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Seroprevalence and Associated Risk Factors of Camel Brucellosis in Selected Pastoral Area of East Bale Zone, Oromia, Ethiopia

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Abstract: *Background and Aim*: Camel brucellosis is a contagious bacterial disease that hinders the productivity of camels, ruminant animals, humans and other suspectable animals. It has public healthy, veterinary and economic importance. However, the status and information of camel brucellosis in the East Bale pastoral area is unknown. *Materials and Method*: A cross-sectional study design was conducted from September 2020-2021 in the Rayitu, Sawena and Lega Hida districts of the Bale zone to estimate the seroprevalence and assess putative risk factors of camel brucellosis. A multi-stage clustering and random sampling technique were employed for the selection of sampling units. A total of 384 sera samples were harvested from blood samples collected by plain vacutainer tubes and tested by complement fixation test at the National Animal Health Diagnostic and Investigation Center laboratory, Sebeta. *Results*: Out of 384 examined sera 1.04% (4/384) were seropositive by Complement Fixation Test. The seroprevalence camel brucellosis was higher (X^2=3.537, P=0.049) in Rayitu (3/128, 2.34%) than Sawena (1/128, 0.78%) and was not recorded in Lega Hida districts (0/128, 0.0%). The history of abortion (X2=23.675, P=0.000) has a strong association with the seropositivity of camel brucellosis. *Conclusions*: The current study showed that camel brucellosis was evident in Rayitu and Sawena districts but was not recorded in other districts. Abortion history strongly associated infection of camel with brucella species. Further study should be designed and implemented to isolate and detect at the molecular level of Brucella strains of camels in the study area.

Key words: East Bale · Brucellosis · Camel · Seroprevalence

INTRODUCTION

Camels (*Camelus dromedaries)* had a great contribution in socio-economic within the pastoral and agricultural systems in dry and semi-dry areas of north-eastern, East, south-eastern and South parts of Ethiopia; namely Oromia (East and West Bale zones, Borana zone), Afar and Somali regional states [1]. Naturally, Camel is known to have specific anatomical and physiological features by which they regulate body temperature to changes with any change in the ambient temperatures, enabling them to survive and produce under severe environmental conditions [2]. Camels are the most important source of income for the nomadic population, milk, meat and hide production, transportation system, draught power and medication purposes [3].

Despite of its high productive potential, camels perform poorly in pastoral area. Management system, insufficient nutrients, slow production, retard reproduction and disease appear to be major constraints faced camels [4]. Also, desert and droughts enhanced stress and made camels more susceptible to many contagious and non-contagious diseases and illnesses. Among contagious and reproductive disease camel brucellosis is the bottle neck that hinder production and productivity [5]. It is a disease caused by the genus

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Brucella, especially species of *Brucella* (*B. abortus, B. melitensis* and *B. ovis*) [6, 7]. Brucella are Gram-negative facultative intracellular coccobacilli that are non-encapsulated, non-spore forming and non-motile belonging to the alpha-2 subdivision of the *Proteobacteria* [8]. *Brucella* species can enter into hosts (animals) through inhalation, ingestion, sexual contact and through the mucous membrane or broken skin [6]. According to different reports *Brucella abortus* and *Brucella melitensis* are the most frequently isolated *Brucella* species from milk, aborted fetal and vaginal swabs of suspected camels [9, 10]. In camel, it is characterized by abortion, infertility, still birth, placentitis, epididymitis and orchitis [11]. Since *Brucella* species favored to the reproductive organs due to the presence of erythritol sugar in the fetal tissues and uterus [12]. In Ethiopia, brucellosis is one of the notifiable diseases associated with reproductive problems in camel production in the pastoral areas. In camels, significant loss of productivity through delayed puberty and calving age, increased calving interval, infertility and decreasing milk production were recorded [13]. Additionally, this disease has imposed a restricted to livestock trade, the free movement animals and retards the exportation of animals. WHO, FAO and OIE ranks brucellosis as the third most important zoonotic disease in the world [14], due to raw milk consumption and contact with discharge of infected animals (animal health workers, butchers, farmers) management factors influencing inter-herd common grazing and watering with mixed ruminant animals, presence of scavengers, lack of vaccination, herd size, methods of housing and use of maternity pens had influenced the probability of exposure to the disease [15].

The seropositivity of camel brucellosis in Ethiopia has been reported, ranging from 0.73 to 11.9% by Rose Bengal Plate Test (RBPT) and 0.4 - 9.6% by complement fixation test (CFT) from different pastoral areas. For instance, it was 7.5% detected by Rose Bengal Plate Test-Enzyme-Linked Immunosorbent Assay (RBPT-ELISA) from both Afar and Somali regional states [16], was 0.9% in Southern Ethiopia [17], was 11.9% detected by RBPT and 7.6% detected by CFT in Afar region [18]; was 5.4% detected by modified Rose Bengal Plate Test (mRBPT) [19]; was 58% detected by RBPT and 47% detected by CFT [20]; was 2.09% detected by RBPT [21]. Somali, Afar and Oromia regional showed 8.2% detected by RBPT and 4.2% detected by CFT [22]. The RBPT detected higher percentage (6.5%) compared to CFT (4.5%) in samples collected from Akaki abattoir [13] and Dire Dawa abattoir 2% (RBPT) and 1.5% (CFT) [23]. In and around Jigjigja and Babile regions, RBPT detected lower cases 2.43% [24], compared to Mehoni district, South-East Tigray determined by either RBPT (5.78%) or CFT (3.56) [25] and Berbera quarantine determined by CFT (2.94%) [26]. These variations in seropositivity of camel brucellosis attributed to the difference in animal husbandry and management systems practiced by pastoral society, sex, age, climatic condition, absence of veterinary services and type of diagnostic methods performed [27].

The status and information of camel's brucellosis in East Bale zone is scarce. In this study area, the traditional habits of raw milk consumption, handling of aborted materials, methods of disposing aborted fetus, manipulation of reproductive excretions with bare hands and others were widely practiced, which enhance the diseases transmission. As the camel brucellosis has public and economic importance. Therefore, the objective of the current study was to estimate seroprevalence of brucellosis in camel and assess its putative risk factors in selected districts of East Bale zone pastoral area, Oromia reginal state, Ethiopia.

MATERIALS AND METHODS

Description of Study Area: This study was conducted in the East Bale zone of the Oromia Regional State Southeastern parts of Ethiopia which is situated 504.5 km South of Addis Ababa. It has mean annual rainfall of 300-800 mm and temperature from 27°C-30°C [28]. Extensive pastoral livestock production system was practiced and it is the basis of livelihood for pastoralists. The rainfall pattern is bimodal with erratic distribution; the main rainy season extends from March to end of June and the short rainy seasons usually extending from September to end of October. The production system in the woreda is pastoral [28]. Three districts were covered and selected purposively based on the basis of study population and accessible to transportation namely, Rayitu, Sawena and Lega Hida districts of East Bale zone pastoral area. The three districts were dominated by hot, dry climate and considered pastoral area. The area experience hot climatic condition with mean annual temperature of 26°C and a maximum of 40°C and the average annual rainfall is less than 300 mL [28].

Study Population: The study population was dromedary type (*Camelus dromedaries*) camels kept under extensive

pastoral production husbandry which allows free browsing. Camels above six months age, not vaccinated against *Brucella* species and both sexes were considered as the study population.

Study Design: A cross-sectional study design was conducted from September 2020 to September 2021 to estimate the seroprevalence brucellosis in the selected districts and assess its potential risk factors related with the seropositivity in the study area.

Sample Size Determination and Sampling Techniques: The study sample size was determined according to Thrusfield [29] formula for an infinite population with 95% confidence level, 5% desired absolute precision by considering expected seroprevalence brucellosis in camel. Accordingly, 384 camels were sampled.

$$N = (1.96)^2 * Pexp*(1- Pexp)/ d^2 \quad (1)$$

where N = sample size, d = desired absolute precision and Pex = expected prevalence; thus, the desired sample size for Pex = 0.5 was N (number of expected sample size) = 384.

Multi-stage cluster and sample random technique was conducted in the study area by considering peasant associations as primary units, camel herds found in each peasant associations as secondary units and selected camel herds as tertiary units. Camels with clusters were selected by simple random sampling strategy after assigning an identification number to each animal. The clustering of the peasant associations was based on accessibility to villages, transportation and camel population. Camels in the herds were divided into adult and young for the purpose of sampling. Early in the morning, herds were visited and samples were taken before they were released into the field. Finally, for the prevalence investigation, 384 camels >6 months, no vaccination history against *Brucella* species and both sexes were chosen from 48 different herds (128 camels from each district).

Sample and Data Collection: About 5mL of blood sample was collected from the jugular vein, using needle and plain vacutainer tube, from each camel and then brought to local laboratory in an icebox. At local laboratory, sera sample was carefully separated from blood samples that were kept overnight to clot at room temperature and put at slant position. The serum was harvested into cryovial and stored at -20°C until transported to the NAHDIC.

A semi-structured questionnaire was prepared in order to assess risk factors for brucellosis in camels. Information for each camel sampled was obtained: age, sex and history of abortion. The animals were classified into two age groups: < 4 years 'young' and > 4 years 'Adult' based on Megersa *et al.* [30].

Laboratory Analysis

Serological Test: Complement Fixation Test (CFT): All the reagents required for the complement fixation test were performed by titration according to the standards given by OIE [31]. A 2% suspension of sheep red blood cells were carefully prepared. The preparation of the reagents and the CFT procedure were conducted [32]. A serum giving 75% complement fixation test of the complement at a dilution ratio of 1:5 and above was taken as positive [33]. The CFT was performed at the National Animal Health Diagnostic and Investigation Center, Sebeta, Ethiopia.

Data Management and Analysis: Data were analyzed using IBM SPSS Statistics 20. Seroprevalence at 95% confidence intervals (CIs) were computed. Potential risk factors related to Brucella-seropositive cases at the individual animal level and pastoral area related risk factors were analyzed via chi-squared test with a 95% confidence interval and a statistical significance variable should take if $P<0.05$.

RESULTS

Overall Seropositivity of Brucellosis in Camel: Out of 384 tested sera samples, 1.04% (4/384) were found seropositive to camel brucellosis by complement fixation test (Table 1).

Animal and Pastoral Area Related Risk Factors Seropositivity of Camel Brucellosis: A higher prevalence was observed in the Rayitu district compared to other districts (Table 1) and the difference was statistically significant ($p<0.05$). Regarding sex of camel's females were more effected than male camels, similarly age of camel adults more affected than young camel (Table 1). Besides history of abortion of camels, camels those had history of abortion showed higher seroprevalence was observed and the difference was statistically significant ($X^2= 23.675$, *P*-value = 0.000; Table 1).

Table 1: Overall camel brucellosis seroprevalence and associated putative risk factors

Variables	Categories	No. sera Tested (camel)	No. sera Positive (%)	X^2 (95%, CI)	P-value
Districts	Rayitu	128	3(2.34%)	3.537	0.049*
	Sawena	128	1(0.78%)		
	Lega Hida	128	0(0.00%)		
	Total	384	4(1.04%)		
Sex	Male	89	0(0.00%)	1.219	0.347
	Female	295	4(1.36%)		
	Total	384	4(1.04%)		
Age	Young	60	0(0.00%)	0.749	0.505
	Adult	324	4(1.23%)		
	Total	384	4(1.04%)		
Abortion history	No	239	0(0.00%)	23.675	0.000*
	Yes	56	4(7.14%)		
	Total	295	4(1.36%)		

*= Statistically Significant X^2 = Chi-Square CI= Confidence Intervals

DISCUSSIONS

Even if camel has yet no own standard set for the diagnostic test protocol and diagnostic titration for brucellosis; however, OIE recommends the diagnostic test should follow the process outline Bovine brucellosis techniques, complement fixation test for confirmation [22, 31]. In the current study all 384 camels sampled from Rayitu, Sawena and Lega Hida districts of East Bale zone Oromia reginal state were clinically normal during sampling and the owners also confirmed that previously they had no shown clinical signs of brucellosis and none vaccinated for brucellosis.

The overall seropositivity of brucellosis in the study area was 1.04%, which indicates the apparently healthy camels might be silent carriers of brucellosis and their products may cause public health, veterinary and production losses. This finding was in line with results of Gumi *et al.* [17] 0.9% reported the seropositivity of camel brucellosis from Borena Ethiopia pastoral area. The findings lower than the current finding were 0.4% Bekele [34] and 0.53% from Borena Ethiopia pastoral area [34]. The findings higher than the current finding were 4.5% seropositivity [13] and 4.2% seropositivity [22] reported from Akaki abattoir, 1.8% from Borena zone [30] and 4.1% [18] and 7.5% [20] seropositivity reported from Afar region, 1.53% from Fafen Zone, Somali Regional State [35], 7.5% from Afar and Somali regional state [16]. Also, the reports from neighboring countries like 2-15.4% in Kenya [36], 19.4% in Jordan[37], 30.15% from Sudan [38], 3.1% in Eritrea [39] and 31.01% from Egypt [40].

The difference reports in the seropositivity of brucellosis in camel between the current and previous studies might be due to agroecological differences of study areas, sample size, herd size, animal management, animal compositions, presence and absence of infectious foci (*Brucella* infected herd), sensitivity of diagnostic methods, nutrition status, body condition, immunity status of animals, sample selection biased and production systems [41].

The higher seroprevalence of brucellosis in camel's female (1.36%), recorded in the current study is in agreement with previous studies conducted in various parts of Ethiopia [17, 22]. On the contrary, Bekele *et al.* [19, 24] reported high seroprevalence of brucellosis in male camels, in Afar and Somali region, respectively. The difference in sex susceptibly for brucellosis due to physiological and behavioral differences [41], the relaxation of immunity associated to pregnancy stress or the presence of erythritol and hormonal which stimulate the growth and propagation of *Brucella* species in reproductive tract of female camels [42]. Furthermore, the increase in concentration of erythritol sugar and different sex hormones with age and sexual maturity, as stated by Radostits *et al.* [41], which might be support the higher seroprevalence (1.36%) recorded in female camels in the current study.

Brucellosis may occur in animals of all age groups, but persists commonly in sexually mature animals of both sexes [41]. This study showed that a higher seropositivity at adult age groups (1.23%), than in the young age groups. This is agree with the observation Scholar, *et al.* [43] who reported a significantly higher occurrence of *Brucella* species in adult camels than young camels from Mehoni district, south-eastern of Tigray region. Dawood [37] and Zewold and Haileselassie [18] stated higher prevalence of brucellosis in adult than in young camels in southern province of Jordan and Afar region in Ethiopia respectively. Madu, *et al*. [44] who reported higher in adult than young camels age groups in three abattoirs

from northern Nigeria. Since, young animals tend to be more resistant to infection and frequently clear infections although few latent infections may occur according to the report of Nowak, *et al.* [45]. The presence of growth factors (erythritol and hormones) favor infections in sexually mature animals [46].

Statistically significant difference in seroprevalence abortion history (Yes: X^2= 23.675, P<0.05), observed in the present study, between female camels with (Yes) and without the history of abortion (No), supports and confirms the fact that brucellosis is one cause of reproductive health problems [47].

CONCLUSION

In the current study, complement fixation test confirmed that camel brucellosis is prevalent among camel managed under extensive pastoral production systems in Rayitu and Sawena districts of East Bale zone pastoral area Oromia Regional State, Ethiopia. Associate risk variables like districts and previous abortion history were showed a strong association with the seropositivity camel brucellosis. So, further study should be designed and implemented at a wider range on isolation and molecular characterization of the *Brucella* species.

Data Sharing Statement: The data used to validate the results of this analysis are available from the first and correspondent authors upon reasonable request.

Ethical Clearance: The best practice guidelines for veterinary care were applied in our study, camel owners were verbal informed and aware about the purpose of the study, blood samples collected and sera were prepared under aseptic conditions by experienced veterinary laboratory technicians without endangering the life the animals. So, ethical clearance was approved by the Animal Research Scientific and Ethics Review Committee of the National Animal Health Diagnostic and Investigation Center, Sebeta, Ethiopia (National referral and reference laboratory).

Consent to Participate

Not Applicable: This manuscript paper didn't include any personal data, images, videos and others that can violate individual participants.

Author's Contributions: All authors made major contributions to the entire research activities, including proposing the project, implementing, collecting data, analyzing and interpreting the manuscript.

Competing Interest: All authors declare they have no competing interests for this work.

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