Prevalence of camel tuberculosis and associated risk factors in camels slaughtered at Akaki Abattoir, Ethiopia.

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Abstract

A cross sectional abattoir based study was conducted from February 2014 to October, 2015 on camels slaughtered at Akaki municipality abattoir to determine the prevalence of Tuberculosis in camels and assess the association of risk factors with the prevalence of Tuberculosis in camels using single intra-dermal comparative cervical tuberculin (SICCT). Of the total of 387 camels investigated, overall camel tuberculosis prevalence of 9.82% (95% CI: 6.84%-12.8%) at a cut off value $\geq$ 4mm and prevalence of 17.05% (95% CI: 13.3%-20.82%) at a cut off value $\geq$ 2mm, were recorded. Pearson chi-square test reveals, there was statistically significant association of prevalence with the origin of camels at a cut off value $\geq$ 2mm (χ^2 = 13.461, P=0.000). However, there was no statistically significant association (P>0.05) of risk factors of age, sex, body condition and origin of camels with the prevalence at a cut off value $\geq$ 4mm.The multivariate logistic regression analysis shows at a cut off value $\geq$ 4mm, being old aged (adjusted OR= 0.999, 95% CI: 0.450-2.22), female (adjusted OR= 2.226, 95% CI: 0.5099-9.719) were identified as risk factors for positive tuberculin reactivity. Similarly, the multivariate analysis at a cut off value $\geq$ 2mm, showed being moderate body conditioned (adjusted OR= 1.583, 95% CI: 0.7399-3.385) was identified as risk factor for higher tuberculin reactivity. The present study aimed to determine tuberculin reactivity of camels and assess associated risk factors with the prevalence. It was concluded that Tuberculosis is an existing phenomenon in camels. It is therefore, recommended that detailed epidemiological investigations should be conducted for the better understanding of the epidemiology of the disease in camels of pastoral communities with particular emphasis to

zoonotic significance in camel rearing areas of Ethiopia.

Keywords: Akaki abattoir, Camel Tuberculosis, Ethiopia, Prevalence, Risk factors, Comparative Tuberculin testing.

Introduction

Dromedary camels (one-humped *Camelus dromedarius*) historically inhabiting the Middle East and the Horn of Africa, have an estimated world population of about 18 million across the arid and semi-arid environments of the African and Asian countries. In Africa the dromedary population of about 15 million accounts for about 74% of the world camel population, and of these, 60% are found in east African countries (Somalia, 6.2 million; Sudan, 2.8 million; Ethiopia, 1.7 million, Kenya, 0.9 million) (Rhodes *et al.*, 2015).

Tuberculosis is a chronic bacterial disease in animals and humans characterized by the progressive development of specific granulomatous lesions of tubercles in affected tissues. The disease affects all age groups of susceptible hosts and is accountable for more deaths throughout the world than any other bacterial disease ever today (Omer and Gaffer*,* 2002). Tuberculosis in cattle and other domestic animals is above all caused by two members of *Mycobacterium tuberculosis* complex (MTC): *M. bovis* and *M. caprae* (Pavlik *et al.,* 2002; Prodinger *et al.,* 2002; Erler *et al.,* 2004). However, occasional occurrence of tuberculosis due to *M. tuberculosis* species with concurrent tuberculous lesions has been reported in pigs (Lesslie and Brin, 1970; Popluhar *et al.,* 1974; Flesja *et al.,* 1978), in cattle (Popluhar *et al.,* 1974; Schliesser, 1976; Thoen *et al.,* 1981; Pavlik *et al.,* 2005), in dogs (Pavlik *et al.,* 2003) and other animals (Pavlik, 2006).

Tuberculosis is a disease that had already been diagnosed around the turn of the century in dromedaries in Egypt (Little wood, 1888; Wernery and Kaaden, 2002). Studies conducted in Egypt, Sudan, India, Somalia, Kazakhstan and Ethiopia has shown that tuberculosis is occurring in camels (Fassi Fehri, 1987). Camel TB has been described in Egypt (Mustafa, 2013), the United Arab Emirates (Kinne *et al.,* 2006; Wernery *et al.,* 2007; 2012), Pakistan, Kazakhstan (Elmossalami *et al.,* 1971), Somalia, Nigeria (Abubaker *et al.,* 2014), and Ethiopia (Mamo *et al.,* 2009; 2011; Zerom *et al.,* 2013). *M. bovis*, *M. tuberculosis* and Non Tuberculous Mycobacteria (NTBC) such as *M. kansasii*, *M. aquae*, *M.*

fortuitum and *M. smegmatis* have all been isolated from Old World Camelids (OWC) as causative agents of camel TB (Kinne *et al.,* 2006; Elmossalami *et al.,* 1971; Mamo *et al.,* 2011; Zerom *et al.,* 2013, Rhodes *et al.,* 2015).

In Ethiopia, few studies have been conducted in the epidemiological investigation of Tuberculosis and identification of the causative agents in camels (Mamo *et al.,* 2009, 2011; Zerom *et al.,* 2013). The present study, therefore, the first of its kind to determine the prevalence of tuberculosis in camels using comparative tuberculin testing of camels slaughtered at Akaki abattoir and assesses associated risk factors with the disease prevalence.

Materials and Methods

Study area

A cross sectional abattoir based epidemiological investigation of tuberculosis in camels was conducted from February, 2014 to October, 2015, to determine the prevalence of tuberculosis in camels and assess the associated risk factors for infection and transmission based on single intradermal comparative cervical tuberculin test, post-mortem examination and mycobcaterial culture isolation on camels slaughtered at Akaki municipality abattoir. Akaki municipality abattoir is located in Akaki town which is one of the districts of Addis Ababa, in central Ethiopia. Camels slaughtered at Akaki municipality abattoir were mainly originated from Borena and Metehara pastoral areas of Ethiopia (Figure 1). Borena is located in the Oromia National Regional State, about 600 kms South of Addis Ababa. The climate of the Borena zone is semi arid. Metehara is also located in Oromia National Regional State, about 250 kms East of Addis Ababa. The prevailing climate in Metehara is arid (NMSA, 1999).
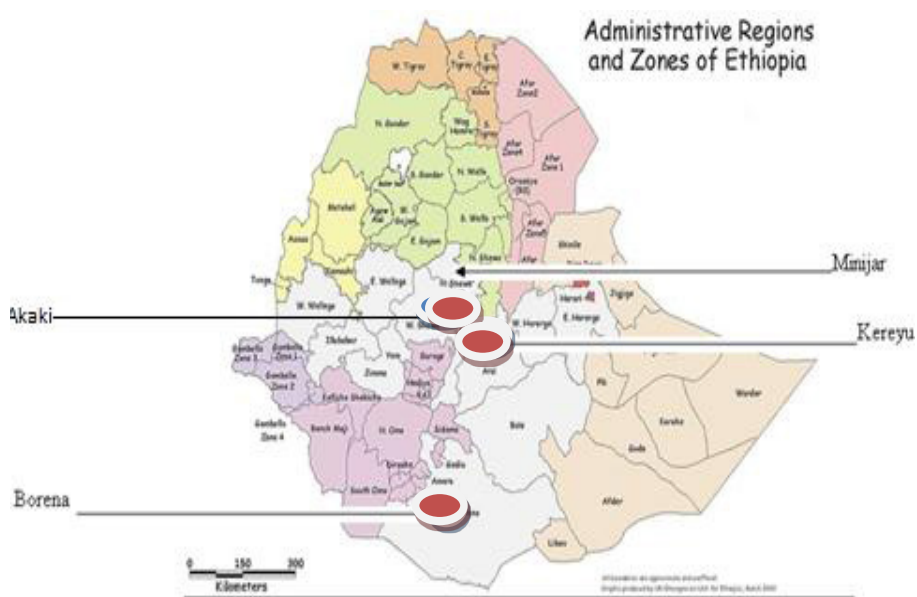
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Figure 1: Map of Ethiopia with zones, showing the origin of camels (Borena and Metehara) slaughtered at the Akaki abattoir (Source: kassaye *et al.,* 2013)

Study population

A total of 387 apparently healthy camels (347 female, 40 male) were included in this study. Frequently, older aged and unproductive female camels were slaughtered in the abattoir during our investigation. Risk factors of age, sex, body condition, and origin of the camels were recorded to assess the association of these risk factors with the prevalence of Tuberculosis. On average, camels which have stayed for at least three days in the holding pen of the abattoir were considered to facilitate re-measuring of Tuberculin skin reaction after 72 hours of injection.

Sample size determination

For cross sectional abattoir investigation to determine the prevalence of Tuberculosis in camels using single intra-dermal comparative cervical tuberculin (SICCT) test, the formula for random sampling of Thrusfied (2005) were used. For this, 95% confidence level, 5% desired absolute precision, and expected prevalence of 10.4% (Mamo *et al.,* 2011) was considered. Thus, the sample size

of 198 camels was calculated; however, to increase the precision of sample estimates, sample size was increased to 387 as much more than the calculated sample size.

Single Intradermal Comparative Cervical Tuberculin Test (SICCT)

Single Intra-dermal comparative cervical tuberculin (SICCT) was carried out by injecting both bovine purified protein derivative (PPD) and avian PPD (Observe™ bovine and avian tuberculin, Asure Quality Company, Mt. Wellington, Auckland, New Zealand). Two sites on the skin of the mid-neck of the camel, 12 cm apart, were shaved, and skin thickness was measured with a caliper. One site was injected with an aliquot of 0.1 ml of 3000 IU/ml bovine PPD into the dermis, and the other was similarly injected with 0.1ml of 2,500 IU/ml avian PPD (Figure 2).

Figure 2: Pictures showing abattoir workers along with field assistants while restraining camels for comparative tuberculin testing and sample collection at Akaki abattoir.

After 72 hours, the skin thickness at the injection sites was measured and recorded. Results were interpreted according to the recommendations of the Office International des Epizooties (OIE, 2009) at ≥ 4 mm cut-off and also at ≥ 2

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mm cut-off (Ameni *et al.,* 2008). Thus, at cut-off ≥ 4 mm, if the increase in skin thickness at the injection site for bovine PPD (PPD-B) was greater than the increase in skin thickness at the injection site for avian PPD (PPD-A) and; If PPD-B minus PPD-A was < 4mm or ≥ 4mm, the animal was classified as negative, or positive for BTB, respectively. At cut-off ≥ 2 mm, if the difference between B and A was ≥ 2mm, the animal was considered as positive, while if the difference is < 2mm, the animal was considered as negative. When the change in skin thickness was greater at PPD-A site, the animal was considered positive for Mycobacterial species other than *Mycobacterium tuberculosis* complex.

Data analysis

Data collected for the study were analyzed using STATA (Intercooled STATA version 12, Stata Corp., Collage station, TX). Statistical tests such as descriptive statistics, Pearson chi-square test, and multivariate logistic regression, were applied to determine prevalence of Tuberculosis in camels, assess association of risk factors considering statistical significance at 95% confidence level and P value of 0.05.

Results

Single intra-dermal comparative cervical tuberculin (SICCT) test result indicated that overall apparent prevalence of 9.82% (95% CI: 6.84%-12.8%) at a cut-off value ≥4mm and prevalence of 17.05% (95% CI: 13.3%-20.82%) at a cut off value ≤ 2mm. Pearson chi-square test showed there was no statistically significant association (P>0.05) of risk factors of age, sex, body condition, and origin of camels at a cut off value ≥ 4mm although higher prevalence of 10.37% and 5.0% was recorded in female and male camels, respectively. However, statistically significant association (x^2 = 13.461, P=0.000) was observed for the origin of camels with the prevalence at a cut off value ≥ 2mm (Table 1).

Table 1: Association of risk factors of camels with tuberculin positive reactivity at a cut off value ≥ 4mm and at a cut off value ≥ 2mm

Variables	No. Examined(%)	No. Positive (%)	COR (95% CI)	AOR (95% CI)
Age				
≤ 6 years	93 (23.5%)			
≥7 years	294(76.5%)	9 (9.68)	1	1
		29 (9.86)		
			1.0213 (0.4649-2.244	0.9998 (0.4503-2.220)
Sex				
Male	40 (10.3%)			
Female	347(89.7%)	2 (5.00)	1	1
		36 (10.37)		
			2.1994 (0.5092-9.500)	2.226 (0.5099-9.719)
Body Cond.				
Poor	209 (54%)			
Moderate	70 (18.1%)	21 (10.05)	0.985 (0.456-2.126)	0.958 (0.4401-2.088)
Good	108 (27.9%)	6 (8.57)	0.8267 (0.2912-2.347)	0.8332 (0.2877-2.413)
		11(10.19)	1	1
Origin				
Borena	323 (83.5%)			
Metehara	64 (16.5%)	31 (9.6)	0.8645 (0.3629-2.059)	(0.3339-1.940)
		7 (10.94)		1
			1	

Note: * Chi-square and P-value with statistically significant association.

The multivariate logistic regression analysis, at a cut off value ≥ 4mm, being old aged (older camels) (COR= 1.0214, 95% CI: 0.468-2.244; adjusted OR= 0.999, 95% CI: 0.450-2.222), being female (COR= 2.199, 95% CI: 0.5092-9.500; AOR= 2.226, 95% CI: 0.5099-9.719). Similarly, the multivariate analysis at a cut off value ≥ 2mm, being moderate body conditioned (COR= 1.863, 95% CI: 0.8972-3.867; adjusted OR= 1.583, 95% CI: 0.7399-3.385) were identified as risk factors for higher tuberculin reactivity (Table 2).

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Table 2: Multivariate logistic regression analysis of risk factors of camel tuberculosis with comparative tuberculin reactivity at a cut off value ≥ 4mm

Variables	No. Examined (%)	No. Positive (%)	COR (95% CI)	AOR (95% CI)
Age				
≤ 6 years	93 (23.5%)			
≥7 years	294(76.5%)	9 (9.68)	1	1
		29 (9.86)		
			1.0213 (0.4649-2.244	0.9998 (0.4503-2.220)
Sex				
Male	40 (10.3%)			
Female	347(89.7%)	2 (5.00)	1	1
		36 (10.37)		
			2.1994 (0.5092-9.500)	2.226 (0.5099-9.719)
Body Cond.				
Poor	209 (54%)			
Moderate	70 (18.1%)	21 (10.05)	0.985 (0.456-2.126)	0.958 (0.4401-2.088)
Good	108 (27.9%)	6 (8.57)		0.8332 (0.2877-2.413)
		11(10.19)	0.8267 (0.2912-2.347)	1
			1	
Origin				
Borena	323 (83.5%)			
Metehara	64 (16.5%)	31 (9.6)	0.8645 (0.3629-2.059)	(0.3339-1.940)
		7 (10.94)	1	1

*Note: COR= Crude Odds Ratio, AOR= Adjusted Odds Ratio

Discussion

The current study based on comparative tuberculin testing, overall prevalence of 9.82% at a cut off value ≥ 4mm and prevalence of 17.05% at a cut off value ≥ 2mm were recorded. The present finding at a cut of value ≥ 4mm is slightly lower but comparable to the previously reported prevalence of Mamo *et al.* (2011) for camels of Afar, and other reports (Mamo *et al.,* 2013). But it is higher than the reports of Mamo *et al.* (2009) at Dire Dawa, and Gumi *et al.* (2011) in Southern Ethiopia. Similarly, it is significantly higher than previously reported prevalence of some other authors at different sites in Ethiopia (Gumi *et al.,* 2011, 2012; Tamiru *et al.,* 2013; Admasu *et al.,* 2014; Ameni and Aklilu, 2007; Romha *et al.,* 2014; Nuru *et al.,* 2015), in Zambia (Pandey *et al.,* 2013), in Ecuador (Proano-Perez *et al.,* 2009) in Bangladesh (Mondal *et al.,* 2014).

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Again the current finding is higher than the findings of Kassaye *et al.* (2013) and Feyissa *et al.* (2014) for camels at Akaki and Easten part of Ethiopia, respectively. It is comparably higher than the incidence reported for camels in Egypt by Manal *et al.* (2008). At the same cut off value ≥ 4mm, the present finding is lower than previously reported prevalence of 23.7% and 30.0% in Ethiopia (Kebede *et al.,* 2008; Firdesa *et al.,* 2012) and in India with 14.3% (Thakur *et al.,* 2010). It is also lower than that of Abubakar *et al.* (2014) for camels slaughtered at Kano abattoir, Nigeria. Similarly, the current prevalence of 17.05% at a cut off value ≥ 2mm is in close agreement with that of Mamo *et al.* (2013) in Afar. But significantly higher than some other previously reported prevalence in different sites of Ethiopia (Tamiru *et al.,* 2013; Gumi *et al.,* 2011; Tschopp *et al.,* 2011). These variations might be due to variations in the study population, study site, animal species, production system, management factors, environmental factors, study design, methodology, and sample size factors. In the present study, prevalence of 9.68% and 9.86% was recorded for young adult (≤6 years) and older aged (≥7 years) camels, respectively at a cut off value ≥4mm and prevalence of 19.35% and 16.33% for young adult (≤6 years) and older aged (≥7 years) camels, respectively at a cut off value ≥2mm, respectively. Age as a risk factor, there was no statistically significant association (P>0.05) between Tuberculosis prevalence and age categories.

This finding was in agreement with that of Nuru *et al.* (2015) for cattle in Amhara region, and Kasaye *et al.* (2013) for camels at Akaki, Central Ethiopia and some other previous studies in Ethiopia (Kebede *et al.,* 2008; Admasu *et al.,* 2014). However, some other previous studies in Ethiopia (Ameni and Aklilu, 2007; Kebede *et al.,* 2008; Gumu *et al.,* 2011; Mamo *et al.,* 2013), from Ecuador (Proano-Perez *et al.,* 2009) and study from India (Thakur *et al.,* 2010) reported significant association of age with positive tuberculin reactivity. In the current study age variation in prevalence might be due to the proportion or the number of camels investigated in the age categories where large number of older camels (76.5%) was slaughtered in the abattoir during the study period than younger age categories (23.5%). Despite very few numbers of young camels in this study, tuberculin reactors were relatively higher than reactors among old age categories of camels investigated.

About 89.7% of the camels investigated were females. The prevalence at the two cut off values were not statistically significant (P>0.05). The current finding is in agreement with that of Nuru *et al.* (2015). However, an Ethiopian

study (Zeru *et al.,* 2014) reported significant association of sex with positive skin reactivity. In the current study, there was large number of unproductive females and older age camels which were slaughtered during the investigation. Moreover, only very few number of male camels were slaughtered that might attribute to the variation in prevalence record between males and females at a cut off value ≥ 2mm although this was not statistically significant (P>0.05). The study of Abubakar *et al.* (2014) at Kano abattoir, Nigeria, reported higher prevalence in male camels (23.9%) than female (21.8%) camels using lateral flow assay and his finding was not statistically significant. As to the body condition of camels investigated, prevalence of 10.05%, 8.57%, and 10.19% were recorded for camels with poor, moderate, and good body conditions, respectively at a cut off value ≥ 4mm and this was not statistically significant (P>0.05). However, at a cut off value ≥ 2mm, higher prevalence of 27.14% was observed in camels with moderate body condition than poor (13.88%) and good (16.67%) body conditions of camels during the investigation and this was statistically significant (χ^2=6.54, p= 0.038) with tuberculin positive reactivity and it is in agreement with the reports of Nuru *et al.* (2015) and some other previous studies (kebede *et al.,* 2008; Zeru *et al.,* 2014) but Firdessa *et al.* (2012) did not report similar findings.

In the current study, about 53% of the camels were with poor body condition, 20% with moderate, and the rest 27% with good body conditions. Animals with poor body condition assumed to have association with high skin reactivity however, poor body conditioned animals have relatively weak immunological responses to TB and subsequently susceptible to infection (Griffin *et al.,* 1993; O'Reilly and Daborn, 1995). Camels with poor body conditions might have in apparent and chronic infectious diseases like Tuberculosis and good body conditioned camels might have strong immune reaction against Tuberculosis infection. Moreover, the proportions or the number of camels in different categories was not comparable and this might have attributed to slight variation in the prevalence with body conditions although it was not statistically significant.

Majority of the camels investigated during the study were Borena camels (83.5%) and some metehara origin camels (16.5%). Prevalence of 9.6% and 10.94% was recorded for camels of Borena and Metehara origin, respectively at a cut off value ≥ 4mm and it was not statistically significant (P>0.05). However, prevalence of 13.93% for Borena and 32.81% for Metehara origin camels

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at a cut off value $\geq$ 2mm was observed and this was statistically significant (χ^2= 13.46, P= 0.000). Some other recent studies reported strong association between breed type and tuberculin positive reactivity for cattle in different sites at a cut off value $\geq$ 4mm (Admasu *et al.,* 2014; Romha *et al.,* 2014; Zeru *et al.,* 2014; Nuru *et al.,* 2015). This prevalence report was significantly higher for Metehara camels than Borena camels.

This might be probably for the reasons of the disproportionate number of camels investigated for Borena and Metehara origin where majority of the camels slaughter at Akaki abattoir during the study were Borena origin camels. Moreover, management and husbandry practices where migration, mixed livestock practices might also contribute to interspecies transmission of TB and other contagious diseases and possibly the pastoral livestock keeping in Borena and Metehara area might have some contribution to this variation in prevalence. To have a better comparison and statistical significance of origin as a risk factor of camel tuberculosis, comparable number of animals should to be taken into consideration to appreciate the significance association of origin with prevalence.

Conclusion

In the present study, the prevalence recorded was comparable to some other previous studies in different sites by different authors in Ethiopia for cattle and small ruminants and very few reports for camels. It was concluded that Tuberculosis is an existing phenomenon in camels although it was assumed that camels are resistant against varieties of diseases affecting other livestock species. It is therefore, recommended that detailed epidemiological investigations should be conducted in camel rearing pastoral communities of Ethiopia with particular emphasis to the epidemiology, molecular epidemiology of strains of Mycobacterium circulating in camels and zoonotic significance of Tuberculosis in camels where the habit of consuming raw camel milk is very common in these pastoral communities.

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