd and of the area in which the herd is situated are of more importance than tests on individual animals. In addition to the above tests, intra dermal test using a defined antigen preparation is the most reliable diagnostic procedure for pigs on both an individual or herd basis (WHO, 2006; OIE, 2009).

Today the indirect and competitive ELISAs, as well as the RBT, CFT and fluorescence polarisation assay (FPA) are the prescribed tests for international trade purposes. The allergic skin test and the buffered plate agglutination test (BPAT) are also useful for identifying infected herds (OIE, 2009).

2.3.7. Treatment

Treatment is unsuccessful because of the intracellular sequestration of the organisms in lymph nodes, the mammary gland, and reproductive organs. *Brucella* species are facultative intracellular bacteria that can survive and multiply within the cells of the macrophage system. Treatment failures are therefore considered to be due not to the development of antimicrobial resistance; but rather to the inability of the drug to penetrate the cell membrane barrier (Greene and Carmichael, 2006).

Several antibiotic therapies have been attempted, but there are no known cures for canine brucellosis. The disease may recrudesce at times of stress and the animal can be a source of infection for other dogs and humans. When treatment is attempted, the patients should be spayed or neutered. Many different antibiotics have been tried, alone or in combination, and none have been 100% effective in eradicating the disease. Bacteriemia has been eliminated in some cases and negative titers of antibodies, especially of those raised against the bacterial cell wall, have been obtained; however, bacteria remain alive in the tissues. Some infected dogs gave negative titers immediately after treatment, but at that same time it was able to isolate the bacteria from their semen. In females, this often happens at estrus and after stressful situations if the animal has not been spayed (Wanke, 2004).

Single-antibiotic regimens are unsuccessful. Combination therapies have better results such as doxycycline (10 mg/kg po q 12 hours), gentamicin (5 mg/kg SC q 24 hours for 7 days and repeated every 3 weeks), and rifampicin (5 mg/kg po q 24 hours) for 3 months. Some success has been reported using entrofloxacin (5 mg/kg po q 24 hours) alone with similar efficacy to that of combination therapy. After antibiotic trial, dogs should repeatedly retest until they have a negative test. Even after reaching a negative serology test, they continue to test every 4 to 6 months and repeat treatment as necessary. It is also important to isolate these treated dogs from other dogs and breeding animals. The cost of antibiotic therapy and diligence of the testing protocol may deter many owners from trying to treat. It is also important to counsel owners and kennel workers that the therapy is not curative and the dog may be a risk to other dogs and humans, especially young children, older persons, and immuno-compromised individuals (Wanke *et al.*, 2006).

No treatments, such as antibiotic therapy, dietary supplements, or other chemotherapy, have been proven effective and economically feasible in curing pigs of brucellosis. Large doses of tetracyclines, streptomycin, or sulfonamides given over relatively long periods have been investigated. In some trials these antibiotics alone or in combination appeared promising. Although treatments have not been effective in eliminating all organisms from the host, chemotherapy in carefully selected circumstances could probably suppress multiplication of *B. suis,* to alleviate clinical manifestations and shedding of organisms. Such approach may have limited practicability, but has beneficial effects in an infected herd and should not be dismissed as useless (Straw *et al.,* 2006).

2.3.8. Prevention and control

i. In dog

Canine brucellosis is usually introduced into a kennel in an infected dog or semen. New animals should be isolated and tested before adding them to the general population. A second serological test, performed before release from quarantine, may detect animals that are in the early stage of the infection and sero-negative on arrival. Periodic testing of all of the dogs twice a year in kennels is recommended. This could be during a heat cycle in the female and then on an every 6 months-period for the males. This kennel screening can help decrease exposure in the event a positive dog is introduced to the kennel and may decrease losses. In infected kennels, brucellosis can be controlled by sanitation and infection control measures, together with the euthanasia. Housing in individual cages reduces the spread of the organism. *B canis* is susceptible to 1% sodium hypochlorite, 70% ethanol, iodine/alcohol solutions, glutaraldehyde, and formaldehyde, and these solutions may be used to clean facilities and equipment to decrease the spread of the disease. Dogs from infected kennels should not be sold or used for breeding. There is no vaccine for *B. canis* (Makloski, 2011; Iowa State Universty, 2012; Bramlage *et al*., 2015).

For outside breeding, artificial insemination will decrease the male dog's exposure to the disease and should be used when possible. Artificial insemination will not protect females from the disease, so testing the male dog prior to breeding is recommended.

Buying dogs from and breeding to dogs in reputable kennels are encouraged, but may not decrease exposure. Repeated testing and removal of infected animals, combined with quarantine and testing of newly added dogs, is the only way to monitor this disease in a population (Makloski, 2011; Bramlage *et al*., 2015).

Canine brucellosis due to *B. abortus, B. melitensis, and B suis* infection will be prevented by preventing dogs from getting access to aborted material. Therefore, proper disposal (burial or burning) of placentas and non-viable fetuses; and disinfection of contaminated areas should be performed thoroughly (WHO, 2006).

ii. In pig

Safe and reliable vaccines that produce serviceable immunity against brucellosis in pigs have not been developed. Strain 19 *B. abortus*, *B. abortus* 'M' vaccine, living attenuated *B. suis* vaccines and phenol and other extracts of *B. suis* are all ineffective (Straw *et al*., 2006; Radostits *et al*., 2007). In herds where the incidence of reactors is high, complete disposal of all stock as they reach marketing age is by far the best procedure because of the difficulty in detecting individual infected animals. This is most practicable in commercial pork-producing herds. Restocking the farm should be delayed for 6 months. The existing serological tests can be used for certifying herds free of infection that can then provide replacement stock (Radostits *et al.,* 2007).

Establishment and maintenance of validated brucellosis-free herds, a particularly purebred herd is important in control of swine brucellosis. Implementation of effective surveillance programs such as identification and testing of market pigs (sows and boars) has been instrumental in locating and eliminating large numbers of infected herds. During swine brucellosis eradication program organism may continue to exist indefinitely in the feral swine reservoir and associated transitional swine population. Transitional swine are defined as those feral swine that are captive or swine that have reasonable opportunities to be exposed to feral swine. Therefore effective separation of commercial production swine from transitional and feral swine, with adequate surveillance and testing of at-risk populations is necessary to assure compliance (Straw *et al.,* 2006). All introductions to farm should be from accredited free herds, clinically healthy and negative to the serum agglutination test;

twice at intervals of 3 weeks before introduction. Eradication of swine brucellosis from an area can only be achieved by developing a nucleus of accredited free herds and using these as a source of replacements for herds that eradicate by total disposal. Sale of pigs for breeding purposes from infected herds must also be prevented (Radostits *et al.*, 2007).

3. MATERIALS AND METHODS

3.1. Description of the study area

The study was conducted from November 2017 to May 2018 in Batu, Alage Agricultural Technical and Vocational Education Training (ATVET) College and Naka (surrounding village). Alage ATVET College is known for its well-developed swine production. The college is positioned at 217 km southwest of Addis Ababa and 32 km west of Bulbula town; near the Abijata and Shala lakes of the Ethiopian Rift Valley. Naka village is located at North east of Alage in Adami tulu jido kombolcha district, Oromia regional state. Both the college and Naka are geographically located at a longitude of 38°30′East and latitude of 7°30'North, with an altitude of 1600 m.a.s.l. The mean annual minimum and maximum temperature range from 11 to 32°C, respectively. The areas have the mean annual rain fall of 800 mm and three distinct seasons; a short rainy season (March to May), a long rainy season (June to September) and a dry season (October to February) (Asgedom *et al.*, 2016; NMSA 2015). Whereas, Batu is a town and separate district, located on the road connecting Addis Ababa to Hawasa in the East Shewa Zone of the Oromia Region of Ethiopia. It has latitude of 7°56′North and longitude of 38°43′East, with an elevation of 1643 meters above sea level (CSA, 2007) (Fig-3).

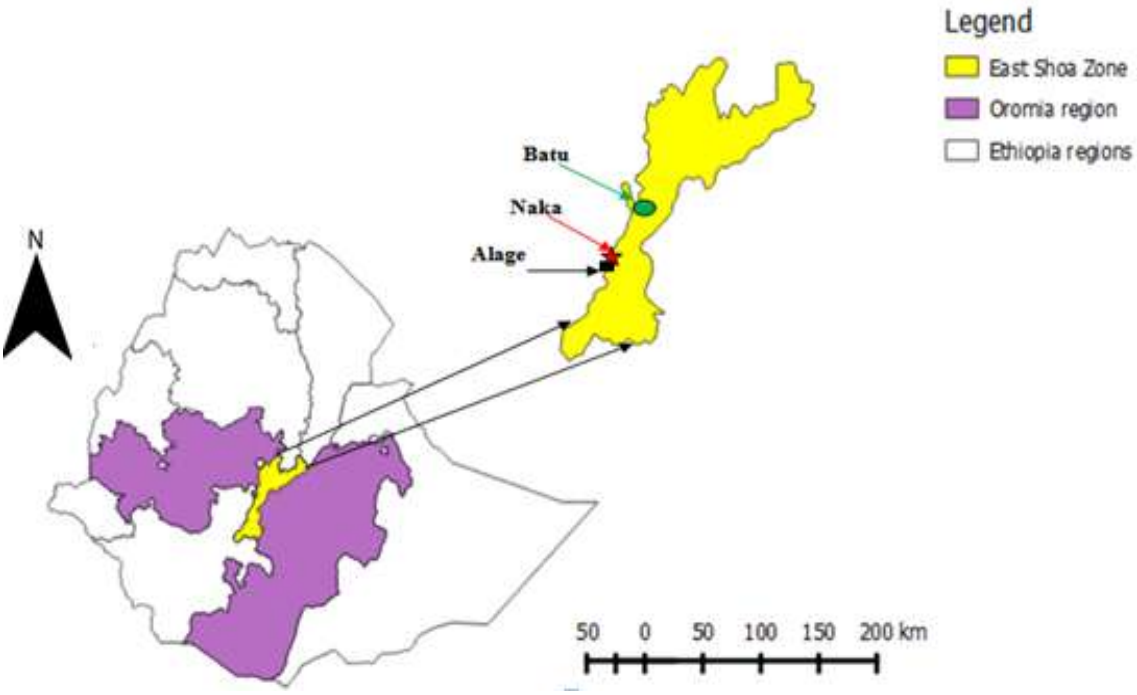

Fig 3: Map showing the study areas

3.2. Study design

A cross sectional study was carried out from November 2017 to May 2018 in Alage, Batu and Naka; using serological tests on dogs and pigs sera; so as to determine the seroprevalence of canine and swine brucellosis, respectively. The areas were conveniently selected based on the abundance of dogs and presence of swine farms. The study had also involved face to face interview.

3.3. Study population

Owned dogs found in Alage, Batu and Naka; and pigs found in Alage and Batu swine farms were the target population. Pigs were ear tagged and both dogs and pigs were older than 6 months. None of the animals were also vaccinated against brucellosis.

3.4. Sample size determination and sampling method

The required sample size of dogs was determined based on expected prevalence of brucellosis and the desired absolute precision stated on Thuresfield (2005).

$$n = \frac{1.96^2 \, Pexp \, (1-Pexp)}{d^2}$$

Where: n = the required sample size; P = estimated prevalence = 0.5; z = level of confidence as 1.96 and d = desired precision level = 0.05.

Canine brucellosis hadn't studied previously in the study area and the country. Therefore, based on an estimate of 50% prevalence, 95% confidence interval, and 5% absolute precision, a sample size of 384 was calculated. There was not any recorded data indicating the total dog population, hence dog owners and their dogs found in each location had registered (sampling frame had prepared). A total of 826 owned dogs (443 in Batu, 224 in Alage, and 159 in Naka) were then identified. Thence, using proportional stratified sampling method, the total population had divided in to three strata based on their locations; and the proportion of minimal number of dogs to be sampled in each location had been calculated, which resulted 206(53.63%) from Batu, 104(27.12%) from Alage, and 74(19.25%) from Naka.

As it was very difficult to take blood sample from those dogs by going to every owners' home, free dog's rabies vaccination campaign had prepared. After that, following establishing many and suitable vaccination centers in all areas, all dog owners had informed to freely vaccinate in nearby vaccination centers (detail of the procedure is described in the following page). However, considering some owners may not vaccinate due to inconvenience, random selection of individual dogs from the sampling frame was not performed. Instead, as described by Stevenson (2005) after calculating the sampling interval (k=2 or from every second vaccinated dog), owners and their dogs had again registered during the vaccination program; and thenceforth a systematic sampling method had been used from the list; to sample individual dogs. Thus, a total of 107 (Alage), 75 (Naka), and 207 (Batu) owned dogs had sampled.

On the other hand, for studying swine brucellosis, as the total number of pigs in the two swine farms were small (n=235), all pigs older than 6 months had sampled. A total of 196 pigs (167 from Alage and 29 from Batu swine farms) were therefore older than 6 months and all of these pigs had included in the study.

3.5. Data collection

Data was collected using serological tests, and by use of interview.

3.5.1. Approaches used to capture and sample dogs

i. Restraining method

For restraining dogs, a portable and safe modified dog crush was employed (Annex III). The crash is invented by the researcher and evaluated and certified by Alage ATVET College (Annex XI). Currently it had registered in Ethiopia Intellectual Property Office and waiting for further examination (Annex X).

ii. Rabies vaccination campaign

In collaboration with Alage ATVET College and National Veterinary Institute (NVI, Ethiopia), the researcher had prepared free and mobile rabies vaccination campaign in the study areas. To do so, consent had made with all administrators of the locations. As vaccinating all dogs in one place and at one time was very difficult, both for the researcher and for the owners who found at distant, suitable time schedule had prepared and different vaccination centers had established in residential areas near to the community. Thence, all residents of the locations were informed by loudspeakers, school mini-medias and notice; to freely vaccinate their dogs on their schedule. Parallel to the vaccination program, after describing the objective of the research, a verbal consent had made with owners of the dogs to take a blood sample. Additionally, a questioner was filled by face to face interview.

3.5.2. *Blood sample collection*

After proper restraining, about 6-8 ml of blood was aseptically collected through the saphenous vein and ear vain of each sampled dog and pig, respectively; using syringe and plain vacutainer tubes. Each sample was coded and had transported to Alage ATVET College Department of Animal Health, Microbiology laboratory. The blood samples were allowed to clot and centrifuged at 3000 rpm for five minutes. Serum

samples were then decanted, transferred in to labeled cryo-vials, and screened by RBPT in the college laboratory. Dogs' and pigs' sera tested positive by RBPT were stored at -20 ^{o}C and lastly sent to NVI (Bishoftu, Ethiopia) and Yulin Animal Disease Prevention and Control Center (Yulin city, China), respectively; for further confirmation.

3.5.3. Serological tests

Rose Bengal's *Brucella* antigens (*B. abortus* and *B. canis*) and their control sera (sourced from China Institute of Veterinary Drugs Control), and CFT *Brucella* antigen, control sera, and complement (Bg vv, Germany and China Institute of Veterinary Drugs Control), were employed each for RBPT and CFT tests, respectively. Samples were then considered as positive for swine brucellosis and canine brucellosis (due to smooth strains), if they were positive using CFT. However, due to the lack of serological tests prepared from rough strains of *Brucella* species in Ethiopian laboratories, samples positive for RBPT with *B. canis* antigen were considered as positive for canine brucellosis due to rough strain (*B. canis* infection).

i. Rose Bengal Plate Test

All dog and pig serum samples were screened by RBPT containing *B. abortus* antigen. Furthermore, to know *B. canis* infection in dogs, a RBPT prepared from *B. canis* antigen was used as described by Nielsen (2002). Using a micropipette, 1 drop (30 µl) of the test serum was placed on one spot of the slide. Using another pipette, an equal volume of RBPT antigen was placed close to the test serum on the slide. Using an applicator stick, the antigen and the test serum were mixed thoroughly; the slide was then hand-rocked for about 4 minutes after which the slide was examined for agglutination under a good source of light. Formation of pink granules (agglutination) was recorded as positive while absence of pink granules (agglutination) was recorded as negative (Annex I).

ii. Complement Fixation Test

Both pig and dog samples positive for RBPT (containing *B. abortus* antigen) were further confirmed by CFT. The confirmation of dog sera was undertaken at NVI, Department of Immunology; whereas for pig sera in Yulin Animal Disease Prevention and Control Center, Veterinary comprehensive laboratory, Yulin city, China. All the reagents required for CFT were evaluated by titration. A sheep Red Blood Cell (SRBC) suspension were prepared before being used in the test proper. The preparation of reagents and CFT procedures were performed according to the protocols of the Federal Institute for Consumer Protection and Veterinary Medicine Service Laboratory, Berlin, Germany (Nielsen and Dunkan, 1990). The CFT test was regarded as positive when the reading is as complete fixation or partial haemolysis and as negative (0) when there is complete haemolysis (OIE, 2013) (Annex II).

3.5.4. Interview

The study had clearly explained to the respondents and informed consent was obtained. A questionnaire was prepared in English and using trained assistant researchers who had a mother tounge of the local language 'Affan Oromo', validation of the questionnaire had conducted by a pre-testing on total of 10 individuals; to analyze and validate the degree to which the questions were properly understood or misunderstood, the degree to which individuals within a group interpreted the questions differently, the effectiveness of the questions in soliciting the proper information, and any areas of information which were neglected by the proposed questionnaire. Once analysis has been completed, some questions were modified. Lastly they were interviewed on their demographic factors, awareness toward the disease, and on history of the animals and possible factors associated with occurrence of brucellosis**.** Questions regarding religion, assisting bitches during whelping, and consumption habits of pork were not included due to being a sensitive topic culturally (Annex V).

i. Variables collected

The information for hypothesized explanatory variables was gathered from the dog owners, swine farm managers/owners, farm employees and farm records. Factors like age, sex, history of obstetrical problems (Abortion, retained placenta, abnormal vaginal discharges, infertility…etc for females; and enlargement of testicle, scotal edema, and scrotum dermatitis for males), and living status (for dogs) were recorded for each animal. Age of dogs were stratified into three categories (≤ 2 years, 2-4 years, and > 4 years); and for pigs in to two (≤3 years and > 3 years). Furthermore, living status (condition) of dogs was categorized as indoors, outdoors, and semi-indoors (modified from Xieng *et al.* (2013) and Momoh *et al.* (2015)).

ii. Definition of terms

Based on the aspect of colonies on agar plates, which is in accordance with the cell surface and LPS structure, *Brucella* may occur either as smooth or rough species (Mancilla, 2015).

Smooth strains of *Brucella* species*:* -** Theses *Brucellae* that express full LPS molecule (S-LPS) that is anchored in the outer membrane. *They carry* complete S-LPS and have a smooth (S) phenotype, so termed after the smooth texture of the colonial surface. This includes the zoonotically more relevant *Brucella* species, *B. melitensis, B. suis, and B. abortus* (Foster *et al.*, 2007; Adone *et al.*, 2011; Mancilla, 2015). To serologically detect *Brucella* infection due to smooth strains, the antigen of the serological test should prepare from smooth strains (Keid *et al.*, 2004; WHO, 2006; Fulya *et al.*, 2014).

Rough strains of *Brucella* species: - They express R-LPS that lack the O-antigen, a trait linked to their reduced virulence and include *B. ovis* and *B. canis*. To serologically detect *Brucella* infection due to rough strains, the antigen of the serological test must prepared from either of the rough strains; as the surface antigens of smooth *Brucella* spp. do not cross-react with these (Keid *et al.*, 2004; WHO, 2006; Fulya *et al.*, 2014). Generally Serologic tests that use suspensions of smooth phase *Brucellae* are useless in diagnosing *B. canis* infections (Wallach *et al.*, 2004).

Living condition/ living status/ maintenance condition: - Dog's living condition varies from owner to owner, which has its own influence for the epidemiology of canine brucellosis (Momoh *et al.*, 2015; Xieng *et al.*, 2013). Researchers had included it as a risk factor and studied about canine brucellosis in association with this factor. They had then further categorized it (eg. as outdoors, indoors, shelter, foster and stray). Therefore, in the present study, considering the existing dog's management system of Ethiopian dog owners, the factors had further categorized in to three sub categories; and are defined below accordingly.

Indoors: - In the present study, the term referred to those confined dogs (i.e they didn't had any contact with outside animals and their food was provided by their owners).

Semi-indoors: - It referred to partially free dogs. They might chained (confined) for half of the day or some hours, but also had freedom to go free, mostly at night. Hence, they had contact with other animals and though their food was principally provided by their owners, they sometimes scavenge outside.

Outdoors: - The term indicated to those always free and mostly scavenging dogs. They differ from stray dogs because they had owners and can rarely feed at home.

3.6. Data analysis

The collected data had entered into Microsoft Excel Spread Sheet program and statistical analysis had computed using Fisher's Exact test and logistic regression using STATA-12 version. The total prevalence was then calculated by dividing the number of animals seropositive for CFT and RBPT (for *B. canis* infection); to the total number of animals sampled. The association between risk factors and seropositivity to anti *Brucella* antibodies was considered as significant at $p < 0.05$ and odds ratio (OR) had used to measure the magnitude of the association between each risk factor. Lastly, the demographics of respondents and their awareness and practices toward the disease were determined using descriptive statistics.

3.7. Ethics approval

All procedures had carried out according to the experimental practice and standards approved by the Animal Welfare and Research Ethics Committee at Addis Ababa University College of Veterinary Medicine (AAU CVMA) in accordance with the international guidelines for animal welfare. Samplings of animals were then held with formal and verbal consent from the animal's owners; who had been informed about the purpose of the study (Annex IX).

4. RESULTS

4.1. Sero-prevalence of canine brucellosis and associated risk factors

4.1.1. Prevalence of anti-B. abortus and anti B. canis antibodies

As the result is summarized in Table 2, out of the 389 owned dogs samples, 21 (5.4%; CI: 3.35, 7.96) were found positive for anti *B. abortus* antibodies using RBPT that had *B. abortus* antigen and 19 of them (4.88%; CI: 2.7, 7.0) were confirmed by CFT. Furthermore, all 389 sera samples were further tested for *B. canis* infection using RBPT that had *B. canis* antigen ($RBPT^{canis}$); and 34 (8.74%; CI: 5.92, 11.56) dogs were positive. Thus, the overall sero-prevalence of canine brucellosis in the study area due to smooth type of *Brucella* species was 4.88% using CFT; whereas 8.74% due to rough type (*B. canis*); using $RBPT^{canis}$.

Table 2: Overall sero-prevalence of canine brucellosis

Study areas	N	Smooth strain positives RBPT (%)	95% CI	Smooth strain positives CFT (%)	95%CI	Rough strain positives $RBPT^{canis}$(%)	95% CI
Alage	107	7(6.54)		6(5.61)		6(5.61)	
Batu	207	11(5.31)		10(4.83)		24(11.59)	
Naka	75	3(4.00)		3(4.00)		4(5.33)	
Total	389	21(5.4%)	3.35,7.96	19(4.88)	2.7,7.0	34(8.74)	5.92,11.56

$RBPT^{canis}$: RBPT containing *B. canis* as an antigen

4.1.2. Analysis of association of risk factors with Brucella seropositivity

i. By Fisher's exact test

Analysis for association between locations of the animals and *Brucella* infection was carried out using Fisher's exact test (Table 3). There was no significant association

observed between the study areas and seroreactivity to both smooth and rough types of *Brucella* infection ($p \geq 0.05$). Even so, using CFT, a relatively higher proportion of anti *B. abortus* antibodies was observed in Alage (5.61%) followed by Batu town (4.83%) and Naka (4%); and using $RBPT^{canis}$, higher proportion of anti *B. canis* antibodies had seen in Batu (11.59%) followed by Alage (5.61%), and Naka (5.33%).

Age groups had only significantly associated with seropositivity to anti *B. abortus* antibodies but not with *B. canis* infection. Other risk factors including sex, history of obstetrical problems and living condition of the dogs were however significantly associated with both rough and smooth *Berucella* specie's infection ($P < 0.05$). Using CFT for detecting anti *B. abortus* antibodies, 9.39% of female and 2.08% of male were tested positive. According to different age groups, 1.25% of ≤2 years old, 4.05% of 2-4 years old, and 13.58% of >4 years old were seroposotive. Moreover, regarding to living condition of the dogs, 13.89% of indoors, 3.88% of semi indoors, and 10.59% of outdoors; and with respect to history of obstetrical problems, 18% of dogs with history of reproductive problems and 2.95% without such history, were positive (Table 3).

For the screening of *B. canis* infection using $RBPT^{canis}$, 14.09% of female and 5.42% of male; 4.17% of indoors, 0.06% of semi indoors, and 18.82% of outdoors; 22% of with history of reproductive problem and 6.78% without that history; were detected positive (Table 3).

Table 3: Association of putative variables with canine brucellosis (Fisher's exact test)

Variables	N	Smooth type positives by CFT (%)	p-value	Rough type positives by RBPT*canis*	p-value
Study area			0.903		0.123
Alage	107	6(5.61)		6(5.61)	
Batu	207	10(4.83)		24(11.59)	
Naka	75	3(4.00)		4(5.33)	
Sex			0.003		0.005
Female	149	14(9.39)		21(14.09)	
Male	240	5(2.08)		13(5.42)	
Age			0.000		0.799
≤2 years	160	2(1.25)		13(8.13)	
2-4 years	148	6(4.05)		15(10.14)	
>4 Years	81	11(13.58)		6(13.58)	
Living status			0.021		0.002
Indoors	72	1(13.89)		3(4.17)	
Semi-indoors	232	9(3.88)		15(0.06)	
Outdoors	85	9(10.59)		16(18.82)	
History of obstetrical problems			0.000		0.002
Yes	50	9(18.00)		11(22.00)	
No	339	10(2.95)		23(6.78)	

N= number of animals tested

iii. Univariable logistic regression analysis

Univariable logistic regression analysis of associations of risk factors with anti *B. abortus* antibodies revealed that among the risk factors considered in the analysis (Table 4), sex, age, living conditions and history of obstetrical problems had statistically significant effect on seroposotivity ($p < 0.05$); while location had not. The

result showed that dogs with history of obstetrical problems had 7.2 times higher odds of getting infection with brucellosis than those hadn't the history ($p < 0.05$). Similarly, dogs >4 years old were 12.4 times higher odds of getting infection with brucellosis than those ≤2 years ($p < 0.05$). Furthermore, according to their living status, outdoor dogs were 8.4 times more likely to be seroposotive than indoor dogs. In contrast, infection of canine brucellosis in males was lower by 0.2 odds ratio than females ($p < 0.05$).

Univariable logistic regression analysis of association of risk factors with anti *B. canis* antibodies also depicted that, age and location had not significant effect with seroposotivity. However, sex, living condition, and history of obstetrical problems had significant association. As it is clearly shown in Table 4, outdoor dogs and dogs with history of obstetric problems had about 5.3 and 3.88 times higher odds of getting *B. canis* infection, respectively than those indoors and those hadn't history of obstetrical problems. However, *B.canis* infection of in males was lower by 0.35 odds ratio than females ($p < 0.05$).

Table-4: Univariable logistic regression analysis of associated risk factors of canine brucellosis.

	Canine brucellosis due to smooth types (CFT)		Canine brucellosis due to *B. canis* (RBPT^{canis})	
Variables	p-value	OR (95% CI)	p-value	OR (95% CI)
Study areas				
Alage	*			
Naka	0.62	0.70(0.17, 2. 9)	0.936	0.95(0.26, 3.48)
Batu	0.77	0.85(0.30, 2.42)	0.094	2.21(0.87, 5.58)
Sex				
Female	*			
Male	0.003	0.21(0.72, 0.58)	0.004	0.35(0.17, 0.72)
Age				
≤2 years	*			
2-4 years	0.144	3.34(0.66, 16.8)	0.541	1.28(0.59, 2.78)
>4 Years	0.001	12.41(2.68, 57.49)	0.845	0.90(0.33, 2.48)
Living condition				
Indoors	*			
Semi-indoors	0.322	2.87(0.36, 45.23)	0.474	1.59(0.45, 5.65)
Outdoors	0.046	8.41(1.04, 68.06)	0.010	5.33(1.49, 19.13)
History of obstetrical problems				
Yes	0.000	7.22(2.77, 18.81)	0.001	3.88(1.76, 8.55)
No	*			

*Reference variable

iii. Multi-variable logistic regression analysis

Table 5 presents final simplified results of multivariable logistic regression analysis of potential risk factors that were significantly associated with seropositivity to anti *B. abortus* antibodies. Explanatory variables in the univariable logistic regression

analysis were included in the full multivariable logistic regression model. Thereupon, final selection of the best potential risk factors that would likely best explain the response of the predictor variable was done; based on a stepwise forward elimination procedure. Accordingly, as it is shown in table 5, location of the animals, semi indoor living condition, and age group between 2 and 4 years were removed from the simplified model of multi variable logistic regression ($p \geq 0.05$).

Thus, male dogs had lower seroposotivity than female dogs (OR=0.26, CI; 0.85, 0.79); and dogs older than 4 years had higher seroposotivity than those ≤2 years (OR=7.78, CI; 2.59, 23.32). Similarly, seropositivity was higher in those with history of obstetrical problems than those without such history (OR=10.27, CI; 3.34, 31.58); and in those outdoor dogs than indoor dogs (OR= 4.72, CI; 1.61, 13.89).

Table 5: Final simplified result of multivariable logistic regression analysis of canine brucellosis due to smooth strains (anti *B. abortus* antibodies).

Canine brucellosis due to smooth strains (CFT)		
Variables	p-value	OR (95%CI)
Study areas	†	†
Sex		
Female	*	*
Male	0.017	0.26(0.09, 0.79)
Age		
≤2 years	*	*
2-4 years	†	†
>4 Years	0.000	7.78(2.59, 23.32)
Living Condition		
Indoors	*	*
Semi indoors	†	†
Outdoors	0.005	4.72(1.61, 13.89)
History of obstetrical problems		
Yes	0.000	10.27(3.34, 31.58)
No	*	*

* Reference variables

† Removed variables up on model simplification by step-wise function (p≥ 0.05)

Similarly, one location (Naka), age, and semi intensive living condition had removed from the final model showing the association of factors with seroposotivity of *B. canis* infection (p≥ 0.05) (Table 6).

Table 6: Final simplified result of multivariable logistic regression analysis of canine brucellosis due to *B. canis.*

Canine brucellosis due to rough strain *B.canis* ($RBPT^{canis}$)		
Variables	p-value	OR (95%CI)
Study areas		
Alage	*	*
Naka	†	†
Batu	0.004	3.86(1.56, 9.54)
Sex		
Female	*	*
Male	0.000	0.38(0.18, 0.82)
Age	†	†
Living Condition		
Indoors	*	*
Semi indoors	†	†
Outdoors	0.000	6.42(2.71, 15.2)
History of obstetrical problems		
Yes	0.005	3.48(1.46, 8.25)
No	*	*

* References

† Removed variables up on model simplification by step-wise function ($p \geq 0.05$)

Consequently, dogs reared in Batu were 3.86 times more likely to be seroposotive compared to those reared in Alage (OR=3.86, CI; 1.56, 9.54). Besides, dogs maintained outdoor were more likely to encounter *B. canis* infection than those maintained indoor (OR=6.42, CI; 2.71, 15.2). Similarly, *B. canis* infection was found to be highly associated with dogs that had history of obstetrical problems than those without such history (OR=3.48, CI; 1.46, 8.25). However, male dogs had lower seroposotivity than females (OR=0.38, CI; 0.18, 0.82).

4.2. Seroprevalence of swine brucellosis

Among the 196 serum samples of pigs, 10(5.1%; CI: 0.95, 6.19) were tested positive by RBPT and 7(3.57%; CI: 1.99, 8.21) of them were further confirmed by CFT. Hence, the overall sero-prevalence of swine brucellosis in the study area was 3.57% (Table 7).

Table 7: Prevalence of swine brucellosis

Farm	N	RBPT positive (%)	95% CI	CFT positive (%)	95% CI
Alage swine farm	167	9(5.39)		6(3.6)	
Batu swine farm	29	1(3.45)		1(3.45)	
Total	196	10(5.1)	0.95, 6.19	7(3.57)	1.99, 8.21

NB. For confidentiality, the name of the farm found in Batu had not used its actual name; instead named by its location.

4.2.1. Association of Risk factors of swine brucellosis

Table 8 present results of *Brucella* seroposotivity with exposure variables using Fisher's exact test. Though the result respectively showed 4.76% and 1.43% seroprevalence in female and male pigs, the difference in the prevalence observed among the two sexes was not statistically significant ($p \geq 0.05$). Moreover, the prevalence was not significantly associated in relation to the farms ($p \geq 0.05$). In contrary to this, significant association ($p < 0.05$) with factors like age and history of obstetrical problems had seen. Swine brucellosis was higher in those >3 years old (10.64%) than ≤ 3 years old (1.34%); and in those with history of obstetrical problems (20%) than hadn't (2.21%).

Table 8: Association of risk factors with seroposotivity by Fisher's exact test

Variables		N	Positive samples by CFT (%)	p-value
Farm				0.723
	Alage swine farm	167	6(3.6)	
	Batu swine farm	29	1(3.45)	
Sex				0.425
	Male	70	1(1.43)	
	Female	126	6(4.76)	
Age				0.009
	≤3 years	149	2(1.34)	
	>3 years	47	5(10.64)	
History of obstetrical problems				0.001
	No	181	4(2.21)	
	Yes	15	3(20)	
Over all		196	7(3.57)	

The magnitude and association of putative variables with *Brucella* positivity by univariable logistic regression analysis (Table 9) indicated that prevalence of swine brucellosis didn't show significant variations among farms and sexes ($p \geq 0.05$). Other risk factors like age and history of obstetrical problems were however highly significant. The odd of brucellosis in pigs >3 years old were 8.75 times higher than those ≤ 3 years old. Similarly, pigs that had history of obstetrical problems had 21.58 times higher odds of getting infection with brucellosis than those without such history.

Table 9: Univariable logistic regression of associated risk factors of swine brucellosis

Varables	p-value	OR (95%CI)
Farms		
Alage swine farm	*	
Batu swine farm	0.969	0.96(0.11-8.27)
Sex		
Male	0.256	0.29(0.03-2.46)
Female	*	
Age		
≤3 years	*	
>3 years	0.011	8.75(1.64-46.73)
History of obstetrical problems		
No	*	
Yes	0.000	21.58(4.29-108.6)

*Reference

The following Table 10 shows the simplified multi variable logistic regression association of risk factors of swine brucellosis. Variables that were not statistical significant in univariable analysis were removed based on step wise function procedure. Thereby, a simplified model with only two significant predictors (age and history of obstetrical problems) was created. Pigs > 3years old had higher infection (OR=9.44, CI; 1.51, 58.92) compared to those ≤ 3 years old; and pigs with history of obstetrical problems had higher seroposotivity (OR=23.22, CI; 3.96, 136.13) in comparison with those hadn't such history.

Table 10: Multi variable logistic regression association of risk factors of swine brucellosis.

Varables	p-value	OR(95%CI)
Farm	†	†
Sex	†	†
Age		
≤3 years	*	*
>3 years	0.016	9.44(1.51-58.92)
History of obstetrical problems		
No	*	*
Yes	0.000	23.22(3.96-136.13

*Reference

† Removed variables following model simplification by step-wise function (p≥0.05)

4.3. Awareness and practices of dog owners towards canine brucellosis

4.3.1. Demographic characteristics of the respondents

Out of the total 389 dog owners, 225(57.84%) of them were males. Age wise, majority (63.24%) were between 30-45 years old and according to their education status 24.16% were elementary (Table 11).

Table 11: Demographic characteristics of dog owners in the study area (n=389)

Demographic characteristics of the respondents	Category	N (%)
Gender	Male	225(57.84)
	Female	164(42.16)
Age	<30 years	87(22.37)
	30-45 years	246(63.24)
	>45 years	56(14.39)
Education status	Illiterates	73(18.77)
	Elementary	94(24.16)
	High school	48(12.34)
	Certificate	56(14.39)
	Diploma	58(14.91)
	Degree and above	60(15.42)
Total		389

4.3.2. Knowledge and practices about the disease

Out of the 389 respondents, 383(98.46%) of them didn't know the disease; and only 20.82% knew that they can got any disease from contact of aborted materials. Besides, 53.47% and 70.18% of them poorly cleaned their dogs' houses and didn't wear any personal protective equipment while in contact with their dogs, respectively. Among the 31 respondents who had reported abortion case in their bitches, 83.7% of them throw away the aborted material to outside environment (Table 12).

Table 12: knowledge and practices of dog owners about brucellosis

Variable	Category	N (%)
Know the disease	Yes	6(1.54)
	No	383(98.46)
Know that they can get any diseases from contact of aborted bitche's foetus	Yes	81(20.82)
	No	203(52.19)
	Not sure	105(26.99)
Wear any protective equipment/ take any precaution before and after handling a dog, disposing aborted material, cleaning house	Yes	116(29.82)
	No	273(70.18)
Disposal of aborted material (**n=31**)	Burning	1(3.23)
	Burying	4(12.9)
	Throw away	26(83.7)
Cleaning of dog's house	Poor	208(53.47)
	Fair	83(21.34
	Good	98(25.19)

4.4. Awareness and practices of swine farm employees towards Swine Brucellosis

4.4.1. Demographic characteristics of the respondents

From the total 13 swine farm employees, majority of them were: males (69.23%), between 30 and 45 years old (46.15%), and elementary (38.46%) (Table13).

Table 13: Demographics characteristics of swine farm employees (**n=13**)

Demographic characteristics of the respondents	Category	N(%)
Gender	Male	9(69.23)
	Female	4(30.77)
Age	<30 years	5(38.46)
	30-45 years	6(46.15)
	>45 years	2(15.39)
Education status	Illiterates	1(7.69)
	Elementary	5(38.46)
	High school	3(23.08)
	Certificate	1(7.69)
	Diploma	1(7.69)
	Degree and above	2(15.38)
Total		**13**

4.4.2. Awareness and practices of swine farm employees towards the disease

As shown in table, 84.62% of swine farm employees didn't know the disease; 53.85% of them assisted farrowing; 46.15% of them gave aborted materials to dogs; only 30.77% knew they could got any diseases from contact of aborted sow's foetus; and 69.23% of them didn't take any precaution while assisting farrowing, disposing aborted material, and clean their house.

Table 14: Awareness and practices of swine farm employees

Variable	Category	N (%)
Know the disease	Yes	2(15.38)
	No	11(84.62)
Know that they can get any diseases from contact of aborted sow's foetus	Yes	4(30.77)
	No	6(46.15)
	Not sure	3(23.08)
Disposal of aborted material	Burning	1(7.69)
	Burying	1(7.69)
	Throwing away	5(38.46)
	Give to dog	6(46.15)
Assist farrowing	Yes	7(53.85)
	No	6(46.15)
Wear any protective equipment/ take precaution before and after handling pig, assisting a birth, disposing aborted material, cleaning house…	Yes	4(30.77)
	No	9(69.23)

5. DISCUSSION

A cross sectional study was done from November 2017 to May 2018 to investigate the seroepidemiology of canine and swine brucellosis; in selected areas of East Shoa Zone, Ethiopia. To the best of knowledge, the study done on canine brucellosis is the first report from Ethiopia; whereas the second report in swine brucellosis. In the study, RBPT containing *B. abortus* antigen was employed for screening both canine and swine brucellosis. However, as RBPT prepared from smooth strains of *Brucella* cannot detect *B. canis* infection, a RBPT containing *B. canis* (rough strain) had used for screening of *B. canis* infection. Consequently, all dog serum samples had screened twice. Complement fixation test containing *B. abortus* antigen had been used for confirmation of swine brucellosis and canine brucellosis due to smooth strains. However, *B. canis* infection had not serologically confirmed. The present finding revealed that, the overall seroprevalence of canine brucellosis due to smooth strains was 5.4% (CI: 3.35, 7.96) and 4.88% (CI: 2.7, 7.0) using RBPT and CFT, respectively. On the other hand, prevalence of canine brucellosis due to rough strain *B. canis* was 8.74% (CI: 5.92, 11.56) using $RBPT^{canis}$ (Table 2). Similarly, an overall prevalence of swine brucellosis was 5.1% by RBPT and 3.57% by CFT (Table 7).

The overall 5.4% (RBPT)and 4.88%(CFT) prevalence of canine brucellosis due to smooth strains of *Brucella* species recorded in the study area is almost equal with the report of 5.46%(RBPT)by Cadmus *et al.* (2011) in Nigeria; and closely similar with the report of 4%(RBPT and iELISA) by Rahman *et al.* (2015) in different parts of Bangladesh. However, lower seroprevalence had reported in Nigeria and China. A seroepidemiological survey done by Xiang *et al.* (2013) from farm dogs, stray dogs and dogs admitted to the Beijing Companion Animal Hospital for immigration and emigration inspection unveiled, 1.42% and 0.42% prevalence of brucellosis due to smooth strains; using RBAT and TAT, respectively. Moreover, no prevalence (0%) had reported by Chidiebere (2015) using RBPT and SAT in Enugu and Anambra states of Nigeria. In contrast to the above findings, higher prevalence had reported with prevalence of 24.24% using RBPT and 7.57% using 2-ME and SAT tests in Brazil by Vieira *et al*. (2016); 32.3% using RBPT and 29.2% using cELISA in

Nigeria by Momoh et al. (2014); 13.33% using RBPT, 6.67 using SAT, and 10% using ELISA in Bangladesh by Talukder *et al.* (2011).

The 8.74% ($RBPT^{canis}$) prevalence of canine brucellosis in the present study owing to the rough strain correspondences with the report of Onkel *et al.* (2005) , who described 7.73% and 7.45% using 2ME-TAT and ELISA, respectively in Turkey. Whereas, it is higher compared with the studies done by: Mosallanejad *et al.* (2009) (4.9%) using Immuno chromatography assay (ICA), in Iran; Vieira *et al.* (2016) (0%) using ICA, in Brazil; Ergene *et al.* (2017) (0.99%) using Microplate Aglutination Test in Northen Cyprus; Xiang *et al.* (2013) (3.58%) and (1.33%) using RBAT and TAT, respectively in China; and Cadmus *et al.* (2011) (0,27%) using RSAT, in Nigeria. Oppositely, it is lower than the reports of: Chinyoka *et al.* (2014) (17.6%) using ELISA, in Zimbabwe; Behzadi and Mogheiseh (2011) (10.62%) using ICA, in Iran; Keid *et al.* (2004) (33.91%) using AGID, in Brazil; and Chidiebere (2015) (27.7%) using Solid Phase Immunoassay technique, in Nigeria.

The difference seen in seroprevalence of canine brucellosis in different countries could be on account of difference in: sampling method used, sample size of the studies, and sensitivity and specifity of serological tests used. It could be also due to the difference in the dog's rearing culture, awareness of the people, population of stray dogs, dogs keeping purpose, contact with other domestic animals, and health, housing, hygiene, breeding, and feeding management system of the dogs. The variation in sensitivity and specifity of diagnostic tests had explained by Talukder *et al.* (2011) and Geresu and Kassa (2016). Difference in prevalence according to keeping purpose of the dogs had also reported by: Talukder *et al.* (2011) and Xiang *et al.* (2013) among different farm dogs; Onkel *et al.* (2005) among different dog shelters kept for different purposes; and by Chidiebere (2015) in dogs kept for slaughter purpose.

In addition to estimating the seroprevalence of canine brucellosis, the association of risk factors had also done. Consequently, no significant association between locations and seroreactivity to both smooth and rough types of *Brucella* infection had detected by Fisher's exact test and univariable logistic regression analysis. The final simplified model of multi variable logistic regression analysis however indicated a significant

association of locations of the dogs with seroposotivity of the rough strain of *Brucella*. As observed in Table 6, dogs in Batu were 3.86 times more infected with *Brucella canis* than those in Alage. Contrary to this, Momoh *et al.* (2014) and Chinyoka *et al.* (2014) described no statistically significant association across study districts. But it is corroborated with the finding of Ayoola *et al.* (2016) who had also described significant association with the location of samplings.

Unlike Alage and Naka, Batu is urban and the higher prevalence of *B. canis* infection in Batu might be because of high population of stray dogs in the town. It is known that stray dogs are the museum of many diseases, including brucellois. In Batu, there is a municipal abattoir, many butcher shops and restaurants; thereby many dogs migrate from neighbor villages in search for food; which could increase the number of stray dogs in the town. Perhaps, the big lake ("Lake Zway") found in the town which produces thousands tones of fish each year, could also be a reason. This is because upon processing the fish, many fisheries dispose the un-edible offals to the shore of the lake; resulting for assembling freely foraging dogs. Thereupon, many dogs come together and those infected could possibly transmit to others during mating and direct contact. This is buttressed by studies of Chikweto *et al.* (2013) and Xiang *et al.* (2013), who had demonstrated a higher prevalence of infection in stray dogs compared with non-stray in India and China, respectively.

A significant association of canine brucellosis among different sexes had shown for both smooth and rough types of *Brucella* infection. A prevalence of 9.39% (in female) and 2.08% (male) was recorded seropositive for anti *B. abortus* antibodies; likewise, 14.09% in females and 5.42% in males were positive for anti *B. canis* antibodies. To know the magnitude of the association, the multi variable logistic regression analysis revealed that, male dogs had lower seropositivity to smooth strains by (OR=0.26, CI; 0.09, 0.79) and to rough strain by (OR= 7.26; CI 2.59-23.32); compared to female dogs (Table 5 and 6). This result is supported with studies of Talukder *et al.* (2011), Bigdeli *et al.* (2011), and Rahman *et al.* (2015). Nonetheless, insignificant difference had reported by Mosallanejad *et al.* (2009), Momoh *et al.* (2014), Keid *et al.* (2004), Behzadi and Mogheiseh (2011), Xiang *et al. (*2013), Chinyoka *et al.* (2014) and Rahman *et al.* (2015). Sexual transmission is one of the principal means of

transmission and hence the reason for higher prevalence seen in females might, because of a single infected male be able to mate many females.

Unlike with anti *B. canis* antibodies, significant association with seroposotivity of anti *B. abortus* antibodies had seen among different age groups. Consequently, 1.25% of those < 2 years old, 4.05% of those between 2 and 4 years old, and 13.58% of those >4 years were seroposotive. The result of of multi logistic regression analysis unveils higher odds of the disease (OR= 7.78, CI; 2.59-23.32) in those > 4 years old compared with those ≤2 years old (Table 5). This is in concordant with many researchers who had reported brucellosis is significantly age dependent and found higher prevalence in adults; like Abubakkar *et al.* (2010), Kebede *et al.* (2008) , Osinubi *et al.* (2004), Aulakh *et al.* (2008), and Talukder *et al.* (2011). This might be due to the fact that sexually mature dogs would have higher potential of contracting canine brucellosis through the venereal mode of transmission and the probability of contracting the disease from carrier and other infected animals and materials raises, as time goes.

Insignificant association but higher prevalence of canine brucellosis in adults had as well reported by Mosallanejad *et al.* (2009), and Momoh *et al.* (2014). However, the result didn't agree with the study carried out by Cadmus *et al*., (2011), who reported more prevalence in young than adult dogs; and on the other way Xieng *et al.* (2013) reported with no differences among the different age groups. This might because brucellosis could be transmitted vertically from pregnant bitches to puppies (transplacental) and furthermore can affect horizontally, irrespective of age difference.

With respect to maintenance (living) condition of the dogs, a very strong statistically significant association with seroposotivity of both types of strains of *Brucella* species had seen. According to the result of the multi logistic regression, the odd of canine brucellosis due to smooth and rough strains in outdoor dogs were 4.72 and 6.42 times higher compared with indoors, respectively (Table 5 and 6). This higher prevalence seen in outdoors might have resulted from the fact that unattended dogs are often in closer contact with infected materials. They had possibilities of getting the infection often in search of their food. Outdoors had a chance of eating infected aborted

materials of domestic animals and abattoir wastes when freely roam; thenceforth they could be infected with any of *B. abortus, B. melitensis, and B. suis* from the materials. This means of transmission in dogs had explained by Chinyoka *et al.* (2014), Hinc *et al.* (2010), Baek *et al.* (2003), Wanke (2004), OIE (2009), Cadmus *et al.* (2011), Lucero *et al.* (2008) and Ramamoorthy *et al.* (2011); as well as by the review of Woldemeskel (2013). It could also because of the higher probability of such outdoor dogs mating with other infected dogs. Many dogs assemble during breeding season and they would have very close contact with other infected dogs, which is suitable for transmission of the disease by mating. This association of the disease with the living condition of dogs is in consistent with Khairani *et al.* (2006) and who got 35% in unattended dogs in Malaysia; and Xieng *et al.* (2013) from China got low prevalence (0.64%) in indoors but high (28.6%) in stray dogs.

Obstetrical problems had strongly significantly associated with seroposotivity of both anti *B. abortus* and anti *B. canis* antibodies in the present study. Higher prevalence was found in dogs with history of obstetrical problems (Abortion, infertility, retained fetal membrane, still birth, scrotum dermatitis, swelling of Scrotum, abnormal vaginal discharge…) than those hadn't (Table 5 and 6). This could be explained by the fact that such reproductive signs are typical outcomes of brucellosis (Lopes *et al.,* 2010; Radositis *et al.,* 2007; Iowa, 2007; Wanke, 2004). As it is described by Holst *et al.* (2012), at abortion the placenta and the discharges can contain up to 1010 colony forming units (cfu) per ml. Thus, 1 ml placental tissue or vaginal discharge is equal to approximately 100,000 infectious doses, and the bitches can have a vaginal discharge for up to 6 weeks after an abortion. Therefore, such dogs will be the source of infection for others. Though insignificant, higher proportion in dogs with history of reproductive problems had reported by Ayoola *et al.* (2016). They found higher seroposotivity in those with history of infertility (OR=2.62 CI: 1.41-4.84). Besides, Gyuranecz *et al.* (2011) had similarly reported.

The an overall prevalence of swine brucellosis 5.1% (CI: 0.95, 6.19) by RBPT and 3.57% (CI: 1.99, 8.21) by CFT seen in the study area (Table 7) is closely related with the previous first report from Ethiopia by Kebeta *et al.* (2015) who found a seroprevalence of 4.5% by RBPT; and with the finding of Rahman *et al*. (2012) who reported a prevalence of 4.8% (RBPT and SAT) in Bangladesh. It is also almost

equal with the study by Sharma *et al.* (2017) who investigated a prevalence of 3.59% from Nepal, using iELISA. However, it is higher than the report of Cadmus *et al.* (2006) and Spicic *et al.* (2016), which didn't got any seroposotive (0%) in Nigeria and in Crotia pig farms in 2009 GC, respectively. Opposite of this, higher prevalence had reported from Nepal (13.59%) using SAT (Poudel *et al.*, 2014). An investigation done in 2008 in Crotia pig farms by Spicic *et al.* (2016) also revealed a prevalence of 21.6%, 21.1%, and 49.0% using RBPT, CFT, and ELISA, respectively.

The difference in seroprevalence of swine brucellosis in the different countries might because of difference in: agro ecological zone, swine production systems used, total pig population in the countries, pork consumption culture of the countries, management systems used, contact with feral pigs/wild pigs/wild boars, sample size used, sampling method, and capacity of the diagnostic tests used. For example, the review of Lopes *et al.* (2010) unveiled that feral swine were rapidly expanding their ranges across the United States. They had then becoming a risk for transmission of *B. suis* to local domestic livestock, including pigs. The review added that brucellosis in swine had re-emerged in different European countries as a result of spillover from the wild boar brucellosis (*B. suis* biovar 2) reservoir, particularly in outdoor reared pigs. Besides, in a study conducted by Munoz *et al.* (2010) in the Iberian Peninsula, high apparent prevalence brucellosis in domestic pigs was found (33%); and wild boar population had found seriously affected by *B. suis* biovar 2 infection; which might be due to contact between free ranging Iberian domestic pigs and wild boar. Other scholars (Godfroid *et al.*, 2010; Bergagna *et al.*, 2009; Cvetniz *et al.*, 2009; Leuenberger *et al.*, 2007) had also reported *B. suis* in wild animals which could be a source of infection to freely foraging domestic pigs.

In relation to the study farms and prevalence of the disease, both Fisher's exact test and univariable logistic regression analysis revealed insignificant association (Table 8 and 9). The prevalence was almost equal among the farms. This similarity may be due to the similar production system, feeding system, and breeding management they follow. Both farms are intensive type and natural mating is their means of breeding system. Besides, they are found in similar agro ecological zone and hadn't contact with other animals. This is correspondent with the report of Rahman *et al.* (2012) who found no significant difference ($p \geq 0.05$) among different study districts. Oppositely,

Kebeta *et al.* (2015) had reported significant association of brucellosis with origins of the pigs. They showed highest prevalence in pigs originated from Adama area followed by Addis Ababa area. However, this may be due to the difference in agro ecological zone and general management system among the study locations. Adama is located at hot lowland of Rift Valley where as Addis Ababa at highland with humid climate.

Sex wise, though higher prevalence in females (4.76%) than males (1.43%) had seen, it was not significantly associated. Similarly, Rahman *et al.* (2012) reported insignificant but high prevalence of brucellosis (5.6%) in female and 2.9% in male pigs in Bangladesh. Other researcher, Poudel *et al.* (2014) had also observed insignificant but higher prevalence in females (15.09%) than that of males (12%). However, significant association of sex with seroposotivity was reported by Kebeta *et al.* (2015) who got higher prevalence in female 8.2% than male 1.6%. In contrary to this Ngbede *et al.* (2013) from Nigeria found relatively higher prevalence of brucellosis in male pigs than female pigs.

Age wise, using multi logistic regression analysis, the odd of the disease in those >3 years old pigs was 9.44(CI=1.51-58.92) times higher compared to those ≤3 years old (Table 10). This is analogous with the researchers conducted by Rahman *et al.* (2012) who found higher prevalence of brucellosis in aged animal (8.1%) than young (0.0%); and with Poudel *et al.* (2014) who observed higher prevalence in adults. However it disagrees with the result of Kebeta *et al.* (2015) who observed in young (5.9%) as compared to adult pigs (3.6%). The present study recorded swine brucellosis higher in sexually matured pigs could be due to increased time for exposure with age and reproductive organs maturity where *Brucella* organisms predilect (Corbel, 2006). Adult animals have more opportunities over time to encounter another positive animal. Because transmission can be associated with mating, the probability of exposure likely increases once an animal is of breeding age.

The odd of the disease in those with history of obstetrical problems was 23.22(CI= 3.96-136.13) times higher compared with those without such history; and was statistically significant (Table 10). This finding fits with a research of Rahman *et al.* (2012) who found that prevalence of brucellosis was significantly higher in aborted or

previously aborted sows than the sows having no record of abortion when the sera samples tested by RBT and SAT. Other researchers like Sandhu *et al*. (2001) and Rahman *et al*. (2006) had also reported similar findings. They reported cows with a history of retained placenta had higher prevalence and was statistically significant. Therefore, it can be said that the principal cause of the obstetrical problems in the studied swine farms was due to brucellosis; as the organisms localizes and replicates in the placenta and the fetuses of sows; and the testis, epididymis, seminal vesicles, and/or bulbo-urethral glands of boars.

The result of questionnaire survey revealed that the knowledge and understanding about brucellosis among the dog owners and swine farm employees was very limited (Table 12 and 14). Only 1.54% of dog owners and 15.38% of swine farm employees knew the disease. This agrees with the study results reported in Nigeria by Momoh *et al.* (2015) who showed low awareness about the disease among dog owners; and with Simon *et al.* (2015) who reported pig traders and dressers at slaughter facilities in Temeke Municipality of Dare-Salaam (Tanzania) had limited knowledge on swine brucellosis. According to their study, the majority of pig traders (66.7%) and pig dressers (94.4%) had limited knowledge on the occurrence of the disease. The low awareness on canine and swine brucellosis in the present study could be attributed to the dearth of health education (especially regarding zoonotic diseases). In Ethiopia, the veterinary medicine is widely recognized important only for the cattle population. The attention given towards these animals by veterinarians is very poor and so much misunderstanding prevails with regard to preserving their healthy conditions. Both of them are also considered as unclean by the Ethiopian society.

Furthermore, only approximately one third of the dog owners (30.77%) and 20.82% of swine farm employees were awared of the risk of transmission of any disease to human from contact of dogs and sows aborted material, respectively. However, a good knowledge about the disease transmission had a precautionary effect; as they could perform better preventive practices to avoid contracting brucellosis. This is supported by Kozukeev *et al.* (2006) who explained that good knowledge about the disease transmission among farmers had a precautionary effect for brucellosis. In a similar way, a case control study done by Sofian *et al.* (2008) in Iran demonstrated that, having awareness regarding modes of brucellosis transmission, was associated

with a reduced risk of human brucellosis infection. This suggests that improving the animal owners' knowledge of the disease and its mode of transmission is likely to reduce their risk of brucellosis transmission from dogs and pigs. It is therefore important to establish an educational campaign in the study areas to enlighten the communities on the disease.

Findings from current study also illustrate that among those reported abortion cases, 83.7% of dog owners and 38.46% of swine farm employee throw out the aborted material to outside environment. Moreover, 46.15% of farm employees gave aborted foetus to dogs. This lack of awareness on proper disposal of the aborted material can lead for the transmission of the disease to other animals and human. Many *Brucella* organisms are shed during abortion, contaminating the environment, and increasing the risk of others (Park *et al.,* 2005). Besides, 53.47% of dog owners poorly maintain the hygiene of their dog's house. As a result, debris (e.g. residues from feces, urine, vaginal or perpetual discharges and other body fluids) might build up and can "hide" the offending pathogen(s). *Brucella* species can then remain viable for several months in these dusts and soil, especially during rainy season and will be the source of infection. This is supported by Abubakkar *et al.* (2011) and Adesokan *et al.* (2013) who had identified unhygienic practices as factors facilitating the spread the *Brucella* infection.

A large proportion of dog owners (70.18%) and 69.23% of farm employees didn't wear any protective equipment/ take any precaution/ such as cover all, hand/rubber gloves, boots, or hand washing /disinfection; before and after handling a dog, disposing aborted material, and cleaning their house. Similarly, Momoh *et al.* (2015) had also reported from Nigeria. According to their report, 84% of respondents neither they used protective wears nor washed their hands, before and after handling of dogs. This is however risky especially for those more than 50% swine farm employees of the present study who assisted farrowing; as the pathogen can enter through intact skin and abrasions (Mantur and Amarnath, 2008).

6. CONCLUSION AND RECOMMENDATIONS

The present study revealed that the overall seroprevalence of canine brucellosis due to smooth strains of *Brucella* species was 4.88% using CFT and 8.74% due to *B.canis* using RBPT containing *B. canis* antigen. On the other hand the seroprevalence of swine brucellosis was 3.57% using CFT. Thus, it can be concluded that the overall prevalence of canine and swine brucellosis was high. The disease had significantly associated with living condition of the dogs and outdoor management system was a key risk factor for the occurrence of the disease. Furthermore, respondants had reported abortion cases and the disease had strongly associated with history of reproductive problems of both animals. Thence, they can be a source of infection to others. The increasing evidence that *B. canis* infections are endemic in the dog population also gives rise to the suspicion that, the zoonotic potential may be greater than suspected. The high prevalence of swine brucellosis also suggests the importance of this zoonotic disease in pig production and public health. Thereon, the awareness of the people toward the disease was the gap in the study area. Therefore, based on the above conclusion the following recommendations are forwarded:

- Constant surveillance program that encompass dogs and pigs should be designed and implemented.
- Public education should be given on transmission, prevention and control methods of canine and swine brucellosis.
- Communal use of boars for breeding purpose should be avoided.
- Test and slaughter policy should implement in the swine farms.
- A strict pre-purchase test has to be advocated among the swine farms to prevent the entry of the disease to native population.
- Control measures should be instituted by dog owners and pig farm employees through constant cleaning of the environment, proper disposal of aborted fetuses, placenta and other contaminated materials.
- Especial precaution should be given by owners and their children who keep dogs in their saloon, so as to avoid being infected with infected secretions.
- Further intensive epidemiological researches that intended to the isolation of causative agent and identification of species should be done.

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8. ANNEXIES

ANNEX-I: Rose Bengal test (RBPT)

a) Materials and Reagents Required

RBPT *Brucella* antigen
Positive control serum
Negative control serum
Test sera
Apparatus plate
Micro pipette tips
Micro pipette
Applicator stick
Magnifying lens

b) Test Procedure

- According to the procedure described by (OIE, 2004), the sera and antigen were removed from refrigerator and left on table at room temperature for at least 30 minutes before the test was conducted.
- 30 µl of the RBPT antigen were dispensed on each of the plate
- 30 µl of test sera were dropped alongside the antigen mixed and gently agitated for 4minutes and then observed for agglutination. For each plate negative and positive controls were included in the remaining two circles of the plate then any visible agglutination of the test sera were considered positive, though the degree of agglutination varies from mild to strong ones.

c) Interpretation

Agglutination were recorded as 0, +, ++, +++ according to the degree of agglutination. 0 indicates absence of agglutination; + indicates barely visible agglutination; ++ indicates fine agglutination; and +++ indicates coarse clumping.

Those samples identified with no agglutination were recorded as negative and others with any degree of agglutination as positive.

Annex-II: Complement Fixation Test (CFT)

a) Materials and reagents required

Veronal buffered diluents (VCM)
Brucella antigen
Complement
Amboceptor solution
1% sheep red blood cell suspension
Alsever solution
Positive control sera
Negative control sera
Micro titer plate
Multi and single channel micro pipettes
Micro pipette tips
Water bath
Centrifuge
Syringe
Glass and plastic beaker
Measuring cylinders
Centrifuge tubes
Graduated glass pipettes
Incubator
Trough
Refrigerator
Test-tube rack

b) The principle of the test

Activation of classical complement system by anti-body bound to antigen results in generation of membrane attack complexes capable of disrupting cell membranes. If the antibody is bound to erythrocyte surfaces, the erythrocyte membranes are disrupted and haemolysis occurs. It is possible to use this reaction to measure serum antibody levels and this test is known as complement fixation test. If complement is fixed by antigen-antibody immune complex, it is unavailable to lyses the target cell in the indicator system. In the case of negative sera samples, the unbound complement will react with the indicator system result in the lyses of sheep red blood cells (SRBC) by activation of complement.

c) Preparation of reagents

i) Preparation of SRBC

- A blood was taken from the jagular vein of male sheep freely flowing in to a syringe containing Alsever's solution (75ml of SRBC in 125ml Alsever solution).
- A small amount of crystalline penicillin was added to avoid bacterial contaminants.
- It was stored at +4°C and this blood can be used for 2 weeks.

ii) Preparation of hemolytic system

- The sheep blood had washed 3 times at a dilution of 1/10 by adding VCM and centrifuged at 2,500 rpm for 5 minutes by discarding the supernatant.
- A tube of identical size was taken and hold next to the centrifuged tube. The packed cell volume of SRBC was measured and then diluted in VCM to 1%.

d) Evaluation of complement

- 25 µl VCM was dispensed in to all well of A, B, C, and D of U-shaped micro plate.

- 25 µl of complement was added at starting dilution of 1:2 in to the first wells of row A, B, and C (i.e. A1, B1, and C1). Row "D" was left as hemolytic system control.
- Two fold dilutions of complement by transferring 50µl of the mixture to other wells were made until A12, B12, C12, and D12 and the final 50µl was discarded after mixing.
- 25µl of the haemolytic system, indicator (Amboceptor + SRBC) was distributed in to all wells of rows A, B, C, and D and incubated at 37°C with constant agitation for 30 minutes.
- It had read and recorded the last dilution's column showing complete hemolysis and 50% haemolysis of SRBC by comparing with the hemolytic system.

e) The test proper

The test was conducted on a 96 well U-shaped micro titer plate and the procedure was

1. 1/5 dilution of serum to be tested was prepared as following.
   - 40 ml of VCM had added to the wells of row A, C, E, and G, and 10ml of serum was transferred from the pre-plate which gave a total of 50ml.
   - It was homogenized by12 multi-channel micropipette and 25 µl of solution was transferred from A1-A12 in to B1-B12, from C1-C12 in to D1-D12…
2. 25 µl of diluted antigen was added to the wells of rows B, D, F, and H.
3. 25 µl of VCM was added to the wells of rows A, C, E, and the plates were incubated at 37°C for 30 minutes, and covered by micro-plate sealer to prevent evaporation.
4. 25 µl of complement was added to all wells and incubated for 30 minutes.
5. 25 µl of haemolytic system was added to all the wells, including control wells and incubated at 37°C for 30 minutes at constant agitation with agitator.
6. The micro plates were put in a refrigerator overnight and the results were read

Annex III: Photos taken while in field and laboratory

(A). Blood collection from a dog

(c). Pigs during feeding (Alage swine farm)

(D). Blood collection from a pig

(E). Collected sera samples in laboratory

(F). "1" while testing samples by RBPT; **"2"** RBPT test result (the arrow indicates positive sera sample); **"3"** CFT test result (the arrow indicates positive sera sample)

Annex IV: A questionnaire format for interview

Addis Ababa Universty College of Veterinary Medcine and Agriculture Department of clinical studies, Bishoftu, Ethiopia

Introduction

Good morning/ Good after noon

My name is **Aregawi Girmay,** a postgraduate student of the department of veterinary Clinical Studies, AAAU-CVMA, Bishoftu, Ethiopia. I am working on a research; **"Epidemiology of Canine and Swine Brucellosis in Selected Areas of East Shoa Zone, Oromia Region, Ethiopia"**. To study this, I seek your permission to interview you, which will assist me in finding the risk factors associated with the disease. This will form the basis for future control of brucellosis in the study areas as well as

encourage advocacy, policy-making and prioritization of the disease in health interventions at a national level. Your positive response will certainly help the research to come up with recommendations that would lead to instituting interventions towards control and prevention. I am hoping that your sincere response to these questions will go a long way in enabling me to achieve these objectives. I appreciate the few minutes you will spend in responding to these questions and sincerely assure you that utmost confidentiality will be maintained.

Thank you for your cooperation.

Client information

A. Personal Identification socio demographics

A.1. Unique Code Number……..……Date..……

A.2. Interviewer's Name……

A2. Name of the interviewee ……

A3.Full Residential Address……

A4. Phone number ……

A.5. Age:- 1. <30 years 2. 30-45 years 3. > 45 years

A.6. Sex ……..

A.7. Education ststus: 1. None 2. Elememtery 3. Highschool 4. Certificate 5. Diploma 6. Degree and above

B. Questionnaire to dog owners

B.1. Dogs' profile

Name of the dog……..

Sex…….

Age……

B.2. General Management system of the dogs

1. Living Condition

- Do your dog most of the time, move freely in the neighborhood?

  A. Yes B. NO

If yes how often roam freely?

A. Very rare B. Sometimes C. Mostly/all the time

2. Feeding habit

A. Mostly Scavenging

B. Both household feed and scavenging

C. Household feed only

D. Sometimes meat purchased from butcher shop/abattoir

E. Mostly/Always meat purchased from butcher shop/abattoir

3. Health Management system

- Do you go to veterinary clinic for treatment? A. Yes B. No

4. Hygiene practice

i. Do you maintain hygiene of the dog's home? A. Yes B. No

If your answer is yes, how often? A. Very Rarel B. Some times C. mostly/Always

ii. Do you maintain Personal hygiene of the dog? A.Yes B. No

If your answer is yes, how-often? A. Very Rarely B. Some times C. Mostly/ always

B.3. Animals' owner ship

iii. Number of dog(s) in the household……

iv. Any other domestic animal(s) in the household (tick which ones apply) indicate.

(a) Goat (b) Sheep (c) cat (d) Cattle (e) draft animals

B.4. History of reproductive problems

i. For males

a. Swelling of scrotum/testicle

b. Scrotum dermatitis

ii. For females

a. Abortion

b. Still birth

c. Retained placenta

d. Abnormal vaginal discharge

B.5. Purpose of dog keeping

a. Companion for children and other members of the family

b. Guard

c. Breeding

B.6. Awareness about the disease

i. Do you know a disease leading abortion in bitches?

a. Yes b. No

ii. Do you know any disease that transmit from contact of aborted dogs to man?
a. Yes b. No

iii. Do you give any aborted material to the dog?
a. Yes b. No

iv. If your bitch had aborted, what did you do for the aborted material?
a. Burn b. Bury c. Throw away to environment

v. If you had disposed aborted material, did you wear any personal protective equipment?
a. Yes b. No

vi. Do you wear any personal protective equipment when washing the dog/ cleaning its home?
a. Yes b. No

vii. Do you help your bitch during whelping? A. yes B. No

viii. If yes, did you wear personal protective equipments? A. Yes B. No

C. Questions for swine farm employee

i. Do you know diseases that transmit to man following contact of aborted material of sows? a. yes b. No

ii. Do you know a disease that lead abortion of sows at late stage of pregnancy? a. yes b. No

iii. If your sow had aborted before, what do you do for aborted material?
a. burn b. bury c. dispose to outside environment d. give to dogs

iv. If yes, did you wear any personal protective equipment when disposing aborted material? a. Yes b. No

v. Did you assist a sow during furrowing? a. yes b. No

vi. If yes, did you wear any personal protective equipment when assisting birth? a. yes b. No

vii. Do you wear any personal protective equipment when cleaning the farm especially farrowing pen? a. yes b. No

viii. History of reproductive problems

i. For males

a. Swelling of scrotum/testicle

b. Scrotum dermatitis

ii. For females

a. Abortion

b. Still birth
c. Retained placenta
d. Abnormal vaginal discharge

Annex V: A format sheet of the sampling frame

Date. _____ Location _____ .

No.	Owners name	Address	Mobile number	Number of household dogs
1				
2				
3				
4				
5				

Annex VI: A Record sheet Format for individual sample dogs

Date_____

No.	Owners name	District	Name of the dog	Sex	Age	Living condition	History of Obstetrical problems
1							
2							
3							
4							
5							

Annex VII: A Record sheet Format for individual sample pigs

Date_____

No.	Ear tag No.	Farm Name	Sex	Age	History of obstetrical problems
1					
2					
3					
4					
5					

Annex VIII: Ethical clearance

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu/Debre Zeit

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/19/05/10/2018

Name of Applicant: Aregawi Girmay (DVM, MVSc fellow)

Address: College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: Epidemiology of Canine and Swine brucellosis in selected districts of East Showa Zone, Oromia Region, Ethiopia

Date of application:	15/11//2017
Nature of the project:	non-invasive
Target animal species:	dog and pigs
Number of animals involved:	585
Study area:	East Showa Zone, Ethiopia

Minutes No. and date of review: VM/ERC/05/10/018, 03/01/2018

The above indicated research project is acceptable from ethical perspective, relevance, originality and technical competence points of view. Hence the project is allowed to be executed provided that:

4. All procedures and conditions stipulated in the proposal are respected and any deviation or changes be reported to the committee
5. The project activities be open for occasional supervision by the committee whenever this is deemed necessary
6. Any major work on human subjects require a separate clearance from concerned authority

Dr Getachew Terefe
Chairman

Signature

The Federal Democratic Republic of Ethiopia

Please quote Our Ref. No. When replying

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