

# Identification of Canine Brucellosis in Three Province, Ethiopia

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## Research article

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# Abstract

**Background:** In Ethiopia, brucellosis has been reported targeted on bovine, occasionally on shoat, and rarely on camels. An investigation of the disease Brucellosis in the neglected companion animals is scarce in Ethiopia. The objective of this study was to identify canine brucellosis in Batu town, Alage and Naka village through cross sectional approaches. A total of 389 serum samples (207 from Batu, 107 from Alage, and 75 from Naka) were collected by restraining dogs with a portable and safe modified dog crush, invented by this author. Blood samples were collected from ear vein and sera were screened for Brucella antibodies using different serological tests. RBPT prepared from the smooth strain *B. abortus* antigen and CFT was used as a screening test and confirmatory test, respectively. Furthermore, all sera samples had also screened by RBPT<sup>canis</sup> antigen (rough strain); and those positive were considered the cause for *B. canis* infection.

**Results:** Using RBPT smooth strains, 21(5.4%; CI: 3.35, 7.96) were positive and 19(4.88%; CI: 2.7, 7.0) were confirmed by CFT. Besides, 34 (8.74%; CI: 5.92, 11.56) were positive for RBPT<sup>canis</sup> rough strains. Relatively, higher proportion of anti *B. canis* antibodies had seen in Batu (11.59%) followed by Alage (5.61%), and Naka (5.33%). Sex, living condition, and history of obstetrical problem were significantly associated with the occurrence of canine brucellosis ( $p < 0.05$ ). 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. This is true the fact that outdoors had a chance of getting infected aborted wastes when roaming freely.

**Conclusion:** This study suggests that canine brucellosis is prevalent in the province. The seropositivity could give an insight that, the awareness of the people toward the disease was also the gap in the study area. Hence, this warrants public education among the community is recommended.

## Background

Nowadays, numerous emerging and re-emerging pathogenic diseases have threatened our globe; these diseases are seriously affecting the wellbeing of human, animal health, and animal production (1,2). Most of zoonotic diseases have a great veterinary and public health impact, particularly in developing countries where people are having daily frequent contact with livestock and animal products (3). Brucellosis 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 (4). It is ranked among the most economically important zoonoses globally, as it can lead international trade ban (5,6).

According to (7), *B. abortus* (cattle and buffalo), *B. ovis* (sheep), *B. melitensis* (sheep and goats), *B. suis* (pigs) and *B. canis* (dogs) are species of *Brucella* that cause disease in domesticated livestock. Brucellosis is primarily a disease of the reproductive tract 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 (8).

Since the first report of brucellosis in the 1970s in Ethiopia, 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 (9,10)

Observably, 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 as 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, feces, 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 (11).

However, despite of these risk factors that 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 (7), Zimbabwe (12), and South Africa (13), there is lack of information on canine brucellosis. This epidemiological investigation was therefore, designed to estimate the sero-prevalence and associated potential risk factors of brucellosis in dogs in selected areas of East Shoa Zone, Ethiopia

## Methods

### Study Areas

The study was conducted in Batu, Alage and Naka village as seen in (Fig. 1). 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) (14,15)

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. According to CSA 2007, the Region had an estimated of 17,214,540 cattle, 6,905,370 sheep, 4,849,060 goats, 959,710 horses, 63,460 mules, 278,440 asses, 139,830 camels, 11,637,070 poultry, and 2,513,790 beehives. However, dog population is not defined except those 300,000 dogs found in Addis Ababa (16).

### 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' sera; so as to determine the seroprevalence of canine brucellosis. The areas were conveniently selected based on the abundance of dogs. The study had also involved face to face interview.

## Study Population

Owned dogs, which were older than 6 months, found in Alage, Batu and Naka; were the target population. None of them were also vaccinated against brucellosis. Study animals were performed through owner's oral consent and study was made with the after obtaining ethical clearance letter from the Board of Research and Ethical Review Committee. The oral-only consent from the owners was approved by the given legal ethics committee.

## Sample Size and Sampling Method

The required sample size of dogs was determined based on expected prevalence of brucellosis and the desired absolute precision stated on (17).

$$n = \frac{1.96^2 P_{exp} (1 - P_{exp})}{d^2}$$

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.

# Data Collection Approaches

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

**Approach 1: Restraining approaches:** For restraining dogs, a portable and safe modified dog crush was employed. The researcher invented the crush also evaluated and certified by Alage ATVET College. Currently it had registered in Ethiopia Intellectual Property Office.

**Approach 2: Rabies vaccination campaign:** In collaboration with Alage 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.

**Approach 3: Blood sample collection:** After proper restraining, about 6–8 ml of blood was aseptically collected through the saphenous vein of each sampled dog using syringe and plain vacationer 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' sera tested positive by RBPT were stored at -20 °C and lastly sent to National Veterinary Institute (NVI), Bishoftu, Ethiopia for further confirmation.

**Approach 4: 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 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 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 (18). 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.

- ii. *Complement Fixation Test*. Positive samples for RBPT (containing *B. abortus* antigen) were further confirmed by CFT. The confirmation of dog sera was undertaken at NVI, Department of Immunology. 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 (19). The CFT test was regarded as positive when the reading is as complete fixation or partial hemolysis and as negative (0) when there is complete hemolysis (20).

**Approach 5: 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 tongue 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 were not included due to being a sensitive topic culturally.

### **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, scrotal edema, and scrotum dermatitis for males), and living status (for dogs) were recorded for each animal. Age of dogs were stratified into three categories ( $\leq 2$  years, 2–4 years, and  $> 4$  years); and for pigs in to two ( $\leq 3$  years and  $> 3$  years). Furthermore, living status (condition) of dogs was categorized as indoors, outdoors, and semi-indoors (modified from (1) and (21)).

### **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 (22).

### **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* (22–24). To detect *Brucella* infection serologically due to smooth strains, the antigen of the serological test should prepare from smooth strains (25–27).

### **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 (25–27). Generally Serologic tests that use suspensions of smooth phase *Brucellae* are useless in diagnosing *B. canis* infections (28).

### **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 (29,30). 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 chain (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.

## **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.

## Results

As the result is summarized in (Table 1), 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<sup>canis</sup>); 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<sup>canis</sup>.

**Table 1. 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 <sup>canis</sup> (%)	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<sup>canis</sup>: RBPT containing *B. canis* as an antigen

### ***Risk factors association with Brucella seropositivity***

Analysis for association between locations of the animals and *Brucella* infection was carried out using Fisher's exact test (Table 2). 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<sup>canis</sup>, 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  $\leq 2$  years old, 4.05% of 2-4 years old, and 13.58% of  $>4$  years old were seropositive. 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<sup>canis</sup>, 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 2. 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 <i>canis</i>	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	81	2(1.25)		13(8.13)	
2-4 years	148		6(4.05)		15(10.14)	
>4 Years			11(13.58)		6(13.58)	
Living status				0.021		0.002
Indoors	72	85	1(13.89)		3(4.17)	
Semi-indoors	232		9(3.88)		15(0.06)	
Outdoors			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

### ***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 in (Table 3), sex, age, living conditions and history of obstetrical problems had statistically significant effect on seropositivity ( $p < 0.05$ ); 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  $\leq 2$  years ( $p < 0.05$ ).

Furthermore, according to their living status, outdoor dogs were 8.4 times more likely to be seropositive 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 seropositivity. 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 3. Univariable logistic regression analysis of associated risk factors of canine brucellosis**

Variables	Canine brucellosis due to smooth types (CFT)		Canine brucellosis due to <i>B. canis</i> (RBPT <sup>canis</sup> )	
	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				
No	0.000	7.22(2.77, 18.81)	0.001	3.88(1.76, 8.55)
	*			

\*Reference variable

### ***Multi-variable logistic regression analysis***

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 as presented in (Table 4 and 5). Accordingly, 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 seropositivity than female dogs (OR=0.26, CI; 0.85, 0.79); and dogs older than 4 years had higher seropositivity than those  $\leq 2$  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 4. A simplified result of multivariable logistic regression analysis 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		
$\leq 2$ 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 ( $p \geq 0.05$ )

Similarly, one location (Naka), age, and semi-intensive living condition had removed from the final model showing the association of factors with seropositivity of *B. canis* infection ( $p \geq 0.05$ ) (Table 5).

Table 5. Final simplified result of multivariable logistic regression analysis of canine brucellosis due to *B. canis*

Canine brucellosis due to rough strain <i>B. canis</i> (RBPT <sup>canis</sup> )		
Variables	p-value	OR (95%CI)
Study areas		
Alage	*	*
Naka	†	†
Batu	0.004	3.86(1.56, 9.54)
Sex		
Female	*	*
Male	0.000	7.78(2.59, 23.32)
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 ( $p \geq 0.05$ )

As illustrated in Table (5), dogs reared in Batu were 3.86 (CI; 1.56, 9.54) times more likely to be seropositive compared to those reared in Alage. However, male dogs had lower seropositivity than females (OR=0.38, CI; 0.18, 0.82). Besides, dogs maintained outdoor were 6.42 times more likely to encounter *B. canis* infection than those maintained indoor (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).

## Survey result on awareness and practices of dog owners towards canine brucellosis

Out of the total 389 interview dog owners, 225(57.84%) of them were males (Table 6). Age wise, majority (63.24%) were between 30-45 years old and according to their education status 24.16% were elementary. About 383(98.46%) of them didn't know the disease; and only 30.77% now that they can get 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.

Table 6: 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

Generally, result of questionnaire survey revealed that the knowledge and understanding about brucellosis among the dog owners was very limited. The low awareness on canine 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 livestock population. The attention given towards dogs by veterinarians is very poor and so much misunderstanding prevails with regard to preserving their healthy conditions. It is therefore important to establish an educational campaign in the study areas to enlighten the communities on the disease.

## Discussion

In Ethiopia, brucellosis has been reported from different localities, commonly targeted on bovine, occasionally on sheep and goats, and rarely on camels (31). To the best of knowledge, canine brucellosis had not studied in the country.

This study investigates the seroepidemiology of canine brucellosis in selected areas of East Shoa Zone, Ethiopia. In the study, RBPT containing *B. abortus* antigen was employed for screening canine 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 were screened twice. Complement fixation test containing *B. abortus* antigen had been used for confirmation of 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<sup>canis</sup> as illustrated in (Table 2).

Detection of canine brucellosis due to smooth strains of *Brucella* species recorded in the study is almost equal with the report by Cadmus *et al.* (7) 5.46% (RBPT) in Nigeria; and closely similar with the report by Rahman *et al.* (31) 4% (RBPT and iELISA) in different parts of Bangladesh. However, lower seroprevalence had reported in Nigeria and China. A seroepidemiological survey done by Xiang *et al.* (1) 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 Anyaoha (32) using RBPT and SAT in Enugu and Anambra states of Nigeria. In contrast to the above findings, higher prevalence had reported with prevalence of 32.3% using RBPT and 29.2% using cELISA in Nigeria by Momoh *et al.* (21), 13.33% using RBPT, 6.67 using SAT, and 10% using ELISA in Bangladesh by Talukder *et al.* (33).

The result of this study due to the rough strain was in agreement with the report of Öncel *et al.* (34) in Turkey, who described 7.73% and 7.45% using 2ME-TAT and ELISA, respectively. Whereas, it is higher compared with the studies done by MOSALANEZHAD *et al.*(35) (4.9%) using Immuno chromatography assay (ICA), in Iran; (36) (0%) using ICA, in Brazil; Ergene *et al.* (37) (0.99%) using Microplate Agglutination Test in Northern Cyprus; Xiang *et al.* (30) (3.58%) and (1.33%) using RBAT and TAT, respectively in China; and Cadmus *et al.* (7) (0.27%) using RSAT, in Nigeria. In contrary, it is lower than the reports of: Chinyoka *et al.* (12) (17.6%) using ELISA, in Zimbabwe; Behzadi and Mogheiseh (2) (10.62%) using ICA, in Iran; Keid *et al.*(25) (33.91%) using AGID, in Brazil; and Anyaoha (32) (27.7%) using Solid Phase Immunoassay technique, in Nigeria.

The difference observed could be due to sampling method, sample size of the studies, and sensitivity and specificity 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.



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 multivariable logistic regression analysis however indicated a significant association of dog's locations with seropositivity of the rough strain of *Brucella*. As observed in (Table 5), dogs in Batu were 3.86 times more infected with *Brucella canis* than those in Alage. In contrary, Momoh *et al.* (21) and Chinyoka *et al.*(12) Sowed no statistically significant association across study districts. However, it is agreed with the finding of Adedoyin *et al.* (38) who had also described significant association with the site 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 brucellosis. 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") in surrounding of the town which produces thousands of tons of fish each year could also be the reason. This is because upon processing the fish, many fisheries dispose the un-edible offal 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 supported by studies of Chikweto *et al.* (3) and Xiang *et al.* (1), 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 multivariable 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 shown in (Tables 5 and 6). This result is supported with studies of Talukder *et al.*, Bigdeli *et al.* and Rahman *et al.* (31,33,39). Nonetheless, insignificant difference had reported by Mosallanejad *et al.* (40), Momoh *et al.* (29), Keid *et al.* (25), Behzadi and Mogheiseh (41), Xiang *et al.* (1), Chinyoka *et al.* (12) and Rahaan *et al.*,(31) Coitus 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 seropositivity 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 seropositive. The result of multi logistic regression analysis reveals higher odds of the disease (OR = 7.78, CI; 2.59–23.32) in those older than 4 years old compared with those ≤ 2 years old as shown in (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.* (42), Kebede *et al.* (43), Osinubi *et al.* (44), Aulakh *et al.* (45),

and Talukder *et al.* (33). 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.

With respect to maintenance or living condition of the dogs, a very strong statistically significant association with seropositivity of both types of strains of *Brucella* species was recorded. 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 as listed in (Tables 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 roaming freely; thereafter they could be infected with any of *B. abortus*, *B. melitensis*, and *B. suis* from the materials. Such means of transmission in dogs had explained by Chinyoka *et al.* (12), Hinic *et al.* (46), Baek *et al.* (11), Hensel (47), OIE (20), Cadmus *et al.* (7), Lucero *et al.* (48) and Ramamoorthy *et al.* (49); as well as by the review of Woldemeskel (50). 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-Bejo *et al.* (51) 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.

In the present study, obstetrical problems had strongly significantly associated with seropositivity of both anti *B. abortus* and anti *B. canis* antibodies. 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 (Tables 5 and 6). This could be explained by the fact that such reproductive signs are typical outcomes of brucellosis (52–54). As it is described by Holst *et al.* (55), at the time of 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 Adedoyin *et al.* (38). They found higher seropositivity in those with history of infertility (OR = 2.62 CI: 1.41–4.84). Besides, Gyuranecz *et al.* (56) had similarly reported.

The present study concluded that the overall prevalence of canine brucellosis was found 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, respondents had reported abortion cases and the disease had strongly associated with history of reproductive problems. 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.

# Abbreviations

RBPT

Rose Bengal Plate Test, CFT: Complement Fixation Test

# Declarations

## Ethics approval and consent to participate

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 with Reference No.VM/ERC/05/10/018,03/01/2018. The oral-only consent from the owners was also approved by the given legal ethics committee.

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## Author Contributions

The idea was conceived, designed and data collected by AG, AS. The experiments were performed, analyzed and written by AG, AS and SB. All the co-authors read and approved the final manuscript for important intellectual content.

## Competing interests

The authors have declared that no interests exist.

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## Consent for publication

Not Applicable

## Availability of data and materials

All data available on the main paper and raw datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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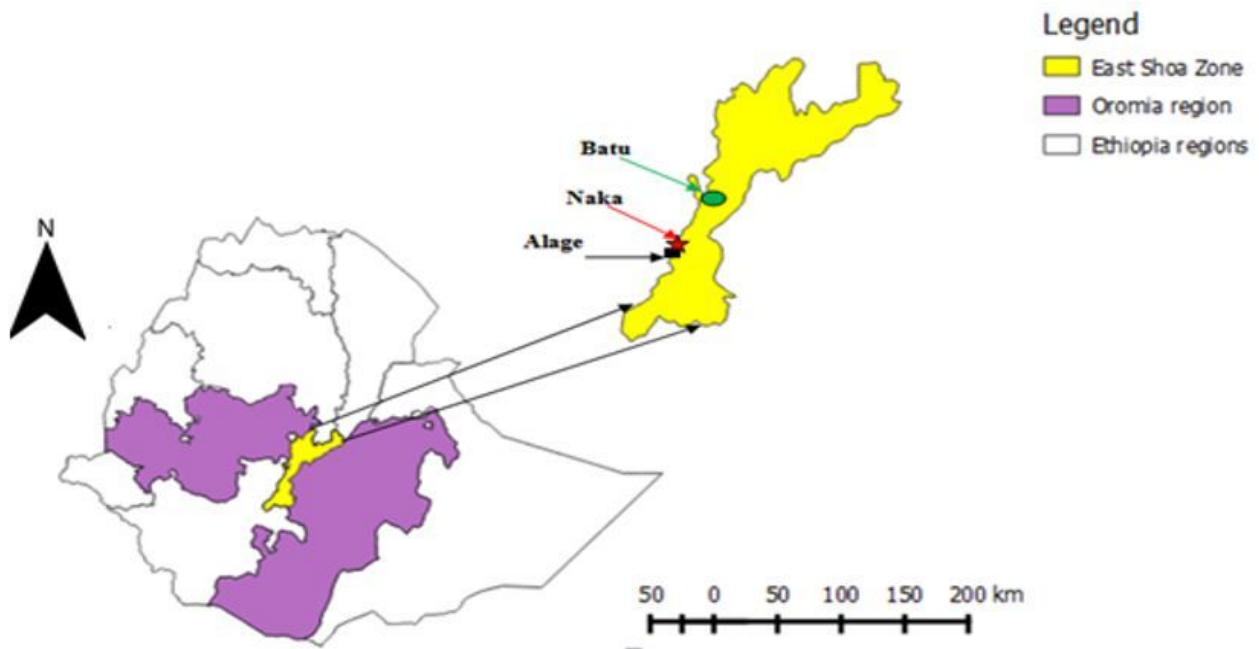
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## Figures



**Figure 1**

Map showing the study areas