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**Original Research**

# Seroprevalence of *brucella* infection in camel and its public health significance in selected districts of afar region, Ethiopia

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**Abstract**
This paper reports the results of a cross-sectional study conducted between October 2010 and May 2011 in two conveniently selected camel-rearing districts of Afar, Awash-Fentale and Amibara, North East Ethiopia with the objective of determining the prevalence and risk factors of camel and human brucellosis. A total of 768 camel blood sera were collected by simple random sampling from eight pastoral and agro-pastoral residences and human sera were collected from 200 purposely selected clinical patients from two health centres of the districts. Sera were screened using Rose Bengal Plate Test (RBPT) and positive samples were then confirmed by Complement Fixation Test (CFT). The overall prevalence of camel brucellosis in the districts investigated was 11.9% by RBPT and 7.6% by CFT and the overall prevalence of human brucellosis was 16% by RBPT and 15% by CFT. The logistic regression on animal level including age, herd size, presence of ruminants and parity of the camels showed statistically significant difference and were the potential risk factors associated with camel brucellosis with significance levels of (P= 0.026, 0.004, 0.0001 and 0.004 respectively. The ownership of Milking Camels, living within the pastoral and agro-pastoral communities, keeping of livestock in close contact, consumption of raw camel milk and milk products, assisting animals during parturition and grooming livestock were potential risk factors associated with human brucellosis. The results of the present investigation indicate that human and camel brucellosis is widely distributed in the study districts of Afar Regional State. Hence, controlling the risk factors, proper hygienic practices, public education and team work between veterinary and health personnel should be improved. An effort to mitigate the economic losses and public health hazard caused by the disease has to be made.



## INTRODUCTION

Camel is an important domestic animal species uniquely adapted to the hot and arid environment. In Ethiopia camels are found in north-eastern, eastern, south-eastern and southern parts of the country [1]. According to the animal population census [2], the camel population in Ethiopia is estimated to be 2.3 million. The major ethnic groups owning camels in Ethiopia are Afar, Somali and Oromo. Camels in the Afar region are mainly kept for milk and meat production and transportation system. They are a means of investment and long-term savings, a source of prestige for their owners and there is also a large market for trade in live camels.

Despite their huge socio-economic importance and adaptation in hot and arid environments, camels are still affected by various diseases. A study on camel husbandry practice in eastern part of Ethiopia by Getahun and Kassa [3] indicated abortion rates and stillbirths of 9% and 4.3%, respectively.. The epidemiology of camel brucellosis in Ethiopia clearly showed that the disease occurrence is endemic and wide spread with significant economic importance with a seroprevalence up to 5.5% reported [4, 5, 6, 7]. No comparable data however is available in the Awash-Fentale and Amibara districts; home to many camel

production system is conducted.

Brucellosis is a zoonotic disease that leads to considerable morbidity resulting in significant loss of working days across the globe and thus perpetuates poverty. The disease presents as an acute or persistent febrile illness with a diversity of clinical manifestations [8]. Camel brucellosis has considerable public health importance as camel milk is consumed raw [9]. Most of the Afar people are pastoralists, camel milk is always consumed either fresh or in varying degrees of sourness in the raw state without heat treatment thus, can pose a health hazard to the consumer. However, the prevalence of human brucellosis is unknown and no available data on camel brucellosis in the study area. Therefore, the present study was carried out with the objective of determining the prevalence of *Brucella* infection in camel and human beings in selected pastoral and agro-pastoral residences of the Awash-Fentale and Amibara districts and to identify potential risk factors associated with the disease.

## MATERIAL AND STUDY METHODS

### Description of the study areas

Afar region is located in the Great Rift Valley, North Eastern Ethiopia. The distance is 170 kms from Addis Ababa, the capital city. Its geographical location is $8^0$ 54' N latitude and $36^0$ 23'- 39 $^0$54' E longitude and is a lowland area with an altitude of 650-1010 m.a.s.l. The annual rainfall is 486 mm, minimum and maximum temperature is $36^OC$ and $42^OC$, respectively. Afar region has 5 administration zones and 29 districts among which *Awash-Fentale* and *Amibara* are the two districts which has pastoral and agro-pastoral communities. The total livestock population of camels, cattle, sheep, and goats in the two districts are 233,015, 640,908, 534,050 and 1,445,690 respectively.

### Study design and sampling method

A cross-sectional study was conducted from October 2010 to May 2011, to determine the prevalence of *Brucella* infection in camel and its public health significance in selected pastoral and agro-pastoral residences of the Awash-Fentale and Amibara districts and to identify potential risk factors associated with the disease. First, two districts, Awash-Fentale and Amibara, were selected based on the easily accessibility of camels. There are 6 peasant associations (PAs) in Awash-Fentale and 18 PAs at Amibara district and 30% of the PAs by proportional allocation of the sample size to each of the study areas on basis of the camel population in each of the districts were included in the investigation. PA indicates the lowest administrative unit within a district that was considered during the survey. Hence, through simple random sampling system two PAs from Awash-Fentale and six PAs from Amibara were selected (Table 1). The average expected prevalence rate was assumed to be 50% for the area within 95% Confidence Intervals (CI) at 5% desired accuracy. Hence, sample size calculation was performed based on Thrusfield [10] using the formula:

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

**Table 1**. Number of camels sampled from each Peasants Association of the study districts

| District | PA | No sampled |
|---|---|---|
| Awash-Fentale | Sabure | 97 |
| | Doho | 127 |
| Amibara | Sheleko | 132 |
| | Halaydeghi | 123 |
| | Melka-Werer | 82 |
| | Algeta | 78 |
| | Hasoba | 68 |
| | Ambash | 61 |
| | Total | 768 |

PA = peasant association, No = number

Where n = sample size, d = desired absolute precision (0.05), $P_{ex}$ = expected prevalence (50%), thus the desired sample size for $P_{ex}$ = 0.5 is n = 384. However, in order to increase the representativeness and randomness of the study animals, the sample size could be inflated by two to four folds which can account for the potentially large variation that may occur among clusters [10]. Therefore, it was inflated the sample size by two-folds and hence, a total of 768 camels were selected by simple random sampling from the herds of the eight PAs. Individual camels above six months of age were randomly selected from the herds till the calculated sample size achieved.

A purposive sampling method was applied to select human patients in the health centres and simple random sampling method was used to include herdsmen from each cluster for the administration of questionnaire. Patients with febrile illness presenting with clinical signs and symptoms resembling brucellosis were included in the study.

### Blood collection

About 10 ml of whole blood sample was collected from the jugular vein, using plain vaccutainer tubes and needles, from each camel aged above six months of age. There was no history of vaccination for brucellosis in the region in general and our area in particular. Similarly, about 5 ml of blood was collected from

potential human risk groups. Each sample was labeled using codes specific to the individual animal and herd information. The tubes were tilted on a table overnight at room temperature to allow clotting. Serum was collected either passively by decanting or using centrifuges at 2500 revolutions per minute for five minutes. The serum was stored at -20$^0$C until it was tested by Rose Bengal Plate Test and Complement Fixation Test.

**Serological testing**

The Rose Bengal Plate Test (RBPT) was used as a screening test for detection of *Brucella* agglutinins and samples giving positive results were then confirmed by the Complement Fixation Test (CFT).

***Rose Bengal Plate Test (RBPT)***

For the RBPT the procedure described by Staak *et al.* [11] was followed. Briefly, 30ml of the sera samples were dispensed onto the plate and 30ml of RBPT antigen was dropped alongside the sera. The plate was rocked by hand for 4 min and the test was read by comparing with the positive and negative control sera by examining for agglutination in natural light. Magnifying glass was used to detect micro-agglutination. Results of RBPT were interpreted as 0, +, ++ and +++ as described by Staak *et al.* [11]. 0 = no agglutination; + = barely visible agglutination (seen by using magnifying glass); ++ = fine agglutination and +++ = coarse agglutination. Samples with no agglutination (0) were recorded as negative while those with +, ++ and +++ were recorded as positive.

***Complement Fixation Test (CFT)***

The CFT procedure was undertaken at the National Veterinary Institute, Department of Immunology at Debre-Zeit, Ethiopia. Preparation of the reagents was performed according to OIE protocols [12]. A titration of hemolysin and antigen was performed before the test. The minimum hemolytic dose was also estimated for each run. As for the interpretation of test results, positive reactions were indicated by sedimentation of Sheep Red Blood Cells (SRBC) and absence of hemolysis. Negative reactions were revealed by hemolysis of SRBC. According to OIE [12] sera with strong reaction, more than 75% fixation of complement at a dilution of 1:10 and at least with 50% fixation of complement at a working dilution (1:5) was classified as positive.

**Ethical considerations**

All experiments in this study were conducted fulfilling the guidelines of the declaration of National Health Research Ethics Guideline of the Ethiopian Science and Technology Commission and hence, consent was obtained from the institutional review board (IRB) office Addis Ababa University College of Health Sciences. All participants were informed about the aim and procedure of the study and were asked for their consent to be clinically examined and for the collection of blood sample and no refusal to participate were recorded. Written informed consent was obtained from all participants in the study.

**Data management and processing**

The data were summarized and compiled by summing up the laboratory findings of human and camel study population. Coded data were stored in Microsoft Office Excel spread sheet and transferred to SPSS Version 15 [13] for statistical analysis. Descriptive and analytic statistics were computed and Logistic regression was employed to see the association of risk factors with that of seropositivity to *Brucella* antibody; the degree of association was computed using Odds ratio (OR) and 95% confidence interval (CI).

## RESULTS

**Overall seroprevalence of camel brucellosis using RBPT and CFT**

From 768 camels collected serum 91 (11.9%) positive reactors for RBPT and 58 (7.6%) for CFT were identified. It was detected in both of the study districts, with district level prevalence rates ranging from 6.3% (n=224) in Awash-Fentale to 8.1% (n=544) in Amibara district (Table 2).

**Table 2.** Distribution of CFT seropositivity to camel brucellosis on the basis of Peasants Association (PAs) of the two districts

| District | PA | No examined | CFT +ve (%) |
|---|---|---|---|
| Awash-Fentale | Sabure | 97 | 4 (4.1%) |
| | Doho | 127 | 10 (7.9%) |
| Amibara | Sheleko | 132 | 9 (6.8%) |
| | Halaydeghi | 123 | 12 (9.8%) |
| | Melka-Werer | 82 | 7 (8.5%) |
| | Algeta | 78 | 7 (9.0%) |
| | Hasoba | 68 | 5 (7.4%) |
| | Ambash | 61 | 4 (6.6%) |
| | Total | 768 | 58 (7.6%) |

***Risk factors and seroprevalence of camel brucellosis***

As indicated in Table 3, there was no any statistically significant association between sex, districts, habitation area and farming system and the occurrence of the disease in the study animals. However herd size (small: 14 - 20, medium: 21- 40 and large: > 40 camels) showed statistically significant difference ($\chi^2$ = 8.47, P = 0.004) in the occurrence of the disease. There was

also high statistically significant difference ($\chi^2$ = 34.74, P = 0.0001) in the prevalence of the disease in these camel population which made contact with goats and cattle on pasture. Likewise, there was statistically significant association ($\chi^2$ = 11.30, P = 0.004) between parity and the seroprevalence of the disease. Those she-camels with the history of more than one parity were more at risk of being seropositive to *Brucella* infection than those with no parturition and those with single parity and those which did not give birth yet.

***CFT Seropositive breeding camels and abortion related factors with dependent Brucella seropositivity***

In the current study female camels were found with a history of abortion at the second half and last stage of pregnancy (gestation period 365-395 days). Of the total 432 she-camels, 46 (10.6%) had aborted and among which 10 (21.7%) were positive for brucellosis. There was higher seropositive and aborted she-camels in Awash-Fentale district than in Amibara with 7 (41.2%) and 10 (21.7%), respectively (Table 4). Regarding the frequency rate of abortion, among the 63 (10.3%) of aborted cases of she-camels in the study districts 50 (79.4%) aborted once and 13 (20.6%) aborted twice.

**Table 3.** Risk factors with dependent Brucella seropositivity in camels of the selected districts of Afar Region, Ethiopia.

| Risk factors | Category | No examined | Prevalence (%) | P-value | OR (95% CI) |
|---|---|---|---|---|---|
| Farming system | Pastoral | 328 | 8.8 | 0.245 | |
| | Agro-pastoral | 440 | 6.6 | | |
| Sex | Male | 159 | 5.7 | 0.295 | |
| | Female | 609 | 8.1 | | |
| Age | Young | 226 | - | 0.026* | 1.70 (1.45-1.95)<br>1.52 (1.24-1.72) |
| | Adult | 355 | 13.8 | | |
| | Old age | 187 | 4.8 | | |
| Herd size | 14-20 (small) | 282 | - | 0.004* | 1.58 (1.40-1.84)<br>1.20 (1.06-1.50) |
| | 21-40 (medium) | 353 | 15.3 | | |
| | >40 (large) | 133 | 3.0 | | |
| Contact | With cattle | 230 | 2.2 | 0.0001* | 1.23 (1.11-1.50) 1.71 (1.55-5.30) |
| | With goats | 219 | 3.7 | | |
| | With cattle & goats | 319 | 14.1 | | |
| Parity | No parturition | 102 | - | 0.004* | 1.25 (1.08-2.32)<br>3.26 (1.53-6.96) |
| | Single parity | 200 | 8.5 | | |
| | More than one | 307 | 10.4 | | |

*Significant at 95% level of significance

**Table 4**.Distribution of CFT seropositive breeding camels and abortion related factors with dependent Brucella seropositivity in the selected districts of Afar Region, Ethiopia.

| Location | | Males | Females | Aborted females |
|---|---|---|---|---|
| District | Peasants Association | Prevalence (%) | Prevalence (%) | Prevalence (%) |
| Awash-Fentale | Sabure | 1 (5.0%) | 3 (3.9%) | 2 (66.7%) |
| | Doho | 2 (7.4%) | 8 (8.0%) | 5 (62.5%) |
| Amibara | Sheleko | 1 (3.7%) | 8 (7.6%) | 2 (25.0%) |
| | Halaydeghi | 2 (8.0%) | 10 (10.2% | 3 (30.0%) |
| | Melka-Werer | 2 (11.8% | 5 (7.7%) | 1 (20.0%) |
| | Algeta | 1 (6.3%) | 6 (9.7%) | 1 (16.7%) |
| | Hasoba | 0 | 5 (9.3%) | 1 (20.0%) |
| | Ambash | 0 | 4 (8.3%) | 2 (50.0%) |
| | Total | 9 (5.7%) | 49 (8.1%) | 17 (34.0%) |

**Table 5**. CFT seropositivity to human brucellosis and spatial risk factors in the study area

| Risk factors | Test result | | Total (%) | $\chi^2$ | P-value | OR (95% CI) |
|---|---|---|---|---|---|---|
| | Positive | Negative | | | | |
| **Farming system** | | | | | | |
| -Patients came from pastoral areas | 4 | 41 | 45 (22.5%) | 28.41 | <0.0001 | 2.1 (1.70-6.30) |
| -Patients came from agro-pastoral areas | 26 | 129 | 155 (77.5%) | | | |
| **Residence area** | | | | | | |
| - Urban | 17 | 89 | 106 (53%) | 0.12 | 0.662 | |
| - Rural | 13 | 81 | 94 (47%) | | | |

**Table 6.** Analysis of potential risk factors from questionnaire survey of qualitative nominal categories related to human brucellosis

| Risk factors | No of Non-brucellosis cases (%) (N= 200) | No of Seropositive to Brucellosis (%) | P-value | OR (95% CI) |
|---|---|---|---|---|
| Ownership of livestock or milking camels | 145 (72.5%) | 28 (14%) | 0.001* | 2.30 (1.78 -8.35) |
| Grooming livestock | 109 (54.5%) | 23 (11.5%) | 0.003* | 1.17 (1.58 -2.32) |
| Milking animals | 107 (53.5%) | 23 (11.5%) | 0.062 | 1.04 (0.51 -2.11) |
| Assisting parturition | 100 (50%) | 19 (9.5%) | 0.0001* | 1.20 (1.60 -2.29) |
| Slaughtering livestock | 72 (36%) | 16 (8%) | 0.264 | 1.00 (0.48-2.50) |
| Consumption of raw camel milk | 73 (36.5%) | 21 (10.5%) | 0.030* | 1.1 (1.51 -2.12) |
| Consumption of fresh cheese | 71 (35.5%) | 13 (6.5%) | 0.0032* | 1.40 (1.68-2.76) |
| Consumption of raw meat | 103 (51.5%) | 22 (11%) | 0.0347* | 1.03 (1.50 -2.08) |
| Consumption of raw milk, cheese and meat | 160 (80%) | 30 (15%) | 0.0003* | 1.20 (1.58 -2.24) |

* Statistically significant association at 95% CI

**Prevalence of human brucellosis**

From the collected 200 human blood samples, 30 were seropositive to *Brucella* antibodies and hence, the overall prevalence rate of human brucellosis was 15%. The overall sex-wise seropositivity was 9% in males and 6% in females.

Those patients who came from agro-pastoral areas were 2.1 times (P < 0.0001, OR = 2.1, 95% C.I. = 1.70-6.30) more at risk of being seropositive to *Brucella* antibodies than those from pastoral areas. Of the 30 (15%) seropositive patients, 4 (13.3%) were from pastoral and 26 (86.7%) were from agro-pastoral areas. On the other hand, there was no statistically significant difference ($\chi^2$ = 0.12, P = 0.662) in the prevalence of human brucellosis between the urban and rural society (Table 5).

***Potential risk factors from questionnaire survey of qualitative nominal categories related to human brucellosis***

The occurrence of human brucellosis in those with the ownership of livestock or milking camels were more at risk of being seropositive to the disease than in those who don't have milking camels or other animals (P = 0.001, OR = 2.3, 95% C.I. = 1.78 -8.35). The likelihood of occurrence of seropositivity to human brucellosis was 1.2 times (0.0003, OR = 1.20, 95% C.I. = 1.58 - 2.24) more in those patients who consumed raw milk, cheese and meat than did not (Table 6). Keeping of livestock in close contact at yard and in pasture, consumption of raw camel milk, assisting animals

during parturition and grooming livestock were potential risk factors which contributed to the occurrence of human brucellosis in the camel or livestock rearing communities of the study districts.

## DISCUSSION

The present study demonstrated that the overall seroprevalence proportion of camel brucellosis in the study districts was 11.9% by the RBPT and 7.6% by CFT. Since none of the animals under study was vaccinated, this seems to reveal a moderate prevalence and natural transmission of *Brucella* organisms in the study areas. The current result is in accord with the results of many previous observations of different countries, including studies in Kenya by Kagunya and Waiyaki [14] and by Wilson *et al*. [15] who reported prevalence rates of 4.6-10.3 and 6.0-38.0, respectively, and in Sudan by Osman and Adlam [16] who reported a prevalence of 8.0%. However, high prevalence was recorded compared to the result recorded by Bekele [1], Teshome *et al*. [7] and Domenech [5] in Borena, Oromia region with prevalence rates of 0.4-2.5%, 4.2% and 4.4%, respectively, and by Richard, [6] who reported prevalence rates of 5.5% in Afar region and in other camel-rearing areas of Ethiopia.

The logistic-regression analysis of risk factors indicated that age, herd size, contact with other animals and parity status of camels were found highly associated with brucellosis seropositivity. Stocking densities are important potential determinants for brucellosis transmission [17, 18]. This concept coincides with the current study that the seroprevalence of brucellosis among three categorized herd sizes showed significant variations with higher seroprevalence recorded in the large herd sizes of camels. Higher seroprevalence was observed in the camel population which made close contact with cattle and goats (14.1%) on pasture than either with goats (3.7%) or with cattle (2.2%). It can be concluded that ruminants had a role in the transmission of the disease to the camel population. Female camels demonstrated higher prevalence (8.1%) than male camels (5.7%) although statistical significant difference was not observed.

Brucellosis causes heavy economic losses in animal production resulting from abortions, sterility, decreased milk production, and the costs of replacer animals [19, 20]. In the current study the association between abortion and seropositivity was interesting. Among the 49 female camels infected with brucellosis 17(34%) were aborted. This finding was in line with Radostits *et al.* [20] who stated that late abortion and premature or full-term birth of dead or weak calves predominated in pregnant animals with brucellosis.

Human brucellosis is a widespread disease in camel producing areas of different countries of Africa and Asia [21]. Data from developing countries in the Mediterranean basin, particularly the Middle East, reported seroprevalence rates of human brucellosis ranging from 8% in Jordan [22] to 12% in Lebanon and Kuwait [23]. Even higher seroprevalence rates have been reported in sub-Saharan countries, with percentages of 18% in Uganda [24] and 13% in Nigeria [25]. The present study is, therefore, about in consistent with the above findings. Out of the 200 human blood samples, 30 (15%) were seropositive to *Brucella* antibodies. The overall sex-wise seropositivity was 9% in males and 6% in females. The higher seropositivity in males in the study area is because of the fact that Afar females of 15-40 years of age are culturally constrained not to drink the camel milk as it is assumed to make them sexily, rude and cheeky in behaviour.

Since there is close contact between humans and their livestock, which sometimes share the same housing enclosures especially in the pastoral areas, brucellosis has found a significant health risk for the entire community of the study areas. On the other hand, there was no statistically significant difference ($\chi^2 = 0.12$, P = 0.662) in the prevalence of human brucellosis between the urban and rural society (Table 5). This was because the consumption of animal and animal products both in the urban and rural is almost similar.

The consumption of contaminated food and an occupational contact remain the major sources of an infection. According to the present findings most of patients had a history of raw milk and cheese consumption or direct contact with camels, goats and cattle. Almuneef *et al*. [26] reported that ingestion of raw milk was considered the likely source of infection among family members and the present study is in accord with it. Keeping of livestock in close contact at yard and in pasture, consumption of raw camel milk, assisting animals during parturition and grooming livestock were potential risk factors which contributed to the occurrence of human brucellosis in the camel or livestock rearing communities of the present study districts.

In conclusion, the results of the present study revealed that camel brucellosis is widely distributed in the study areas. The rate was higher in the pastoral areas. Risk factors like herd size, contact with other animals and the parity status of camels were found important risk factors associated with *Brucella* seroreactors. The existing scenario of brucellosis in camels of the study area calls for urgent capacity building of regional laboratories. Co-ordinated nationwide epidemiological surveillance is urgently required together with typing of infecting strains, thus enabling the transmission dynamics to be elucidated and informing upon control and eradication strategies. In case of human case the

transmission of the disease occurs by direct or indirect contact with infective excretions; and *Brucella* contaminated milk presents a potential threat to human beings as it can spread through ingestion and equally important, teamwork between veterinary and human health personnel is of extreme importance to create awareness through educational campaigns among human risk groups.

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