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Research Article

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Seroprevalence of Brucellosis in Camels *(Camelus dromedaries)* in South East Ethiopia

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Abstract

The present study was delineated to investigate the seroprevalence and risk factors of brucellosis in camels at Adama town brought for export purposes from Bale and Borena zones of Oromia region, Ethiopia. Also questionnaire survey was conducted among camel owners to assess the knowledge-attitude-practice (KAP) among these farmers towards brucellosis. A total of 1500 camel blood sera were randomly collected in the period from December, 2011 to April, 2012. Two serological tests were used to screen all serum samples, Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT). Multivariate logistic regression was constructed to study the risk factors associated with *Brucella* seropositive cases. Based on CFT, the estimated overall survey adjusted true animal level seroprevalence was 0.53% (95% CI: 0.2- 1.05). The sensitivity and specificity of RBPT was 100% (95% CI: 62.91- 100.00) and 99.80% (95% CI: 99.41- 99.96) respectively. The kappa statistics showed that there was almost perfect agreement between RBPT and CFT (k=0.8411).Multivariate logistic regression on animal level showed that rearing with other ruminants was the only potential risk factor (χ^2=8.3, p< 0.05). Questionnaire survey among the camel owners established that raw camel milk was consumed by 78% of the respondents, 3.3% of the respondents confirmed of consuming raw liver and hump of camel. Almost one fourth of interviewees (23.33%) knew of the disease brucellosis and only 19% knew that camel can transmit brucellosis to humans. The results of the present investigation indicate that *Brucella* spp. exists within the camels in Oromia region. Coordinated nationwide epidemiological surveillance in camel and other ruminants is required together with typing of infecting strains, thus enabling the transmission dynamics to be elucidated and initiating immunization campaigns, public health education and eradication strategies.

Keywords

Camel brucellosis; Seroprevalence; Risk factors; Oromia; Questionnaire

Introduction

The camel (*Camelus dromedarius*, one-humped camel) play an important socio-economic role within the pastoral and agricultural system in dry and semi dry zones of Asia and Africa [1]. Camels are a subset of huge livestock resources in Ethiopia with the population estimated to 2.3 million [2]. This number ranks the country third in Africa after Somalia and Sudan and fourth in the world. In Ethiopia camels are reared in arid and semiarid areas in Borena, Somali, and Afar regions by pastoralists and agro-pastoralists [3].Camels in the Oromia region are mainly kept for milk and meat production and transportation system. Camels like other domestic animals are susceptible to different diseases including brucellosis [1].

In Ethiopia, brucellosis has been reported in camels by various workers [4-9]. Camels are not known to be primary hosts of Brucella, but they are susceptible to *B. abortus, B. melitensis* and *Brucella ovis* [10]. Consequently, the prevalence depends upon the infection rate in primary hosts being in contact with them [11]. Infected animals show clinical signs of abortion and stillbirth in female and orchitis and epididymitis in male animals and infertility in both cases [12].

The economic and public health impact of brucellosis remains of concern in developing countries [13]. In Ethiopia, annual losses from brucellosis among cattle have been estimated to be 88,941.96 ETB at Chaffa State Farm Wollo [14]. However no information is available regarding economic losses due to camel brucellosis. The disease can generally cause significant loss of productivity through late first calving age, long calving interval time, low herd fertility and comparatively low milk production in camels. The disease poses a barrier to export and import of animals constraining livestock trade and is an impediment to free animal movement [15]. *B. melitensis* is considered to have the highest zoonotic potential, followed by *B. abortus*, and *B. suis*. The disease presents as an acute or persistent febrile illness with a diversity of clinical manifestations in humans [16]. Brucellosis is transmitted to humans mainly by direct contact with infected livestock and the consumption of unpasteurized contaminated milk and dairy products [11].

Difficulties can arise in diagnosis of camel brucellosis, especially as this disease provokes only few clinical signs in contrast to its clinical course in cattle [17]. Rose Bengal Plate Test (RBPT) is widely used as a screening test [18] in Ethiopia for diagnosis of the acute and chronic forms of the brucellosis [19]. Due to high specificity of Complement Fixation Test (CFT) than any other conventional test, it has been recognized as a confirmatory serological test for brucellosis [20]. This test is a "prescribed test for trade" by the OIE [21]. The epidemiology of brucellosis in cattle and small ruminants in different geographical areas has been investigated extensively. In spite of its vital importance, studies on this disease in camels are limited particularly in Oromia region. The aims of the present study were to estimate the seroprevalence of camel brucellosis in Adama Town (originated from Borena and Bale) by using serological tests RBPT and CFT and to elucidate risk factors associated with it and to assess the knowledge-attitude-practice (KAP) among camel owners towards brucellosis.

Material and Methods

Study areas

A cross-sectional study was carried out from December, 2010 to April, 2011 in Adama Town of eastern Showa zone of the Oromia region, Ethiopia. Oromia represents the largest regional state

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covering 32% area of the country. According to regional estimates, the camel population in Oromia is 139,830 which represent 30.6% of Ethiopia's total camel population. The Oromia Regional State has 14 administrative zones, which are further sub-divided into 192 wards as (districts). Of the 14 zones, Bale and Borena account for 45.7% of the state's area. Pastoralism and agropastoralism are the two major livelihood ways practiced in the region. The camels originated from Borena and Bale zones of Oromia region and were brought to Adama for export purpose. Adama is located at a distance of 99 Kms South East of Addis Ababa at a longitude of 39.7°N and 8.33°Eand with an altitude of 1622 meters above sea level. The town gets an annual rain fall of 400-800 mm and the mean annual temperature is about 21°C [22]. Borena, the origin of study animals is situated at 600 km south of Addis Ababa on altitudes ranging from 500 to 2500 meters above sea level. The climate of Borena is semi arid. It has an annual rainfall of 450-650 mm in bimodal pattern with long rainy season between March and May and the short rainy season between October and November. The mean annual temperature varies from 19°C to 25°C with moderate seasonal variation [22]. Bale, the other origin of study animals is situated 430 km South of Addis Ababa located on altitude of 2500 meters above sea level. It has mean annual rainfall of 300–800 mm and the mean annual temperature varies from 27°C to 30°C [22]. Extensive pastoral livestock production is the main system and the basis of livelihood for millions of pastoralists in the study area.

Study animals

The study was carried out on 1500 apparently healthy one humped male camels (*Camelus dromedarius*) which were designated for export. Of the camels, 1149 were brought from Borena and 351 from Bale areas to Adama feedlot at different times during the period from December 2011 to April 2012. The animals were given numbers for the purpose of identification as soon as they reach the feedlot. They were fed on green grass, hay, and wheat bran and had free access to water. Since the animals were brought for export purposes, all were clinically healthy and in good body condition.

The animals were not vaccinated against brucellosis. They were allotted to three groups according to their ages based on Abebe et al. [23]. Group 1 (G 1) consisted camels 1 to 3 years old (375 camels), group 2 (G 2) 3 to 6 years old (937 camels), group 3 (G 3) 6 and above years old (188 camels). Serum samples were collected for serological examination from selected camels; information of each camel sampled was recorded including its location, age, physiological status and co-existence with other ruminants.

Sample size: The sample size of the study animals were determined by using the formula given for simple random sampling methods [24]

$n = 1.96^2 [p_{exp} (1-p_{exp})]/d^2$

Where:

n = Required sample size

P_{exp} = Expected prevalence of brucellosis (50%)

d = Desired absolute precision level at 95% confidence level (5%)

1.96 = The value of Z at 95% confidence level

Thus the desired sample size for P_{exp} = 0.5 is n = 384, however, 1500 camels have been included in the study to increase accuracy, representativeness and randomness in the study animals.

Blood sample collection: Blood samples were collected aseptically from either jugular vein or milk vein using disposable needles and vacutained tubes and then brought to local laboratory in an icebox. At local laboratory blood samples were kept overnight to clot at slant position at room temperature. Then the separated serum was carefully collected in cryovial without mixing with the clotted blood. The serum was stored at -20°C until further processing took place.

Serological examination: All the serum samples were screened by RBPT for the presence of Brucella agglutinin (Rose Bengal stained *B. abortus* antigen obtained from BIO-RAD, Marnes-la-Coquette, France) according to standard procedures described by Nilson and Dukan [25]. All sera samples were further tested using CFT as per the method described by Alton et al. [26]. *Brucella* serposiitve camels are camels with both positive RBPT and CFT results. Both tests were performed at National Veterinary Institute, Debreziet Ethiopia.

Questionnaire survey

A questionnaire survey was administered to one-hundred fifty willing respondents out of the total 600 camel owners whose camels were included in the study. The information gathered relates to livestock structure, composition of camel herds, camel rearing experience, camel management, milk consumption habits and purpose of camel rearing and knowledge about brucellosis and its zoonotic importance.

Data management and analysis

The data collected from field and serological test result were entered into Microsoft Excel spreadsheet and descriptive statistics were summarized. Animal-level prevalences were also calculated and were adjusted for the sensitivity and specificity of the serial testing system. True prevalence was then calculated using the formula of Rogan and Gladen [27]: TP = (AP + Sp – 1) / (Se + Sp – 1), where AP = apparent prevalence, Se = sensitivity of the test series, Sp = specificity of the test series.

Efficiency of routine RBPT to detect brucellosis in camels (with CFT as reference tests) was assessed based on computation of sensitivity, specificity, positive and negative predictive values. Further evaluation of the validity of RBPT procedure was carried out by computing the likelihood ratio (LR). The kappa test was used to assess the degree of agreement between the tests. All the necessary diagnostic statistical analyses were carried out using the on-line software SISA (Simple Interactive Statistical Analysis- Diagnostic statistics) [28]. Similarly, the pre-and posttest infection probabilities were illustrated graphically by Fagan's nomogram using diagnostic test calculator software [29]. The relationship of associated risk factor with positive serological test result was analyzed by logistic regression using Stata software (version 9). A test value was considered as statistically significant when $p \leq 0.05$.

Results

Brucella seroprevalence

1500 camels brought to Adama from Borena and Bale zones of Oromia region for export purposes were screened for brucellosis by RBPT and CFT serological tests. Out of total 1500 samples tested for RBPT, 11 (0.73%) were positive for brucellosis. Of these, 8 (72.73%) were confirmed to be seropositive for brucellosis upon further testing by CFT, giving 27.27% false positive (Table 1). Based on CFT, the estimated overall survey adjusted true animal level seroprevalence was 0.53% (95% CI: 0.2- 1.05) (Table 1). The sensitivity and specificity of

Table 1: Comparative analysis for the results of RBPT and CFT tests.

Test properties		CFT		
RBPT		+ve	-ve	Total
	+ve	8	3	11
	-ve	0	1489	1489
	Total	8	1492	
Sn[a](%)[95%CI]	100[62.91-100]			
Sp[b](%)[95%CI]	99.80[99.41-99.96]			
Kappa[c](%) 95%CI]	0.8411[0.81-.89]			
PLR[d][95%CI]	497.33[160.58-1540.29]			
NLR[e][95%CI]	0.00			
TP[f][95%CI]	0.53%[0.23-1.05]			
PPV[g][95%CI]	72.73%[39.08-93.65]			
NPV[h][95%CI]	100.00%[99.75-100.00]			

[a]Sensitivity of RBPT test with respect to CFT for detection of brucellosis in camels
[b]Specificity of RBPT test with respect to CFT for detection of brucellosis in camels
[c]Signifies degree of agreement between the different methods of detecting camel brucellosis
[d]Positive Likelihood Ratio
[e]Negative Likelihood Ratio
[f]True Prevalence
[g]Positive Predictive Value
[h]Negative Predictive Value

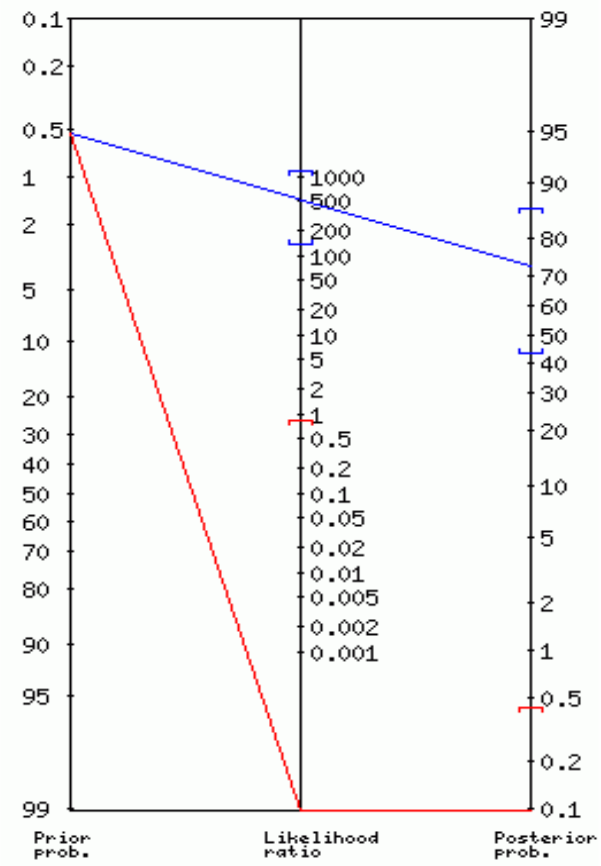

Figure 1: Fagan's nomogram for post-test probabilities of brucellosis occurrence in camels based on Rose Bengal Plate test (Comparison was made with Complement Fixation test).

Table 2: Associations with risk factors for *Brucella* seropositivity as screened by CFT.

Risk Factors		No. of camels tested	No. of positive camels (%)	X^2 & P values
Origin	Bale	351	2(0.6)	X^2=0.01, P=0.915
	Borena	1149	6(0.5)	
Age	1-3years (G1)	375	2(0.5)	X^2=0.00, P=1.00
	3-6years (G2)	937	5(0.5)	
	>6years (G3)	188	1(0.5)	
Contact	Reared with ruminants	1045	7(0.67)	X^2=8.3, P=0.03
	No contact	455	1(0.21)	

RBPT was 100% [95% CI: 62.91- 100.00] and 99.80% [95% CI: 99.41 to 99.96] respectively. The kappa statistics showed that there was almost perfect agreement between RBPT and CFT (k=0.8411).The positive likelihood value was 497.33[95% CI: 160.58 -1540.29], while negative likelihood value was 0.00.The positive predictive value was estimated to be 72.73% [95% CI: 39.08- 93.65]. Figure 1 shows nomographic depiction to estimate post-test probability of brucellosis from pre-test probability and likelihood ratio. The post-test probability of brucellosis (predictive value for positive result of RBPT) was found to decline to 72.73% when compared to Complement Fixation Test (CFT).

Risk factors

The univariable logistic analysis of the putative risk factors indicated no significant difference of brucellosis in camels from Bale (0.6%) and Borena zone (0.5%) (χ^2=0.011, p>0.05). Similarly there was no significant difference in seroprevalence of brucellosis between camels of different age groups (χ^2=0.130, p>0.05). However, statistically significant difference on seroprevalence of brucellosis between camels reared with other ruminats like cattle/ sheep/goat than camels reared alone (χ^2=8.3, p< 0.05) (Table 2).

Questionnaire survey

A total of 150 camel owners, who brought their animals to Adama for export purposes were interviewed, based on their willingness to participate in the survey. The owners revealed that extensive management system was exercised in both the zones. Camels are kept alone as well as together with other species of animals mainly for milk production (110/150=73.3%), transport (33/150=22%) and export purposes (7/150=4.7%). The highest proportion (60/150=40%) of the camel herds kept together with cattle, sheep and goats, while (31/150=20.6%) of camel herds were kept only with cattle, (15/150=10%) with sheep and goats, (2/150=1.5%) with cattle and equine and (42/150=28%) camel herds alone. The mean camel herd size was 18.4 with the maximum and minimum values being 182 and 3, respectively. Although over two third of the interviewed owner's (101/150=67.33%) stated that they drank fresh raw milk regularly, however habit of consuming raw camel meat was not found in the area. Only few (5/150=3.3%) respondents confirmed of consuming raw liver and hump of camel. Most (147/150=98%) of the milk originated from their own camels, the rest was purchased. Almost one fourth of interviewees (35/150=23.33%) knew of the disease brucellosis and 82.86% (29/35) of those who knew the disease did not know that camel can transmit brucellosis to people. Two interviewees stated that they were treated in clinics for brucellosis.

Discussion

Brucellosis is a serious zoonotic disease affecting man and all domestic animals including camels. It is considered as one of the

great public health problem all over the world [30]. In Ethiopia, brucellosis in camels has not been extensively studied as compared to other livestock. Little attention has been paid to this disease in camels as it provokes only few clinical signs in contrast to its clinical course in cattle [17]. Control of brucellosis in livestock and humans depends on the reliability of the methods used for detection and identification of the causative agent.

In the present study, all 1500 camels were clinically normal at the time of sampling and according to the owners, none had previously shown clinical signs of brucellosis. The true seroprevalence of camel brucellosis in two zones of Oromia region as adjusted to the RBPT and CFT sensitivities and specificities is 0.53%. The 0.53% prevalence of brucellosis in apparently healthy camels in the present study indicates that many infected camels might be silent carriers for brucellosis and their products may pose a serious health problem for consumers. This finding is in agreement with the results of Bekele [31], and Gumi et al. [9] who reported in Borena, Oromia region with prevalence rates of 0.4-2.5% and 0.9% respectively in Borena, Oromia region. The seroprevalence result of the present study is lower than many of the earlier reports in Ethiopia [4.2% in Borena, Oromia region [6], 1.8 % in Borena, Oromia region [5], 1.7% in Tigray [31], 7.6 % in Afar [7], 2.43 % in Jijiga [32,33]; 5.7% in Afar and 2.8% in Somali regions [6]. The findings in the present study were also lower than reported in other African countries. For instance a prevalence of 2.0 to 15.4% was reported in Kenya [34], 3.1% in Eritrea [35] and 3.1% in Somalia [36] 3% Iraq [37], 19.4% in Jordan [20], 30.5% in Sudan [38], 7.61% in Egypt [39]. Teshome et al. [6], Tefera [40], Gyles and Prescott [41] reported higher prevalence of brucellosis in female camels which may be associated to erythritol. Erythritol, a sugar alcohol synthesized in the ungulates placenta stimulates the growth of virulent strains of *B. abortus* [42]. Also relaxation of immunity in females associated to lactation, pregnancy and other reproductive stress may also contribute to higher prevalence in female camels [41]. In the present study, since only male camels were included that might have contributed to low prevalence. Differences in seroprevalence observed in this study, as opposed to those recorded by previous researchers, might also be due to differences in herd size, sample size, tests used, agro ecological and management conditions, and the presence or absence of infectious foci, such as *Brucella*-infected herds, which could spread the disease among contact herds.

In this study we also evaluated the efficacy of RBPT to screen camels for brucellosis. Of the 1500 camels tested by RBPT, 11 (11/1500=0.73%) were found to be positive for brucellosis. When CFT procedure was applied, the proportion of positive animals decreased to 8 (8/1500=0.53%). The sensitivity and specificity of RBPT was 100% [95% CI: 62.91-100] and 99.80% [95% CI 99.41 -99.96] respectively. RBPT recorded only 0.2% (3/1500) false positive camels (with CFT method considered as test comparison), however no false negative camel were reported by RBPT. Almost perfect agreement between RBPT and CFT was proven by calculating Kappa values (0.84). This observation needs further evaluation of both tests, to validate their diagnostic use in camels.

Even though, brucellosis was detected in both the two zones with slight variation in prevalence, it was not statistically significant difference ($p>0.05$). This could be attributed to the similarity in agro-ecological conditions and livestock management system in the zones. The camels are herded together with sheep and goats and to a lesser extent with cattle and they share the same watering points and pastures. The camels might have contracted the infection through close contact between infected and susceptible animals and cross transmission between species, through the alimentary tract from contaminated feed or water, through the respiratory system via contaminated dust or droplets, or through the genital system from infected semen [43].

Age had no significant effect ($p>0.05$) on animal level seroprevalence suggesting existence of susceptibility to brucellosis in camels of different age groups which is in agreement with the previous reports from Ethiopia [6,33]. Prevalence of *Brucella* antibodies in all age groups in camels indicates that brucellosis infection started early in life probably through sucking and persisted into adulthood as shown in Table 2 However Radostits et al. [30] and Quinn et al. [44] reported that younger animals tend to be more resistant to infection. This may be due to the fact that sex hormones and erythritol, which stimulate the growth and multiplication of *Brucella* organisms, tend to increase in concentration with age and sexual maturity [29]. But in the present study since no she camel was included for the study therefore no difference was observed between different age groups. Seroprevalence of brucellosis was significantly higher ($\chi^2 = 8.3, p<0.05$) in camels reared along with other ruminants than those reared alone. This finding is in agreement with Ghanem et al. [36], Al-Majali et al. [45], Hadush et al. [8], Zewold and Haileselassie [7]. Such animal species distribution and diversification is common to other areas in Ethiopia and has economic and ecological advantages. However, it increases the chance of brucellosis and other disease transmission from other infected ruminants to dromedaries. The endemic nature of brucellosis in small ruminants, large animals in Oromia region [6,9,46,47] and lack of adequate *Brucella* control program including vaccination may contribute to this prevalence of camel brucellosis in this region as the Infection rate in camels depends upon the infection rate in primary hosts animals in contact with them [13].

The questionnaire survey has provided information regarding the knowledge and practices of Camel keepers about brucellosis in Southeast Ethiopia. Knowledge of diseases is a crucial step in the development of prevention and control measures [48]. Despite huge efforts of the government and non-government institutions to promote and improve animal production in the areas, our study highlighted that general knowledge of brucellosis among the farmers was poor. Camel owners in the Bali and Borena zones of Oromia regional state practiced a high degree of ruminant diversification, i.e., in addition to camels, they kept cattle, sheep and goats. Mixing of animals although having its own economic importance increases the chances of transmission of brucellosis to the camels [36,45]. In most of the areas in the study zones, animals had direct acess to water sources like pond/dam water and contaminated it through discharges. However, the exposure rate may not be very high due to the fact that camel herds are mobile; this does not restrict them to a specific category of the water resources [31].

Most of the camel owners believed that camel milk to possess superior storage life, medicinal properties (against dropsy, jaundice, diabetes, glycaemia) and has an aphrodisiac effect. However, most of them didn't have any knowledge about the transmission of brucellosis from consumption of raw milk. Camel owners (pastoralists) of the study area consume raw camel milk and do delivery assistance, clean newborns, assist suckling and carry the young from field to home without any protection and share the same housing enclosures. These farmers from nomadic areas believe that raw camel milk has a curative effect on their digestive system and boiling is considered to remove its "goodness". In these areas, milk is usually preserved by

souring, which does not destroy brucellae as they are preserved in the milk fat [49].

Unfortunately, infected farmers with symptoms of undulating fever and joint pain very rarely seek medical help, and if they do, the fever is usually ascribed to malaria or typhoid, therefore human brucellosis is likely to be greatly under-diagnosed. In Ethiopia, the difficulty in diagnosis is compounded by hospitals lacking adequate laboratory diagnostic methods. This is true of the regional hospital of Oromia, which does not keep records of brucellosis cases and cannot currently confirm clinical diagnosis with laboratory methods. Ragassa et al. [50] reported 30.8% incidence among people with clinical signs compatible with brucellosis in Borena. This study highlights the need for improved diagnostics for human brucellosis in health facilities.

Conclusion

The low seroprevalence of brucellosis in apparently healthy male camels indicate that these animals are reproducing normally and serve as permanent carriers of brucellosis. Since this low seroprevalence of camel brucellosis is not the result of informed policy, there is no guarantee that it will continue unchanged. It is therefore an important period of consolidation for these camel owners and local authorities to keep the disease burden low. Co-ordinated nationwide epidemiological surveillance is required together with typing of infecting strains, thus enabling the transmission dynamics to be elucidated and initiating immunization campaigns, public health education and eradication strategies. That will be possible only by including camels in the national program for control and eradication of brucellosis in Ethiopia.

Conflict of Interest

We declare that we have no conflict of interest.

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