Seroprevalence and Risk Factors of Brucellosis among Camels Belonging to Selected Districts of Afar, Ethiopia: Need for Public Awareness

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Abstract Brucellosis is a highly infectious bacterial disease of global significance, which affects humans as well as a wide variety of animals. A cross-sectional study was conducted in three selected districts of Afar region of Ethiopia to determine the seroprevalence of camel brucellosis. A total of 245 camels from selected districts were included in the study. All serum samples were tested and screened serologically using Rose Bengal plate test and confirmed using a complement fixation test. Associated risk factor analysis was also conducted using chi-square and logistic regression analysis. As a result, 30 (12.2%) were Rose Bengal plate test reactors in which 10 (4.1%) were confirmed to be positive using complement fixation test. The statistical analysis indicated that herd size ($\chi2$=13.206; p=0.001 and OR=17.053; 95% CI=1.988-146.254), contact with other ruminants ($\chi2$=13.524; p=0.00 and OR=12.774; 95% CI=1.560-104.625) and history of abortion ($\chi2$=28.022; p=0.00 and OR=38.000; 95% CI=4.694-307.657) were statistically significant and the major risk factors for the presence and transmission of the disease between animals. But age, body condition score, physiological status, parity and geographical location were found statistically insignificant (P>0.05). Public awareness towards the diseases was interviewed with the structured questionnaire format and it was noted that most of the pastoralists had no knowledge about zoonotic disease transmission, consequences of consuming raw milk, and handling aborted animals without any protective material. In general, camel brucellosis is prevalent in this area of study and public awareness towards zoonotic importance is low. Therefore, fruitful and sustainable work is required from the government, animal health professionals, and other stakeholders in the prevention and control of the disease. Improved public awareness is inevitable to reduce the prevalence and spread of the camel brucellosis.

Keywords: camel brucellosis, complement fixation test, pastoralist, risk factors, rose bengal plate test, seroprevalence, zoonotic importance

Cite This Article: Fikru Gizaw, Gizachew Fentahun, Semu Mersha, Hailegebriel Bedada, Mahendra Pal, and Venkataramana Kandi, "Seroprevalence and Risk Factors of Brucellosis among Camels Belonging to Selected Districts of Afar, Ethiopia: Need for Public Awareness." *American Journal of Microbiological Research*, vol. 5, no. 5 (2017): 94-100. doi: 10.12691/ajmr-5-5-1.

1. Introduction

Pastoralism is extensively practiced in hot, arid and semi-arid desert regions of Ethiopia, and Ethiopian farmers greatly rely on local livestock for their livelihood [1]. Camels are an important livestock species in the arid and semi-arid zones in Africa and Asia that accounts for 60% of the land area in Ethiopia and inhabited by pastoralists. 95% of the 18.9 million camel populations of the world are dromedary camels, which live in Africa, the Middle East and 5% are Bactrian camels residing in central Asia, China, and Mongolia [2]. 82% (15.4 million) of the world's camel population is found in the African continent. The camel population of Ethiopia has been estimated to be 2.3 million heads, which places it 3rd in the world next to Somalia and Sudan [2]. Camels contribute significantly to the livelihood of the pastoralists and agro-pastoralists living in the fragile environments of deserts of Africa and Asia as sources of milk, meat, transportation, leather and wool [3].

Camel production could be a profitable venture for utilizing the vast arid and semi-arid areas of Ethiopia, where other animals survive with difficulty, especially due to the recurring drought conditions. Under such environmental conditions, camels thrive and form a source of milk and meat. But, complete exploration of camels for milk and meat production would only be possible when their reproductive performance is properly understood and improved. Unfortunately, dromedaries are reported to have low reproductive efficiency compared to other domestic species [4].

Brucellosis is a re-emerging bacterial zoonotic disease of high economic and public health importance, prevalent throughout the world [5,6]. Brucellosis is still endemic in countries of the Mediterranean basin, the Middle East and Central Asia [5,7]. Camel brucellosis is widely prevalent among African countries such as Ethiopia, Eritera, Somalia and Sudan. Brucellosis is caused by Gram-negative coccobacilli belonging to the genus *Brucella*. They are short, non-motile, aerobic, facultative intracellular bacteria [8]. Previous research has revealed that the ability of *Brucella* to replicate and persist in host cells could be directly associated with its capacity to cause persistent disease and to circumvent innate and adaptive immunity [9].

In the pastoral and agro-pastoral areas of Ethiopia, brucellosis has been noted to be endemic causing infections in cattle, camels and other small ruminants [10,11,12]. Although endemic in nature in many developing countries, brucellosis remains under diagnosed and under-reported [13]. Prevalence of brucellosis in Ethiopian camels has not been extensively investigated in comparison with those of other domesticated animal species. It was observed that brucellosis can be transmitted among cattle, sheep, goats, camels and other species [7]. Camels were found to be at greater risk of developing brucellosis, especially when they were in contact with infected large and small ruminants [7].

Brucellosis can generally cause significant loss of productivity in camels through late first calving age, long calving interval time, low herd fertility and comparatively low milk production [7, 14]. Moreover, it should be noted that human brucellosis represents a major public health hazard, which can affect social and economic development in various countries. Human brucellosis is commonly referred to as undulant fever or Malta fever or Mediterranean fever [8]. It is an important occupational zoonosis of livestock handlers, abattoir workers, veterinarians and dairy farmers [12,15]. Currently, over five-lakh cases of human brucellosis are reported annually the world wide [13].

In Ethiopia, various surveys have been carried out on the prevalence of brucellosis on livestock in different regions of the country by various investigations. However, the detailed status of brucellosis and its associated risk factors are not thoroughly studied in camels' particularly in Afar regional state and information is so far scanty. Therefore, the objectives of this study were to determine the seroprevalence of camel brucellosis, to assess the associated risk factors for camel brucellosis and to also to know the public awareness towards zoonotic importance of diseases in the study area.

2. Materials and Methods

2.1. Study Area

Afar regional state is in the Great Rift Valley, comprising semi-arid range land in Northeastern Ethiopia. According to regional estimates, the livestock population of Afar is about 10.12 million and out of this, comprising of 2336488 heads of cattle, 4267969 goats, 2463632 sheep, 852016 camels and 187287 equines that support the region and contributes to the national economy [16].The annual temperature and rainfall in the region is 30-50 ^{0}C and 200-600mm, respectively. The altitude of the region ranges from 100-1000 meter above sea level. It is located at 80 40' 13" to 140 27' 29" N and 390 51' 13" to 420 23' 03" E latitude and longitude, respectively. The Afar Regional State has five administrative zones, which are further subdivided into 32 districts. Pastoralism and agro-pastoralism are the two major livelihood ways practiced in the region. The population of the region is estimated to be about 1.5 million of which 90% are pastoralists and 10% agro-pastoral [17].
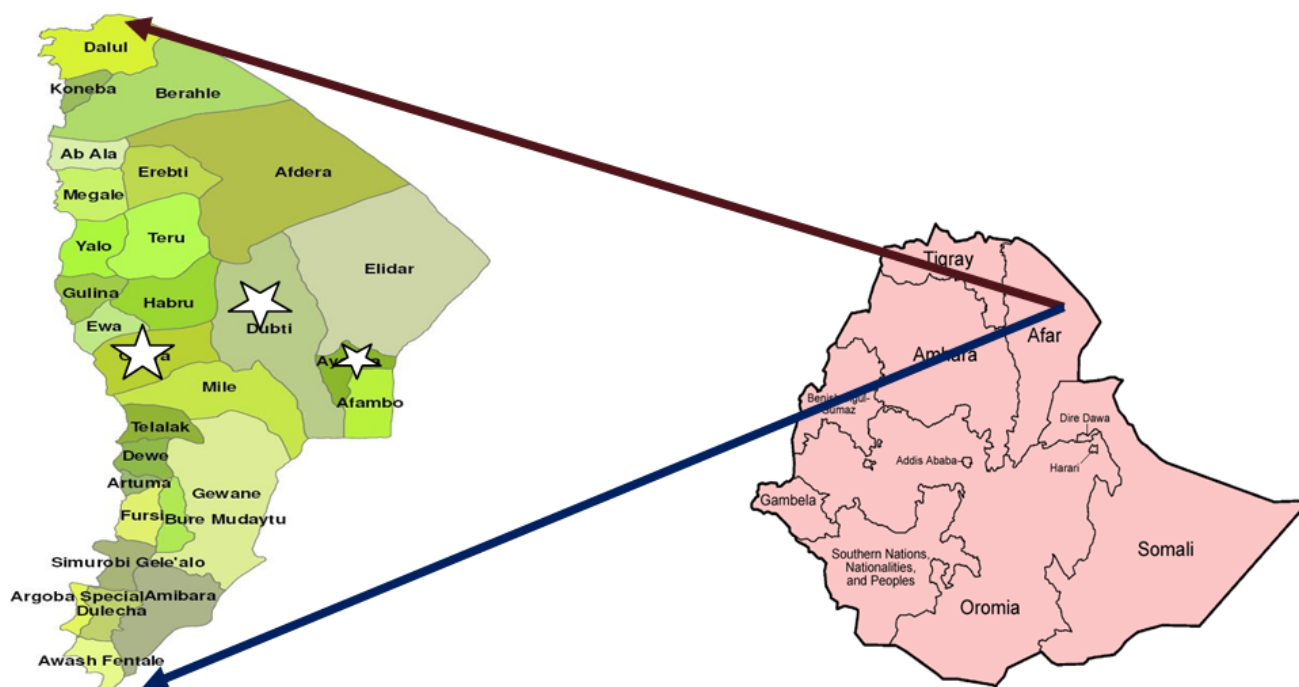

Figure 1. Geographical map of the selected study district in afar regional state, Ethiopia [17]

2.2. Study Animals

The study populations were dromedary type (*Camelus dromedaries*) camels kept under extensive husbandry which allows free browsing. Camel's age, herd size, body condition score, herd composition, physiological status, history of abortion and parity were recorded.

2.3. Sampling Method and Sample Size Determination

Camels were sampled using a combination of multistage and simple random sampling to select peasant associations (PAs), villages and herd. The zone and woredas (they are the third-level administrative divisions of Ethiopia) were selected purposefully based on camel abortion outbreak history, camel population, and willingness of pastoralists and accessible to vehicles. Three districts (Aysaita, Chifra and Dubti) from the zone, four PAs from the woredas, and three herds from each PAs and maximum ten (proportional allocation) camel from each respective herd was included as the study population. The total numbers of camels were proportionally sampled from all districts. Accordingly, a total of 245 camels (100 camels from Chifra, 87 camels from Aysaita and 58 camels from Dubti) were included in this study.

The sample size for this study is determined by the following formula given by Thrusfield (2007) [18]. $n= [1.96^2 \, Pexp \, (1-Pexp)]/d^2$, Where: n= sample size, Pexp= minimum expected prevalence, 1.96= the value of Z at 95% confidence interval d= desired accuracy level at 95% confidence interval. Therefore, by using the above formula and taking the previous prevalence of 4.1%, the minimum sample size at 95% confidence interval and at 5% precision or accuracy level, the sample size was calculated to be 61 [6]. This study included 245 samples, around four times more than the calculated sample size to increase the significance.

2.4. Study Design

A cross-sectional study design was conducted from November 2016 to April 2017 by using serological tests, the Rose Bengal plate test and complement fixation test to determine the prevalence of *Brucella* infection in camels in the selected districts and to identify the potential risk factors associated with the sero-positivity.

2.5. Study Protocol

2.5.1. Blood Sample Collection

Approximately 6 to 8 ml of blood sample was collected from the jugular vein of each camel using plain vacutainer tubes. The collected blood samples could clot at room temperature and serum was separated from clotted blood by decanting to plastic cryo-vials. A separated serum was stored at –20°C for further serological testing in the National Veterinary Institute (NVI).

2.5.2. Serological Test

Two types of serological tests were employed as a screening and confirmatory test for the detection of *Brucella* antibody: The Rose Bengal Plate Test (RBPT) and the Complement Fixation Test (CFT), respectively.

Figure 2. Images of NVI Serology laboratory

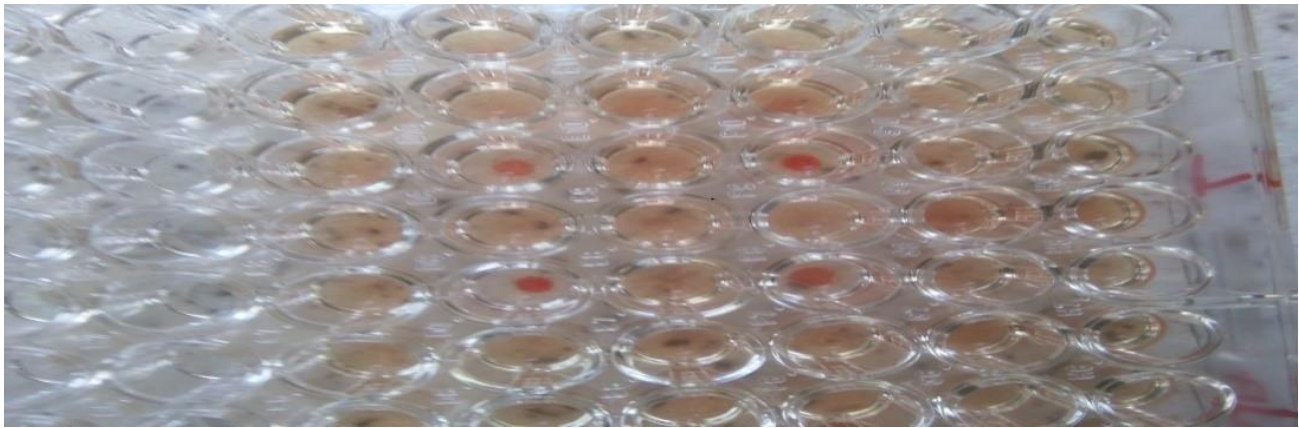**Figure 3.** Image showing rose Bengal precipitation test (RBT) result

Figure 4. Image showing complement fixation test result

2.5.2.1. Rose Bengal Plate Test (RBPT)

The RBPT is a rapid agglutination test effective in diagnosing brucellosis. It is used as a screening test on the serum samples collected for the presence of *Brucella* agglutinins. The results were interpreted according to the presence and degree of agglutination. Samples with no agglutination (0) were recorded as negative, while those with + (weak reactive), ++ (reactive), and +++ (strong reactive) were recorded as positive as shown in Figure 3. The test was conducted as described previously by other authors [19].

2.5.2.2 Complement Fixation Test (CFT)

Sera tested positive by RBPT was further confirmed using CFT. The complement systems consist of a series of protein that, if triggered by an antigen antibody complex, react in a sequential manner to cause cell lysis. The test has two steps. The first step is antigen; test serum and complement are mixed and incubated. The second step is an indicator system that consists of sheep red blood cells (SRBC) and an amboceptor that sensitizes red blood cells to the action of the complement. If the test serum contains antibodies to *Brucella*, an antigen-antibody complex is formed; the complement is used up, and no lysis of SRBC occurs. If the test serum does not contain *Brucella* antibodies (negative reaction), the complement will not be fixed and lysis of CRBC would occur as shown in Figure 4 [20].

2.5.2.3. Questionnaire Survey

Verbal consent was obtained from the respondents, and the objectives of the survey were explained to them before the start of the interview. The interviews were conducted in the local language (Afarigna). Two questionnaire formats, one for the serum sampled individual animal history and the other with a structured questionnaire format for the herders, was developed and used in this study. The questionnaire focused on animal husbandry and housing practices, knowledge about zoonotic diseases, the habits of animal product consumption and handling, and dead-animal/aborted fetus disposal practices. In total, 45 pastoralists 15 from each district whose animals tested for brucellosis were interviewed. In doing so, the risk factors that have possible associations with the occurrence of brucellosis were investigated and used to support the serological results. The collected data were tabulated, classified and further categorized for systematic and suitable statistical analysis.

2.6. Data Analysis

The data collected through questionnaires and laboratory results of the collected samples were entered into Excel databases and analyze using the SPSS software package (SPSS 20.0 for Windows 7, SPSS Inc, Chicago, Illinois). Descriptive statistics such as percentages, proportions and frequency distributions were applied to compute some of the data or were used to describe the nature and the characteristics of the data. The seroprevalence was calculated as the number of serologically positive samples divided by the total number of samples tested.

The difference between the effects of some risk factors on prevalence was analyzed using the Pearson chi-square (χ^2) test. Logistic regression analyses were used to assess the strength of association and were also calculated to quantify the association of different risk factors with the prevalence of Brucellosis disease. A statistically significant association between variables was said to exist if the calculated P-value is less than 0.05 and if the 95% confidence interval (CI) for OR doesn't include 1.

3. Results

3.1. Prevalence and Distribution of Camel Brucellosis in Zone 1 Afar

In this study, out of 245 camels examined 30 (12.2%) were positive on RBPT screening test. but, on further confirmation by CFT only 10 camels (4.1%) were seropositve to *Brucella* infection. Therefore, the true prevalence of camel brucellosis in the selected pastoral area was 4.1% (Table 1).

3.2. Association between Some of the Risk Factors WITH Occurrence of Brucellosis

The prevalence of brucellosis seems higher in Dubti (6.9%) than Assyita (3.4%) and Chifra (3.0%) but the difference was statistically insignificant (χ^2=1.562; P=0.458) (Table 2). Table 2 shows that risk factor at animal level herd size, herd composition and history abortion were found statistically significant (χ^2=13.206; p=0.001, χ^2=13.524; p=0.00, χ^2=28.022; p=0.00, respectively) while age, body condition score, physiological status, parity and geographical location were found not statistically significant.

Table 1. Prevalence and distribution of camel brucellosis in Zone 1 Afar

District	No.tested camel	Positive (inRBPT)	prevalence	Positive (in CFT)	prevalence
Dubti	58	9	15.5%	4	6.9%
Chifra	100	11	11.0%	3	3.0%
Assayita	87	10	11.5%	3	3.4%
Total	245	30	12.2%	10	4.1%

Table 2. Association between some of the risk factors with occurrence of brucellosis

Variables	Category	No. tested	Positive (%) RBPT	Positive (%)CFT	X^2	p-value	OR(95%CI)
District	Dubti	58	9(15.5%)	4(6.9%)			
	Chifra	100	11(11.0%)	3(3.0%)	1.562	0.458	
	Assaiyta	87	10(11.5%)	3(3.4%)			
Age	Young(< 4years)	32	3(10%)	1(3.1%)			
	Adult(4-10 years)	179	21(11.7%)	6(3.4%)	2.271	0.321	
	Old(>10years	34	6(17.6%)	3(8.8%)			
Body condition	Poor	81	13(16.0%)	4(4.9%)			
	Medium	80	8(12.0%)	3(3.8%)	0.228	0.892	
	Good	81	7(8.6%)	3(3.7%)			
Parity	No parity	32	3(9.4%)	1(3.1%)			
	1st parity	61	5(8.2%)	1(1.6%)			
	2nd parity	57	5(8.8%)	2(3.5%)	2.983	0.561	
	3rd parity	60	11(18.3%)	3(5.0%)			
	4th and above	35	6(17.1%)	3(8.6%)			
Herd size	<20	108	10(9.3%)	1(0.9%)			3.640(0.372-35.609)
	21-30	93	7(7.5%)	3(3.3%)	13.20	0.001	17.053(1.988-146.254)
	>30	44	13(29.5%)	6(13.6%)			
Herd composition	Camel alone	99	4(4.0%)	1(1.0%)			1.338(0.082-21.741)
	Camel and bovine	75	4(5.3%)	1(1.3%)	13.52	0.001	12.774(1.560-104.625)
	Camel, bovine and small ruminant	71	22(31.3%)	8(11.4%)			
History of abortion	No	191	8(4.2%)	1(0.5%)	28.02	0.000	38.000(4.694-307.657)
	Yes	54	22(40.7%)	9(16.7%)			
Physiological status	Pregnant	73	9(12.3%)	4(5.5%)			
	Dry	101	14(13.9%)	2(2.0%)	1.940	0.379	
	lactating	71	7(9.9%)	4(5.6%)			

Prevalence of camel brucellosis in animal level and associated risk factors were analyzed by using chi-square test(X^2) and binary logistic regression test analysis for statistically significant variables to measure the degree of association as shown in Table 2.

Camels kept in herds which had >30 camels were 17.053 times (OR=17.053; 95% CI=1.988-146.254) higher at risk for *Brucella* infection than the herd's size of <20 camels. Camels, which had kept together with small and large ruminants were12.774 times (OR=12.774; 95% CI=1.560-104.625) higher at risk for *Brucella* infection than camel alone. According to camel herder's information, she-camels had a history of abortion were 38.000 times (OR=38.000; 95% CI=4.694-307.657) higher at risk of contracting *Brucella* infection than she camels with no history of abortion.

3.3. Results of Questionnaire Survey

Forty-five animal owners were interviewed as input for serological testing sample and assessing public awareness towards diseases and zoonotic importance with associated risk factors. It was observed that 80% Afar pastoralists kept different species of animal along with a camel. Moreover, the mixing of the different species during migration, at watering or in night enclosures (resting), between camels and small ruminants was recorded. 100% of the respondents consumed raw milk and in the Afar pastoralist traditionally, it is not applicable at all to boil camel milk, and all the 45 respondents said they never boiled camel milk. Ninety percent of the animal owners had no awareness about zoonotic importance. Abortion material and other excreta were handled with bare hands, and they do not destroy these materials.

4. Discussion

Previous serological surveys in Ethiopia showed that camel brucellosis is an endemic and widespread disease in Ethiopia [6,21,22,23,24,25]. In this study, the overall seroprevalence of camel brucellosis in selected districts recorded was 12.2% by the RBPT and 4.1% by CFT. The true prevalence of 4.1% recorded in the present study

agrees with the results recorded by Teshome *et al.* (2003) in Borena with a prevalence of 4.2% and Hadush *et al.* (2013) in the Afar with a prevalence of 4.1% [6,21]. However, the result of this study was higher than the observation recorded by Mohammed *et al.* (2011) in Dire Dawa (1.6%), Tilahun *et al.*, 2013 in Jijiga and Babile districts, Eastern Ethiopia (2.43%) and Zeru *et al.* (2016) in the Afar (2.09%) [23,25,26]. Our study result was noted to be lower than the observation by Zewolda and Wereta (2012) and Sisay and Mekonnen (2012) who reported a prevalence of 5.7% and 7.6%, respectively in different districts of Afar region [22,27]. Our results were found to be significantly lower when compared to a study by Musa *et al.*, 2008 in Darfur, Western Sudan, who reported a prevalence of 23.8% [28]. Such increased prevalence rates could be due to variations in topography, and management system. The results of the present study were between 1.6% and 7.6%, similar to those observed by other studies from Ethiopia. This may be due to the unpredictable nature of the environmental condition of pastoral and agro-pastoral area of arid, semi-arid, and drought resulted stress, and reoccurrence of disease contributes to fluctuation of prevalence.

Age was classified as young (< 4 years), adult (4-10years) and old (>10) according to most previous survey and based on sexual maturity with modification of addition old age by asking owners to see the distribution of diseases in each age group. Accordingly, young she-camels examined 32 with seropositivity of 1(3.1%), adult she-camels examined 179 with seropositivity of 6(3.4%) and old she-camels examined 34 with seropositivity of 3(8.8%). This indicates that more seropositivity to camel brucellosis was seen in adults and old than young camels as it is a disease of sexually matured animals. Hence, the presence of seropositive breeding males and she-camels were considered as risk factors playing a role in the transmission of the disease to other animals [29]. Although no statistically significant difference (P>0.05) was observed between each age groups, slightly higher seroprevalence was found in those groups with age of greater than 4 years than those groups with age of less than 4 years . Sexually matured animals are more prone to Brucella infection than sexually immature animals since sexually matured animals are at risk of infection and diseases transmission due to sexual mating and sugar erythritol development, which favors the multiplication of pathogen [7].

Body condition of the camels was considered in this study to see the distribution of the infection in different body condition scores since underfed animals are expected to have a poor body condition that is manifested by decreased immunity against various infections [7,30]. even though, in this study body condition score was statistically not significant (P>0.05), high seropositivity was found in camels with poor (4.9%) and medium(3.8%) body condition score than camels with good (3.7%) body condition score.

Herd size was highly statistically significant associated with brucellosis in camels (P=0.01). This is in accordance with the findings of Bekele (2004) and Zewolda and Wereta (2012) in Borena and Afar, respectively and Adugna and co-workers (2013) in Afar [22,29,31]. As herd size increases, the chance of contact between animals increases leading to more chances of infection particularly during calving and abortion.

Herd composition (contact with other ruminant) in the present study, there was highly statistically significant difference (P=0.001) in the prevalence of the disease in the camel population which had contact with other ruminants. It increases the chance of brucellosis transmission from other infected ruminants to dromedary camels. The results of our study goes parallel with the findings of Adugna *et al.*(2013) and Zeru *et al.* (2016) in Afar and Tassew and Kassahun, 2014 in Mehoni District, South Eastern Tigray, Ethiopia [23,24,29].

The seropositivity of she-camels with the history of parity with 3rd and 4th were 5% and 8.6%, respectively which is slightly higher than those with 2nd, 1st and no parturition (3.5%,1.6% and 3.1%) respectively. But here was no statistically significant association (P=0.561) between parity and the seroprevalence of the disease. Therefore, this is consistent with the previous study by Hadush *et al*. (2013); Zewolda and Wereta (2012) in Afar and Tassew and Kassahun, 2014 in Mehoni District, South Eastern Tigray, Ethiopia [6,22,24].

Physiological status showed no statistically significant difference (P=0.379) in this study and also in accordance with the findings of Hadush and co-investigators (2013) in Afar. In this study, lactating and pregnant camels were found higher (5.6% and 5.5% respectively) in seropositivity than dry (2%) consistent with scientific view since pregnant and lactating camels more prone to stress and immune suppressed than dry and concentration of sugar erythritol contributes to variation in seropositivity [7].

In this study history of abortion was concerned and found highly statistically significant difference (p=0.000) that having history abortion is 38 times more associated with the brucellosis than with no history of abortion this implies the most classical symptoms of brucellosis in camels is abortion [28]. This finding also in accordance with the finding of Zeru *et al.* (2016) in Afar, even though, the statistical significant difference is very high in this finding [23]. This happened due to selection of study districts with history abortion.

5. Conclusion and Recommendations

Brucellosis is a widespread zoonotic disease, with increased interest among veterinarians. It is closely associated with public health and economic concern in many developing countries including Ethiopia .The results of this study revealed that camel brucellosis is widespread and established in pastoral areas of Afar, including the three camel rearing districts. The associated risk factors contributing to the presence and transmission of the disease from animal to animal were age, body condition, herd size, contact with other ruminants, parity, history of abortion and physiological status. However, according to the statistical analysis, the major risk factors for the transmission of the disease from camel to camel included large herd size, contact between large and small ruminants and having history of abortion. Moreover, higher seropositivity was recorded in sexually mature and old, pluriparous, pregnant, and lactating camels which contributed for transmission of the disease. Poor management practices, mixing with other animals and unrestricted movement of camels were thought to support spread of the disease in the study area.

Lack of awareness about brucellosis together with existing habit of raw milk consumption and close contact with animals may exacerbate the zoonotic importance.

Therefore, based on the above conclusion we recommend that improved management practices and strategic plans should be developed to minimize the chance of contact between animals at different situations. Public health education should be given on modern animal husbandry and disease prevention techniques. Public awareness should be given on the economic and zoonotic importance of the disease. Further investigation on the zoonotic importance, economic impact and etiology of diseases to molecular level should be conducted. Adequate brucellosis control programs in small ruminants would contribute to the reduction of the disease prevalence in camels. Adequate veterinary service should be established in the lowland area pastoral community.

Acknowledgements

Conflict of Interest

Nil.

Research Grant

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