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Prevalence of camel tuberculosis at Akaki abattoir in Addis Ababa, Ethiopia

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A cross sectional study was conducted on 420 apparently healthy camels slaughtered at Akaki abattoir from October 2011 to March 2012 to determine the apparent prevalence of camel tuberculosis and to see its association with sex, age, origin and body condition of camels. Camels were examined for the presence of gross tuberculosis lesions and further cultured to isolate members of the genus *Mycobacterium*. The overall apparent prevalence of camel tuberculosis was 4.52% (95% confidence interval: 2.53, 6.51) based on gross tuberculosis lesion detection; 4 of them were culture positive for *Mycobacterium bovis*. The apparent prevalence of camel tuberculosis was not influenced by sex, age, origin and body condition (p>0.05). In relation to distribution of the tuberculosis lesions in body organs, 57.14% of the tuberculosis lesions were localized in the lungs and associated lymph nodes, 28.57% in the retropharyngeal lymph nodes and 14.29% in the mesenteric lymph nodes. In conclusion, this study revealed that the occurrence of tuberculosis in camels at Akaki abattoir is a common phenomenon, hence at present a public health threat. As a result, public awareness and appropriate control and prevention measures should be implemented to reduce the public health and economic burden of the disease in the country.

Key words: Akaki abattoir, culture, dromedary camel, *Mycobacterium bovis*, apparent prevalence, tuberculosis lesion.

INTRODUCTION

The dromedary camel (*Camelus dromedarius*), which is a versatile animal capable of living in harsh semi-arid and arid areas of the world, is an extremely important animal in the livelihoods of pastoral communities through provision of milk, meat and draft power for transportation of goods. In pastoral communities of Ethiopia such as Afar, Somali and Borena, camels are kept almost entirely for milk production (Getahun and Belay, 2002).

In recent years, camels have become one of the national export animals for Ethiopians. Despite its role in the livelihoods of pastoral communities and national economy, little attention has so far been given to camel production in general and health care in particular. Prevalent diseases and inaccessible/hostile environments in which camels bred are major constraints (Tegegne and Gebrewold, 1997).

Tuberculosis occurs worldwide in people, wild and domesticated or captive animals (Krauss *et al*., 2003). Tuberculosis (TB) in dromedaries has been documented since 1888 (Littlewood, 1888). *Mycobacterium tuberculosis*, *Mycobacterium bovis* and atypical Mycobacteria (*Mycobacterium kansasii*, *Mycobacterium aquae*, *Mycobacterium fortuitum*, *Mycobacterium smegmatis*) have been isolated in camels as causative agents of camel TB (Kinne et al., 2006).

The two major pathogenic species among these are *M.*

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tuberculosis and *M. bovis*, the causative agent of TB in humans and cattle, respectively. Tuberculosis caused by *M. bovis* is the most common form of tuberculosis in camels. The organism causes granulomatous abscesses in various tissues with a predilection for lymphoid tissues and lungs (Wernery and Kaaden, 2002). However, it is well known that *M. bovis* is zoonotic, while infection with *M. tuberculosis* has been sporadically reported in domestic and wild animal species, most frequently in animals living in prolonged, close contact with humans (Ameni et al., 2010).

Tuberculosis, as a zoonosis from camel to human also plays an important role among nomadic people where milk and milk products are consumed raw (Seifert, 1992). The principal agent of zoonotic tuberculosis is *M. bovis* (Krauss et al., 2003). Tuberculosis in humans remains one of the major global reportable diseases, and a rise in its incidence has caused the World Health Organization (WHO) to declare the disease a global emergency (Nakajima, 1993).

There are different modes of spread of tuberculosis between camelid herds. The introduction of an infected animal into a non-infected herd is one among others (Bush et al., 1990). The disease occurs more frequently when camels are kept in close quarters with other camels or in close contact with cattle. It is rare among camels kept under nomadic conditions (Kinne et al., 2006).

Predisposing factors for the occurrence of TB could be environmental, host and pathogen risk factors. The environmental risk factor includes housing, sharing the same shelter with humans and the stocking intensity of animals. The host risk factor: all species including human beings, body conditions, sex and age groups are susceptible to *M. bovis* (Radostits et al., 2007; Mamo et al., 2011). Also the pathogen risk factor: the causative organism is moderately resistant to heat, desiccation and many disinfectants; the virulence of *M. bovis* relates to its ability to survive and multiply in host macrophages (Quinn and Markey, 2003).

Diagnosis of tuberculosis infection in camels is often based on clinical signs, necropsy findings and specific immune response (Alvarez et al., 2011). Prevention and control of tuberculosis in livestock and wildlife species relies on timely detection and removal or slaughter of infected animals and/or herds. In camelids, this strategy is difficult to conduct because of the lack of adequate tests for live animals (Wernery et al., 2007; Alvarez et al., 2011). Measures to prevent transmission of TB should be the primary objective to be achieved with trained public health personnel, public education and proper hygienic practices (Cosivi et al., 1998).

In the past, some preliminary research work on camel TB was carried out in certain parts of Ethiopia. Cross-sectional studies conducted in Dire Dawa, Akaki and Metehara abattoirs indicated prevalence of camel TB ranging from 5 to 10% (Mamo et al., 2009; Mamo et al., 2011). Further investigation on camel TB and the identification of its risk factors are thus justified as important steps towards supporting ongoing global and national disease control efforts and to reduce the risk of zoonosis in pastoral communities in Ethiopia. Hence, the objectives of the present study are: determining the apparent prevalence of TB in camels at Akaki abattoir based on post mortem gross pathological findings, understanding the association between different risk factors and camel tuberculosis and assessing the distribution and frequency of tuberculosis lesion in different tissues or organs of slaughtered camels.

MATERIALS AND METHODS

Study area

The study was conducted at Akaki abattoir which is located in the southern outskirts of Addis Ababa. Addis Ababa is the capital city of the Federal Democratic Republic of Ethiopia. It lies in the central highlands of Ethiopia. Camels slaughtered at the Akaki abattoir originated from Borena, Kereyu and Minijar areas of Ethiopia (Figure 1). Borena is located in the Oromia National Regional State, about 600 km South of Addis Ababa. The climate of the Borena zone is semi arid. Kereyu is located in Oromia National Regional State, about 250 km East of Addis Ababa. The prevailing climate in Kereyu is arid. Minijar is located in Amhara National Regional State, North Shoa administrative Zone about 130 km East of Addis Ababa. The prevailing climate in Minijar is dry (NMSA, 1999).

Study animals

The study animals were constituted from apparently healthy camels slaughtered at Akaki abattoir during the study period. A total of 420 camels slaughtered at the abattoir from October 2011 to March 2012 were examined for tuberculous lesions. The figure comprises 166 male and 254 female camels. The camels slaughtered at the abattoir were transported from their areas of origin to the Akaki abattoir on trucks and kept at the lairage for 1 to 7 days.

Study design

A cross sectional study was undertaken to determine the apparent prevalence of camel TB at Addis Ababa Akaki Abattoir by postmortem examination and mycobacterial culture. All camels slaughtered on each visiting day were examined and sampled. A total of 420 camels were slaughtered during the study period. Four visits were made to the abattoir in each week of the study period. The visiting days were selected randomly. In each visiting day 8 to 10 camels were examined and sampled.

Individual camels were carefully identified, and the sex, age, origin and body condition score (BCS) were recorded. Body condition score was categorized into three groups: poor, medium and good which is determined by hump structure of the camel (CACIA, 2001). Age category was determined by using the dental eruption and wear as described by Schwartz and Dioli (1992). These parameters (sex, age, origin and body condition score) were assessed for the presence of possible association with the presence of TB lesion.

Postmortem examination

Thorough postmortem examination was performed following the

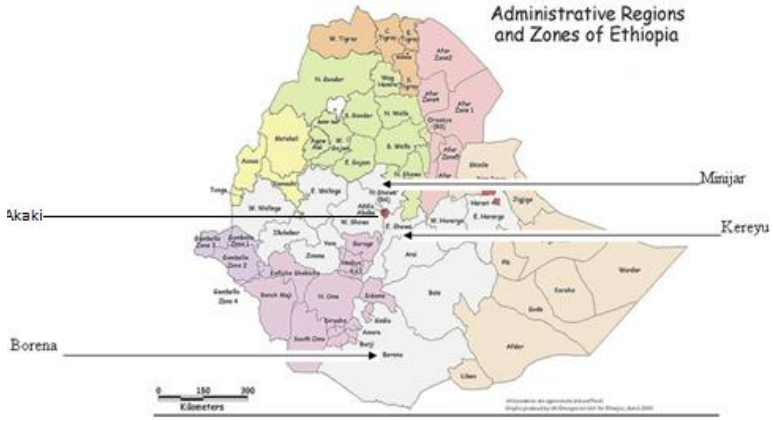

Figure 1. Zones of Ethiopia Map, showing the probable origin of camels (Borena, Kereyu and Minijar) slaughtered at the Akaki abattoir.

procedure as previously described by Corner (1994) and Asseged et al. (2004). Mandibular, retropharyngeal, bronchial, mediastinal, mesenteric and hepatic lymph nodes were examined and organs including lungs, liver, small intestine and kidneys were closely examined. Camels with macroscopic lesions varying from firm or hard white, grey, or yellow nodule with a yellow, caseous, necrotic centre that was dry and solid to thin walled suppurative abscesses were classified as post mortem positive (Smith, 2009). Tuberculous lesions from slaughtered camels were aseptically collected into sterile universal bottles containing 5 ml of 0.9% saline solution for mycobacteriological isolation. The samples were kept in an icebox with solid packs, transported to Aklilu Lemma Institute of Pathobiology (ALIPB) and stored at +2 to +8°C until mycobacteriological culturing was carried out in the TB laboratory.

Microbiological culturing and Ziehl-Neelsen (Z-N) staining

In ALIPB TB laboratory, tissue samples were cut in a sterile petridish using sterile blade and forceps to get fine pieces, and then each sample was homogenized using sterile mortar and pestle for 10 min in 5 ml of normal saline. Then, 2 ml of the homogenate was transferred to a centrifuge tube, decontaminated by adding an equal volume (2 ml) of 4% NaOH and centrifugation at 3 000 rpm for 15 min. The supernatant was decanted, while the sediment was neutralized with 1% (0.1N) HCl with phenol red as an indicator. Neutralization was achieved when the color of the solution changed from purple to yellow (OIE, 2000). Thereafter, 0.1ml of suspension from each sample was spread on to a slant of Lowenstein-Jensen (L-J) medium. Duplicates of L-J medium were used; one was enriched with 4% sodium pyruvate while the other was enriched with glycerol. Cultures were then incubated aerobically at 37°C for up to 8 to 12 weeks with weekly observation for growth of colonies. When visible colonies were observed, Ziehl-Neelsen staining was performed to confirm the presence of acid-fast bacilli (WHO, 1998; Quinn et al., 2002). Mycobacterium species were identified based on their cultural characteristics and biochemical reactions (Quinn et al., 2002).

Data management and analysis

Data collected from the study was entered in to MS Excel spread-sheets and analyzed using Stata 11 software package. The apparent prevalence was calculated as the number of camels harbouring TB lesion in their tissue and organ divided by the total number of camels examined. The degree of association between each risk factor and the occurrence of camel TB lesions was assessed using the Pearson Chi-square (X^2) and Fisher's exact tests. For all analysis, a p-value of less than 0.05 was considered as significant.

RESULTS

The overall apparent prevalence of TB in camels slaughtered at Akaki abattoir during the study period was 4.52% (95% confidence interval (95% CI): 2.53, 6.51) based on gross tuberculosis lesion detection. Macroscopically the common lesions seen in many of the affected organs and/or lymph nodes were circumscribed caseous yellowish masses of various sizes and numbers. The lesions were more conspicuous in cranial mediastinal and retropharyngeal lymph nodes. Miliary lesions were observed in the lung and mesentery in 2 of the 19 (10.53%) positive cases.

The number of male camels examined (166) was far lower than their female counterparts (254). Of these, 4 of the males (2.41%) and 15 of the females (5.91%) were tuberculose camels (Table 1). The greatest majority of examined camels were aged more than 10 years. None of the 22 camels aged less than 5 years were harboring detectable TB lesions. Of the 95 camels aged between 5 and 10 years, 7 (7.37%) had TB lesions in their visceral organs. In the older age category (>10 years), of the 303 camels examined, 12 (3.96%) were also harboring TB lesions in their lungs and visceral lymph nodes (Table 1). There was no discernable statistical association between age (p=0.265) as well as sex (p=0.293) of animals and apparent prevalence of TB lesions.

Relatively a higher TB apparent prevalence rate of camel tuberculosis was found in camels originated from Kereyu (20%) as compared with those from Borena (4.79%). None of the camels from Minjar presented any TB

Table 1. Apparent prevalence of camel tuberculosis in relation to sex, age, origin and body condition.

Variable	Category	No of camels Examined	No of camels with TB lesions	Proportion of camels with TB lesions (95% CI)	P value
Sex	Male	166	4	2.41 (0.08-4.74)	0.293
	Female	254	15	5.91 (3.01-8.81)	
Age (years)	<5	22	0	0.00	0.265
	5-10	95	7	7.37 (2.12-12.62)	
	>10	303	12	3.96 (1.76-6.16)	
Body condition	Poor	121	7	5.79 (1.63-9.95)	0.501
	Medium	210	7	3.33 (0.90-5.76)	
	Good	89	5	5.62 (0.84-10.40)	
Origin	Borena	376	18	4.79 (2.63-6.95)	0.113
	Minjar	39	0	0.00	
	Kereyu	5	1	20.00 (0-55.06)	

Table 2. Distribution of TB lesions in the lymph nodes and lungs of inspected camels and their culture results.

Organs/tissues inspected	No (%) of camels with gross TB lesions	No (%) of camels positive for culture
Lymph Nodes		
Cranial mediastenal	5 (23.81%)	1 (20.00%)
Retropharangeal	6 (28.57%)	1 (16.67%)
Mesentric	3 (14.29%)	0 (0.00%)
Right bronchial	2 (9.52%)	0 (0.00%)
Lung lobes		
Right cardiac	3 (14.29%)	2 (66.67%)
Right apical	1 (4.76%)	0 (0.0%)
Left apical	1 (4.76%)	0 (0.0%)

lesions. Once again, there is no statistically significant association (p=0.113) among the three areas in terms of apparent prevalence of TB lesions (Table 1). Regarding body condition, the highest (5.79%) apparent prevalence was recorded in camels with poor body condition score, whereas the lowest (3.33%) apparent prevalence was detected in the medium body condition score category. However, there was no statistically significant (p=0.501) difference in TB apparent prevalence among camels of different body condition scores (Table 1).

The distribution and frequency of tuberculous lesions in different organs is presented in Table 2. Tuberculosis lesions were detected in the lung lobe (5 cases) and lymph nodes (16 cases). In the lung, the lesions occur more frequently in the cardiac lobe (14.29%). The frequency of occurrence of TB lesions in lymph nodes is also variable: retropharyngeal lymph nodes (28.57%), cranial mediastenal lymph nodes (23.81%), mesenteric lymph nodes (14.29%) and bronchial lymph nodes (9.52%). This means that TB lesions in camels are most frequently (57.14%) found in the lungs and associated lymph nodes (Table 2).

From the total of 21 TB lesion samples cultured into L-J medium only 4 (19.05%) showed growth in pyruvate-enriched L-J medium, which indicates the presence of Mycobacterium species. High culture positivity was observed, in descending order of importance, from right cardiac lung lobe (66.67%), cranial mediastenal (20%) and retropharangeal lymph nodes (16.67%) (Table 2). Positive cultures were confirmed with Ziehl-Nelseen staining indicating the presence of Acid-fast bacilli and by identifying *Mycobacterium bovis*.

DISCUSSION

In general, the information on camel TB is scanty. Few reports have been published on camel TB in Ethiopia and

other countries. In the present abattoir-based study TB lesions were detected in 4.52% of camels. This apparent prevalence is in agreement with the findings of previous studies done with similar diagnostic methods (Mamo et al., 2009) at Dire Dewa Abattoir and additionally the apparent prevalence coincides with previous work done by Zubair et al. (2004). The current apparent prevalence was found lower than that of 10.04% (Mamo et al., 2011) at Akaki and Metehara Abattoir. The reasons for this might be due to the decrease in proximity or contact between camel and cattle at the pastoralist area (Kinne et al., 2006) and consideration of non-tubercle lesions as tubercle during post mortem examination in the previous studies. Though the apparent prevalence shows a tendency to decrease when compared with the previous studies, the figure reported in this study is still high enough to be a public health concern, since milk and milk products are often consumed raw or unpasteurized in pastoralist (camel rearing) areas (Seifert, 1992).

Tuberculosis lesions were found most frequently in the lungs and associated lymph nodes (57.14%), retropharyngeal lymph nodes (28.57%), and mesenteric lymph nodes (14.29%). Similar findings were previously reported by Kinne et al. (2006), and Windsor (1999). Other studies on bovine TB in Ethiopia have also reported similar results (Regassa, 1999; Asseged et al., 2004). This finding indicates that inhalation might be the principal route of TB infection in camels. The presence of TB lesions in mesenteric lymph nodes also indicates the existence of infection through ingestion (Radostits et al., 2007).

In the present study, in general, low culture positivity rate (19.05%) was observed. This is not in agreement with the findings of Mamo et al. (2009) and Mamo et al. (2011), who reported 28.6% and 34% positivity rate respectively in their previous studies. Such low culture positivity rate in the present study might be due to the absence of viable mycobacteria in calcified TB lesions. It has been established that in completely calcified lesions, tubercle bacilli are dead and, therefore, there will be no growth up on L-J media (Quinn et al., 2002). The lower culture positivity might be also related to the non-optimal condition of the culture (Mamo et al., 2011). The other reason might be that the cultured lesions might be healed and mycobacteria could not be isolated from healed lesions (Bush et al., 1990).

Absence of statistically significant difference between TB's apparent prevalence on the one hand and sex, age and BCS of examined animals, on the other hand, is consistent with the findings of other scholars (Mamo et al., 2009) in the field. Although, the difference is not statistically significant, higher TB infection was observed in camels within the age group of 5 to 10 years (7.37%) and no infection in the age group of < 5 years. This might be due to longer exposure and life span of the older animals than the younger ones. It might be also due to the chronic nature of the disease in which the animals might have acquired the infection at young age and developed the clinical signs at old age (Radostits et al., 2007).

In this study, TB pathological lesion was more frequently observed in female (5.91%) camels as compared to male (2.41%) camels. This finding is in agreement with previous studies in Ethiopia by Mamo et al. (2009) who reported a higher prevalence (6.25%) in female camels. This could be due to the fact that female camels were brought for slaughter at an older age after completion of their reproductive life (Inangolet et al., 2008; Munyeme et al., 2009) and this has directly related to long time exposure for TB.

There was no statistically significant difference (P=0.501) in the occurrence of camel tuberculosis among the three BCS categories, with the apparent prevalence being higher in poor body condition (5.79%) compared to medium BCS (3.33%). This finding is consistent with previous reports, which indicated that the animals with poor body condition score have relatively low resistance to infectious agents (Radostitis et al., 2007).

It is obvious that tuberculosis is a huge problem in Ethiopia where there is a high incidence of the disease in human, cattle, camel and other populations. A test and slaughter program for livestock is not really feasible and there is no effective vaccination, though bacille Calmette-Guérin (BCG) vaccine is used in humans and domestic livestock. Yet there is no attempt to vaccinate camels against tuberculosis. Therefore future research shall aim at the finding of an effective vaccine against camel tuberculosis.

Conclusion

The 4.52% apparent prevalence of camel tuberculosis recorded in this study is relatively lower than the previous study on the same or other abattoirs in Ethiopia. However, given the habit of raw milk consumption in pastoral communities the result is still important, in view of its public health implications. The result also revealed that the occurrence of camel TB does not have association with any of the factors (sex, age, BCS and origin of examined animals) considered in the study. The fact that most of the TB lesions detected were in the lungs and associated lymph nodes signifies the importance of the respiratory route for the entrance of mycobacteria.

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