

## Full Length Research Paper

# Prevalence of bovine tuberculosis in dromedary camels and awareness of pastoralists about its zoonotic importance in Eastern Ethiopia

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The aim of this study was to investigate bovine tuberculosis in camels which was conducted in Eastern part of Ethiopia using post mortem examination, *Mycobacterium* isolation, tuberculin skin test and questionnaire based interview. Tuberculosis (TB) is an important disease for pastoralist, particularly due to prevailing habit of consuming raw milk and sharing house during night time with their animals. This study has showed prevalence rate of 8.3% (33/398) based on the post mortem examination and 6.0% (29/480) at cut off >4 mm based on the tuberculin test. From 33 camels with suspected tuberculosis compatible lesions, 12% (4/33) showed mycobacterial growth on Lowenstein-Jensen (LJ) media supplemented with pyruvate and 18% (6/33) on LJ media supplemented with glycerol. 94.7% (36/38) of the interviewed pastoralists knew about TB while only 50% (19/38) were aware about its zoonotic importance. However, only 18.4% (7/38) of the interviewed camel owners usually boil camel milk before consumption. The result of this study highlights the potential public health risk posed by TB from camels. Hence, prompt measures are required to control the possible zoonotic transmission of the disease-prioritizing on educating the pastoralist to consume camel milk after boiling.

**Key words:** Dromedary camel, tuberculosis-compatible lesions, tuberculin test, zoonotic importance.

## INTRODUCTION

Thirty-four percent of the land surface distributed worldwide is deserts and semi-deserts (Roger, 2006). The dromedary camel (*Camelus dromedarius*) is an important multipurpose livestock species uniquely adapted to these harsh environments that can be used for meat, milk, wool and hide production and transportation and as a source of entertainment, celebration and competition (Abdelhakim

and Youcef, 2012). There are 25.89 million dromedary camels in the world with 80% of them in Africa. Ethiopia takes the 3rd place in Africa next to Somalia and Sudan in possessing 2.40 million dromedary camels (FAO, 2011). Ethiopia camels are kept in arid and semi-arid lowlands such as Borana, Somalia and Afar regions (Teshome et al., 2003). They serve as an important source

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of milk, meat, draught power and transportation for the pastoralists (Bekele, 1999). Zoonotic infections, transmissible between humans and animals, are closely associated with pastoralists, because of their close contacts with their domesticated animals (Macpherson, 1995; Schelling et al., 2003; Li et al., 2005) and habit of raw camel milk consumption (Farah et al., 1990; Radwan et al., 1992; Younan, 2004).

Animal tuberculosis (TB) is a zoonotic disease caused by members of the *Mycobacterium tuberculosis* complex, including *M. tuberculosis*, *Mycobacterium africanum*, *Mycobacterium microti*, *Mycobacterium bovis*, *Mycobacterium caprae*, *Mycobacterium canettii* and *Mycobacterium pinnipedii* (Ayele et al., 2004). In dromedary camels, *M. tuberculosis* (Elmossalami et al., 1971; Zubairet et al., 2004), *M. bovis* (Kinne et al., 2006), *M. caprae* (Pate et al., 2006) and *M. microti* have been isolated from tissue lesions (Wernery and Kaaden, 2002; Kinne et al., 2006). *M. bovis* strains were also isolated by Donchenko et al. (1975) in Russia from bulked samples of raw dromedary milk. Recent studies in Ethiopian abattoirs also reported isolation of *M. bovis* (Mamo et al., 2011) and *M. tuberculosis* from tissue lesions (Gumi et al., 2012; Zerom et al., 2012). Five to twelve percent abattoir-based prevalence of tuberculosis compatible lesions (TCL) in dromedary camels slaughtered at Dire Dawa abattoir in Eastern Ethiopia and in Addis Ababa abattoir were recorded (Mamo et al., 2009; Mamo et al., 2011; Zerom et al., 2012).

The incidence of human TB in Ethiopia is high with an annual incidence of 261 cases/100,000 population (WHO, 2011). TB lymphadenitis in cervical lymph nodes (TBLN) accounts for ≈33% of all new cases in the country (WHO, 2011), raising the possibility of orally acquired infection. Thus the zoonotic risk arising from dromedary camel milk should be considered, because camel milk is mostly consumed in its raw state (Farah et al., 1990; Radwan et al., 1992; Younan, 2004).

This study was designed to estimate the prevalence of bovine TB among dromedary camels of Eastern Ethiopia based on post mortem examination and comparative tuberculin test; and to assess the awareness of camel holders about the zoonotic nature of dromedary TB.

## MATERIALS AND METHODS

### Study area

This study was carried out in Dire Dawa City Administrative Council (DDAC) and Somali pastoral region from June 2007 to June 2008. Dire Dawa is located between 9°49' North latitude and between 41°38' and 42°19' East longitude at 520 km East of Addis Ababa. It comprises of diversified topographic features with altitude varying from 950 m above sea level (m.a.s.l.) in the north-east to 2260 m.a.s.l. in the southwest. The mean annual rainfall in the area varies from 550 to 850 mm. The monthly mean maximum temperature ranges from 28.1°C, which is recorded in the months of December and January, to 34.6°C recorded in the month of June (Agricultural Development Office of DDAC, 2001). The livelihood of the rural

residents of DDAC is based on agriculture in which raising livestock (such as goats, camels and cattle) plays a great role.

The Somali pastoral regional state is situated in the Eastern part of Ethiopia at 4 to 11° North latitude and 40°48' East longitude. The altitude ranges between 500 and 1600 m.a.s.l. The average annual rainfall is about 560 mm. The annual daily minimum and maximum temperature is 13 and 27°C, respectively (National Meteorology Service, 2001). Eighty five percent of the population of the region makes up pastoral community. Two zones, namely Jijiga and Shinile, were selected for the purpose of this study.

### Study design

This study was a cross sectional study using post mortem examination of camels slaughtered in Dire Dawa municipal abattoir and sampling of suspicious cases for bacterial isolation and laboratory tests; a Single Intradermal Comparative Tuberculin Test (SICTT) carried out in rural parts of Dire Dawa, Shinile and Jijiga; and interview with camel holders about the awareness of the zoonotic nature of bovine TB.

### Abattoir survey

Age, sex, body condition and origin of each camel were recorded prior to slaughter. Age estimation and body condition scoring (BCS) were done according to Wilson (1984) and Faye et al. (2001), respectively.

Post mortem inspection was conducted according to procedures mentioned by Croner (1994). Thorough examination was conducted on the mandibular, parotid, retropharyngeal, bronchial, mediastinal and mesenteric lymph nodes, together with the thoracic and abdominal organs. The lobes of the left and right lungs were palpated and inspected externally then, each lobe was sectioned into about 2 cm-thick slices to facilitate the detection of lesions with sterile surgical blades. Similarly, lymph nodes were sliced into thin sections (about 2 mm-thick) and inspected for the presence of visible lesions. TB suspected tissue samples were collected in to sterile bottle in normal saline (0.9%), labelled and kept in a deep freeze (-20°C) in Dire Dawa Veterinary Diagnostic and Investigation Laboratory until being transported to Akilu Lemma Institute of Pathobiology (ALIPB), Addis Ababa University. The tissue samples were transported in cold chain using an ice box packed with ice.

### Comparative tuberculin test

Peasant associations (PA's) were randomly selected from Dire Dawa, Shinile and Jijiga, and where security was in doubt and transport was not convenient, PA's with similar conditions were replaced. Proportional number of households was randomly selected from each PA. Cluster sampling was conducted for the SICTT, a household was considered as a cluster. Camels which were younger than six months, in late pregnancy and recently calved dams were excluded from the study. The tuberculin test was done using purified protein derivatives (PPD), which was produced by Central Veterinary Laboratories, Weybridge, UK. Volumes of 0.1 ml 30,000 iu/ml and 25,000 in/ml of bovine and avian PPD were administered to the middle of the cervical skin at 10 cm apart. Before the PPD was injected each site was shaved, cleaned with alcohol and marked with ink. The skin thickness was measured before injection and after 72 h using a digital calliper. The SCITT was considered positive if the reaction to the bovine PPD was >4.0 mm greater than that to the avian PPD, inconclusive if it was only from 1.0 to 4.0 mm greater, and negative result was recorded if the reaction to the bovine PPD was equal to or smaller than that obtained at the avian site of injection (OIE, 2004).

### Questionnaire survey

Questions about the management of camels and public health importance of bovine TB were collected using a structured questionnaire. The questionnaire was administered by 'face to face' interviews with camel owners by local languages (Oromifa and Somaligna) side to side with the tuberculin test.

### Laboratory test

Direct smears were made from the centrifuged sediments of 33 suspected tuberculous tissues for direct microscopic examination and stained using the Ziehl-Neelsen staining technique (Dekanter et al., 1987). Tissue samples were macerated in a sterile Petri-dish to get fine pieces by using a sterile blade and forceps and then each sample was homogenized using a sterile mortar and pestle for 10 min in 5 ml of normal saline. Two milliliters of the homogenate was transferred to centrifuge tube and decontaminated by adding an equal volume (2 ml) of 4% NaOH by centrifugation at 3,000 rpm for 15 min. The supernatant was discarded, while the sediment was neutralized with 1% (0.1 N) HCl with phenol red as indicator. Neutralization achieved when the color of the solution changed from purple to yellow (OIE, 2004). Therefore, 0.1 ml of suspension from each sample was spread on to a slant of Lowenstein-Jensen (LJ) medium. Duplicates of LJ media were used; one was with 4% sodium pyruvate while the other was enriched with glycerol. The cultures were then incubated aerobically at 37°C for up to 12 weeks with weekly observation; Ziehl-Neelsen staining was performed to confirm the presence of acid-fast bacilli (AFB) (Quinn et al., 1994; WHO, 1998). Initial identification of mycobacterial species were based on the rate of growth, pigment production and colony morphology. Species belonging to the *M. tuberculosis* complex show a slowed growth rate (Quinn et al., 1994).

### Data management and statistical analysis

The data generated from the abattoir and the tuberculin test were analysed using STATA (Version 9) and Win Episcope epidemiologically software. The animal level prevalence was expressed as the number of camel positive to the post mortem examination and the SICTT per 100 separately. The chi-square ( $\chi^2$ ) test was carried out to study the association between bovine TB status of camels and host factors, and a test of agreement (Kappa) to compare the direct microscope and the bacterial growth (Thrusfield, 1997). The confidence level was set at 95% ( $\alpha=0.05$ ).

## RESULTS

### Post mortem examination

The detailed post mortem examination carried out at the abattoir revealed that 33 (8.3%) of the 398 examined carcasses showed TCL. Large proportions of the lesions (57.5%) were found in the thoracic lymph nodes and the lungs followed by the lymph nodes of the head (27.2%) and the complement in mesenteric lymph nodes. The average number of lesions per infected animal was 1.12, and about 87.8% of carcasses with TCL possessed only a single lesion. Out of 33 camels with TCL, 4/33 (12%) showed growth on LJ media supplemented with pyruvate and 6/33 (18%) on LJ media supplemented with glycerol.

Ziehl-Neelsen staining of centrifuged sediments of the 33 lesions smears demonstrated only 11 acid fast bacteria (AFB) positive slides. Out of these 11 AFB positive smears, only six proved positive upon bacteriological examination culture (Table 1).

The association of risk factors like sex, age and body condition with the TCL status of camels was studied using  $\chi^2$  test (Table 2). The age category showed significant difference ( $p<0.05$ ) as regard to the abattoir-based prevalence of bovine TB. The prevalence of bovine TB varies among the various origins of camels slaughtered in Dire Dawa (Figure 1). The highest was found in camels originated from PA's predominantly inhabited by Oromo ethnic group such PA's are Bishan Bahe, Mermersa and Dujuma.

### Tuberculin test

Of the total 480 examined camels by the SICTT, 29 (6.0%) of them were positive to bovine TB (Table 3). The lowest prevalence was observed in Shinile zone; though the difference among the three study sites was not statistically significant ( $p>0.05$ ). Likewise, the differences in prevalence between the sex and between different age groups were not significant ( $p>0.05$ ).

### Questionnaire survey

Thirty eight dromedary camel owners (households) were interviewed to gather information on public health risk factors of camel TB. Six (15.8%) of the interviewed respondents, house their camels during night along with the family, and only 18.4% consume milk usually after boiling (Table 4). Strikingly, none of the respondents consume raw meat. The awareness of the zoonotic nature of camel TB is 50%.

## DISCUSSION

The prevalence of bovine TB in dromedary camels in Eastern Ethiopia was 8.3 and 6.0% based on the post mortem examination and the SICTT, respectively. The abattoir-based prevalence is a little higher than a similar work done in the same place one year earlier, which was 5.1% (Mammo et al., 2009). The camels that were slaughtered in the abattoir originated from the pastoral areas where the pastoralists depend on the milk of their camels for subsistence and where no treatment of milk is practiced before consumption, either it is consumed raw or when just soured. A previous study shows that the raw camel milk plays a role in transmission of TB into human (Younan, 2004). Therefore, this finding shows the possible public health risk of the disease on the pastoral community.

Based on the culture on the LJ media, only 4 camels

**Table 1.** Comparison of direct microscopic and bacteriological examinations of samples taken from camels with tuberculous compatible lesions slaughtered in Dire Dawa municipal abattoir, 2007-2008.

Direct microscopy	Culture		Total
	Positive	Negative	
Positive	6	5	11
Negative	4	18	22
Total	10	23	33

Sensitivity= 60% (95% CI: 30-90.4), Specificity= 78.3% (95% CI: 61.5-95.2), Kappa ( $\kappa$ ) = 0.37.

**Table 2.** Association of macroscopic tuberculous lesions with host factors in dromedary camels in Dire Dawa, 2007-2008.

Variable	Category	Number of examined camels (n)	Number of positive camel (%)	$\chi^2$ (P-Value)
Sex	Male	301	24 (7.97)	4.5 (0.49)
	Female	97	9 (9.3)	
Age	≤ 5 years	11	0	6.9 (0.03*)
	6-10 years	200	17 (7.9)	
	>10 years	188	22(11.7)	
Body condition score	Poor	97	15(15.5)	8.5 (0.14)
	Medium	212	13(6.13)	
	Good	88	5(5.7)	

\*Statically significant.

**Table 3.** The result of single intradermal comparative tuberculin test carried out in Eastern Ethiopia to study the status of bovine tuberculosis in dromedary camels, 2007-2008.

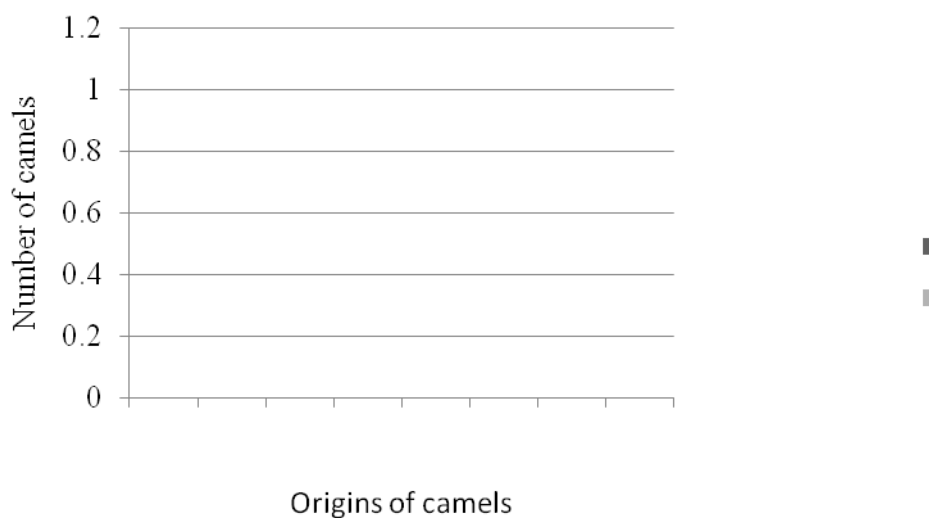
Variable	Tested camels [n (%)]	Reactors [n (%)]	$\chi^2$ (P-value)
Site	Dire Dawa	118	8 (6.8)
	Shinile	208	6 (3.9)
	Jijiga	154	15 (7.2)
Sex	Male	121	4 (3.3)
	Female	359	25 (7.0)
Age	< 4 years	120	4 (3.3)
	4 - 10 years	259	19 (7.3)
	> 10 years	101	6 (5.9)
Total	-	480	29(6.0)

(12%) showed growth on the pyruvate supplemented media and 6 camels (18%) on the glycerol supplemented media. The small number of culture positive samples

when compared with the number of macroscopic tuberculous lesions may arise from classification of non-tuberculous lesions as TCL due to similarity with parasitic

**Table 4.** The awareness of camel owners about zoonotic importance of bovine tuberculosis in camels in Eastern Ethiopia, 2007-2008.

Factor	Category	Number of respondents (%)
Housing of camels during night	Separate barn	32 (84.2)
	Along with families	6 (15.8)
Ways of milk consumption	Usually boiled	7 (18.4)
	Usually raw	21 (55.3)
	Occasionally boiled	9 (23.7)
	Occasionally raw	1 (2.6)
Ways of meat consumption	Usually cooked	38 (100)
	Usually raw	0 (0)
	Occasionally cooked	0 (0)
Awareness about TB	Occasionally raw	0 (0)
	Aware	36 (94.7)
	Non-aware	2 (5.3)
Awareness about zoonotic nature of TB	Aware	19 (50)
	Non-aware	19 (50)

**Figure 1.** Origins of dromedary camels slaughtered in Dire Dawa municipal abattoir and their status of the post mortem examination for macroscopic tuberculous lesions, 2007-2008.

granulomas and abscess and decontamination process. Nevertheless, it could also be due to the absence of viable mycobacteria in calcified lesions. In completely calcified lesions tubercle bacilli are dead and no growth will be obtained up on culture (Gracey, 1986; Quinn et al., 1994; Tekluet al., 2004) or due to inappropriate handling of samples.

TCLs were found most frequently in the lungs and associated lymph nodes (57.7%) followed by lymph nodes of the head (27.2%), which is in consistence with the findings of Mammo et al. (2009) and Zerom et al.

(2012) in camels. Croner (1990) reported that up to 95% of cattle with visible TB lesion could be identified by examination of lungs and associated lymph nodes. This finding suggests inhalation as the principal route of TB infection in cattle which might also be true in camels. The presence of lesions in the mesenteric lymph nodes (15.3%) indicates infection through ingestion (Radostitis et al., 1994). The average number of infected tissues per infected camel was 1.12, while 87.8% of the camels had only a single lesion. This finding complies with previous report on camel (Mammo et al., 2009).

The low agreement ( $\kappa=0.37$ ) between direct microscopy and bacteriology (Table 1) complies with a previous report from Ethiopia (Teklu et al., 2004) and indicates that AFB other than *Mycobacteria* (e.g. *Nocardia*, *Actinomyces*) may have produced the spurious results. Irrespectively, direct microscopy is considered to be the least sensitive of all diagnostic methods for the detection of AFB (Baron et al., 1994).

The difference in TB prevalence between male and female based on macroscopic tuberculous lesions was not statistically significant, which is consistent with Mammo et al. (2009) in camels and Assegid et al. (2004) and Teklu et al. (2004) in cattle. In contrast to sex based study, the difference in TB prevalence among different age groups is statistically significant ( $\chi^2=6.9$ ,  $P=0.03$ ). Animals older than ten years had the highest prevalence (11.6%) compared to other age groups. This is consistent with Abdurrahman and Bornstein (1992); they reported TB as chiefly disease of old camels. This may be the fact that, in spite of their susceptibility to infection, camels are very resistant to the effects of the disease and a long period elapses before they become cachectic and emaciated (Manson, 1917) and may also to develop tuberculous lesion.

Higher prevalence of the bovine TB was observed in camels that originated from PAs inhabited by Oromo society like Legaoda, BeshanBahe, Mermersa and Dujuma when compared to the camels that originated from areas inhabited by Somali society like Shinile (Table 3). The reason for this might be the difference in production system in the two camel rearing societies where mixing of camels with other livestock possibly contribute for the higher prevalence in the former ones. In Eastern Africa, the gradual spread of the camel to tribes not owning it in ancient times resulted from a pre-existing nomadic or semi nomadic way of life (Wilson, 1989) might cause change in production system that can have a great role in transmission of TB to camels. The Ethiopian Somalia camel rearing community in Shinile such as Issa is almost pure camel herders and keep their camels in open land just making a fence with thorny plants around it during night time, but in case of Oromia camel rearing areas where most of the camels are kept in close confinement with cattle, especially during night camels and cattle are kept together. Close confinement of camels and cattle together might be the source of cross infection (Mamo et al., 2011).

Based on tuberculin test, the difference in prevalence among the different age groups is insignificant, though the highest rate was observed in the middle age group (4 to 10 years). It is an established fact that TB is a chronic disease (Radostitis et al., 1994) and so the older an animal, the higher the probability of establishment of infection. But this finding does not favour this fact.

The questionnaire survey reveals that the camel rearing people are at high risk of contracting TB if their camels get infected by *Mycobacterium* species. Because,

in the society which lack general awareness of TB and its zoonotic importance and family members share the same living house with their camels during the night time, there is high possibility of transmission of the infection between humans and camels. On top of that, only few of them have the habit of boiling milk before consumption. Raw camel milk consumer of the camel rearing society and their urban customers are in higher health risk. Personal communication with health workers at Dire Dawa and Jijjiga indicated that extra-pulmonary TB is one of the common problems of the regions.

## Conclusion

The livelihood of camel rearing pastoralists is closely related with the health of their camels. Camels are kept mainly for milk production and transportation in camel rearing areas of Eastern Ethiopia. However, the finding of 8.3 and 6.0% prevalence of bovine TB in dromedary camels based on detail post mortem examination and SICTT in the present study is alarming in the population where only 50% have awareness of the zoonotic importance of TB and only 18% usually boil milk before consuming. The result of this study shows the presence of high public health risk posed by bovine TB in dromedaries up on Eastern Ethiopia camel rearing communities. Hence, prompt measures are required to control the possible zoonotic transmission of the disease; prioritizing on educating the pastoralist to consume camel milk after boiling and avoid sharing night accommodation with their camels.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

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