Tuberculosis in dromedaries in eastern Ethiopia: Abattoir-based prevalence and molecular typing of its causative agents

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A B S T R A C T
Although tuberculosis is endemic in cattle in Ethiopia, little information is available on tuberculosis in dromedaries. Thus, this study was designed to investigate the epidemiology of tuberculosis and its causative agents in dromedaries slaughtered at four representative abattoirs in eastern Ethiopia. A total of 293 dromedaries were examined by detailed post-mortem examination and the prevalence of tuberculosis-compatible lesion (TCL) was 12.3% (36/293), and occurrence of lesion was significantly associated with female dromedaries (95% confidence interval [CI] [0.19–0.97]). Mycobacteria were isolated in 61% (22/36) of the dromedaries with gross lesions. Further characterization of the isolates using PCR showed that 68% (15/22) of the isolates were non-tuberculosis mycobacteria (NTM) while 13.6% (3/22) were Mycobacterium tuberculosis (M. tuberculosis) and 18% (4/22) were not members of the genus Mycobacterium. Spoligotyping of the three M. tuberculosis isolates revealed that one of the three isolates was SIT 21 while the remaining two isolates with octal values of 77357776763671 and 773357777763661 were not reported to the SITVIT database. The isolation of large proportion of NTM from tuberculosis-compatible lesions in dromedary is suggestive of these bacteria being pathogenic to the species, while the isolation of M. tuberculosis from dromedary carcasses highlights the zoonotic risk represented by consuming the meat.

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1. Introduction

Tuberculosis is a chronic disease caused by bacteria of the genus Mycobacterium that affects several animal species. It is characterized by the development of tubercles in the organs of most species (FAO, 2000). The disease had long been diagnosed in dromedaries in Egypt (Littlewood, 1888) and in India (Lingard, 1905; Leese, 1908). Acid-fast organisms from lung lesions of dromedaries resembling miliary tuberculosis were isolated in 1910 by Archibald in Egypt. Members of the Mycobacterium tuberculosis complex including M. tuberculosis (Elmossalam et al., 1971; Zubair et al., 2004), Mycobacterium bovis (M. bovis) (Kinne et al., 2006; Mamo et al., 2011), and M. capre (Pate et al., 2006) and non-tuberculosis mycobacteria including M. avium, M. kansasii (Elmossalam et al., 1971; Strauss, 1995), M. auque, M. fortuitum and M. smegmatis (Elmossalam et al., 1971; Wernery et al., 2002) have been isolated from tuberculosis like lesions in dromedaries. Furthermore, M. bovis strains were also isolated by Donchenko et al. (1975) in Russia from bulked samples of raw dromedary milk.

As in most dry lands of Africa and Asia, in Ethiopia dromedaries are the principal source of income and food
for millions of pastoralists (Getahun and Belay, 2002). They serve as an important source of milk, meat, draught power and transportation for the pastoralists in eastern and southern Ethiopia (Bekele, 1999). In these communities, the risk of acquiring zoonotic tuberculosis from dromedary milk should be considered because dromedary milk is usually consumed raw (Radwan et al., 1992; Younann, 2004) or when it just fermented (Farah et al., 1990). Ethiopia is one of the African countries where tuberculosis is widespread in both humans and cattle and the endemic nature of tuberculosis in humans and cattle has long been documented (Shitaye et al., 2007). Regarding tuberculosis in dromedaries in Ethiopia, there are only very few studies conducted so far. Richard was quoted by Mustafa (1987) and Wernery et al. (2002) reporting the existence of dromedary tuberculosis in Ethiopia for the first time. Additional reports of the occurrence of tuberculosis in dromedaries were published in recent years (Mamo et al., 2009, 2011).

However, although the findings of earlier studies are useful indicators of the occurrence of tuberculosis in dromedaries, additional studies are required to provide the true epidemiological picture of the disease in the whole population of dromedaries in the country. The present study was designed to estimate the prevalence of tuberculosis-like lesions, and to isolate and characterize any mycobacteria from tuberculosis-like lesions found in dromedaries slaughtered in eastern Ethiopia.

2. Materials and methods

2.1. Sample selection

The study was carried out on 293 apparently healthy dromedaries slaughtered at four abattoirs located 500–600 km east of Addis Ababa, the capital of Ethiopia. After arriving the abattoirs the dromedaries were subjected to ante-mortem examination. Samples were collected for five consecutive months starting from the end of November 2010 until the end of April 2011. During this period the numbers of dromedaries examined per slaughterhouse were 92 in Dire Dawa, 11 in Harar, 16 in Aweday and 172 in Jigjiga abattoirs. Identifying a precise geographical origin for each investigated dromedary was not possible. The dromedaries slaughtered at these abattoirs were both male and female, and originated from eastern pastoral parts of the country.

Census sampling was used to collect all the tissue samples from all dromedaries which were slaughtered in the abattoirs. On average, four dromedaries were slaughtered per week in Dire Dawa, 2 in Aweday and Harar, and 14 in jiggiga abattoirs. The meat of these dromedaries is used for local consumption. Age estimation and body condition scoring (BCS) was done during ante-mortem examination according to Australian Camel Industry Association Guidelines, which uses teeth eruption for age estimation, and hump and superficial body conformation for body condition scoring (CACIA, 2010).

2.2. Post mortem examination and bacteriological culturing

The procedure described by (Croner, 1994) was followed during post mortem examination. Mandibular, parotid, retropharyngeal, bronchial, mediastinal and mesenteric lymph nodes, together with thoracic and abdominal organs were examined in details under a bright-light source. In particular, 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. Then, lesions which were indicative of tuberculosis were collected and placed in 50 ml tubes with screw caps containing 5 ml of sterile 0.9% saline solution. The specimens were transported to the laboratory in fridge temperature. During mycobacterial culturing, the OIE (2004) procedure was followed. The specimens were sectioned using sterile blades and then homogenized with a mortar and pestle. The homogenate was decontaminated by adding an equal volume of 4% NaOH and centrifugation at 3000 rpm for 15 min. The supernatant was discarded, and the sediment was neutralized by 1% (0.1 N) HCl using phenol red as an indicator. Neutralization was achieved when the color of the solution changed from purple to yellow. Next, 0.1 ml of suspension from each sample was spread onto a slant of Lowenstein–Jensen (LJ) medium. Duplicate slants were used, one enriched with sodium pyruvate and the other enriched with glycerol. Cultures were incubated aerobically at 37°C for about 10 weeks with weekly observation for growth of colonies. Cultures were considered negative if no visible growth was detected after 10 weeks of incubation. In the presence of visible growth of colonies, microscopic examination of cultures using the Zielh–Neelsen (ZN) staining method was performed to select acid-fast bacilli (AFB) positive isolates.

2.3. Multiplex polymerase chain reaction

Acid-fast colonies were harvested and heat-killed at 80°C for 1 h and then after processed for molecular typing. Mycobacterial genus typing was conducted as described by Wilton and Cousins (1992). Heat killed AFB positive samples were used as source of DNA template. DNA amplification was done with 20 μl reaction volumes consisting of: 5 μl of genomic DNA as a template; 8 μl of Hotstar Taq Master Mix; 0.3 μl of each of the six primers; and 5.2 μl of water (Qiagen). The primers used for amplification were MYCCEN-F, 5’ AGA GTT TGA TCC TGT CTC AG 3’ (35 ng/μl); MYCCEN-R, 5’ TCC ACA CAG GCC ACA AGG GA 3’ (35 ng/μl); MYCAV-F, 5’ ACC AGA AGA CAT GCC TGT CG 3’ (35 ng/μl); MYCAV-R, 5’ CCT TTA GGC CCA TGA TGT CTT TA 3’ (75 ng/μl); TB1-F, 5’ GAA CAA TCT GCA GTT CAC AA 3’ (20 ng/μl); TB1-R, 5’ AGC ACG CTG TCA ATC ATG TA 3’ (20 ng/μl). M. bovis strain 2122/97 and M. tuberculosis were used as positive control while water (Qiagen) was used as negative control. The reaction mixture was heated in Thermal Cycler (Applied biosystem; PTC-100TM) using the following amplification program: 95°C for 10 min for enzyme activation, and 35 cycles consisting of 95°C for 1 min, 61°C for 0.5 min, 72°C for 2 min and finally the reaction mixture was kept at 72°C for 10 min.

The PCR product was electrophoresed in 1% agarose gel in 1XTAE running buffer. Ethidium bromide at a ratio of 1:10 in 1% agarose gel, 100 bp DNA ladder, and orange 6x loading dye were used during gel electrophoresis. The gel was visualized in Multi-image TM light cabinet using Alpha Innotech version 1.20.1 (Alpha Innotech Corporation). All members of the genus Mycobacterium produce a band of 1030 bp, M. avium or subspecies such as M. avium subspecies paratuberculosis produces a band of 180 bp, while members of M. tuberculosis complex produce a band with 372 bp.

2.4. Spoligotyping

Spoligotyping was performed on genomic DNA following the procedure described by Kamerbeek et al. (1997). A total volume of 25 ml of the following reaction mixture was used for the PCR: 12.5 μl of Hotstar Taq Master Mix (Qiagen: this solution provides a final concentration of 1.3 mM MgCl₂ and 200 mM of each deoxynucleotides triphosphates), 2 μl of each primer (20 μM each), 5 μl suspension of heat-killed cells (approximately 10–50 ng) and 3.5 ml distilled water. The mixture was heated for 15 min at 96°C and then subjected to 30 cycles of 1 min at 96°C, 1 min at 55°C and 30s at 72°C. The amplified product was hybridized to a set of 43 immobilized oligonucleotides, each corresponding to one of the unique spacer DNA sequences within the DR locus. After hybridization the membrane was washed twice for 10 min in 2 × SSPE (1 × SSPE is 0.18 M NaCl, 10 mM Na₂HPO₄ and 1 mM EDTA [pH 7.7]) and 0.5 percent sodium dodecyl sulfate at 60°C and then incubated in 1:4000 diluted streptavidin-peroxidase (Boehringer) for 60 min at 42°C. The membrane was washed twice for 10 min in 2 × SSPE and 0.5 percent sodium dodecyl sulfate at 42°C and rinsed with 2 × SSPE for 5 min at room temperature. Hybridizing DNA was detected by the enhanced chemiluminescence method (Amersham) and by exposure to X-ray film (Hyperfilm EC) as specified by the manufacturer.
Data were entered into Excel spread sheet, and transferred to STATA software version 11 and analyzed. Pearson chi-square was used to evaluate the statistical significance of variables. Bivariate and multivariate logistic regression analyses were performed to assess the strength of associations of selected factors and prevalence of tuberculosis compatible lesion of dromedary. A probability value $P<0.05$, and the 95% confidence interval for adjusted odds ratio (OR) that does not include 1 was considered statistically significant.

3. Results

3.1. Lesion prevalence and analysis of different risk factors

The prevalence of tuberculosis-compatible lesions was 12.3% (36/293) on the bases of detailed post mortem examination. Mycobacteria were isolated in 61% (22/36) of the dromedaries with suspicious TB lesions. The association of risk factors and prevalence is presented in Table 1. The prevalence was affected by sex (95% confidence interval (CI) [0.19–0.97]).

3.2. Lesion distribution

Out of 36 camels with suspicious tuberculosis lesion, 24 (66.7%) harbored lung lesions, 8 (22.2%) lymph nodes lesions while the remaining 4 (11.1%) had lesions on both lungs and lymph nodes. Macroscopically, most of the lesions were encapsulated caseous masses and circumscribed grayish to white calcified masses of various sizes. Thick, grayish white exudates was also found with some area of consolidation in retropharyngeal, submandibular, and prescapular lymph nodes and lungs.

3.3. Mycobacterial isolation

From the 36 dromedaries with visible tuberculosis-compatible lesions, mycobacterial growth was observed in 61% (22/36). Mycobacterial isolation was highest in the bronchial lymph node followed by retropharyngeal and the mediastinal lymph nodes while the yield was low in the lung and mandibular lesions. Mycobacterial growth could not be obtained from mesenteric and prescapular lymph node lesions.

### Table 1

Association and logistic regression analysis of host risk factors with post mortem tuberculosis-compatible lesion.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. (%) Examined</th>
<th>No. (%) PM Positive</th>
<th>$\chi^2$</th>
<th>$P$-value</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
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<tbody>
<tr>
<td>Sex</td>
<td></td>
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<tr>
<td>Female</td>
<td>141 (48.1)</td>
<td>25 (17.7)</td>
<td>7.474</td>
<td>0.006</td>
<td>1</td>
<td>0.36 (0.17–0.77)</td>
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<tr>
<td>Male</td>
<td>152 (51.9)</td>
<td>11 (7.2)</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
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<td></td>
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<tr>
<td>≤5 years</td>
<td>19 (6.5)</td>
<td>1 (5.3)</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>6–10 years</td>
<td>111 (37.9)</td>
<td>13 (11.7)</td>
<td>1.125</td>
<td>0.565</td>
<td>2.4 (0.29–19.40)</td>
<td>1.33 (0.15–12.13)</td>
</tr>
<tr>
<td>&gt;10 years</td>
<td>163 (55.6)</td>
<td>22 (13.5)</td>
<td></td>
<td></td>
<td>2.8 (0.37–22.10)</td>
<td>1.29 (0.142–11.69)</td>
</tr>
<tr>
<td>BCS</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Poor</td>
<td>126 (41)</td>
<td>23 (18.2)</td>
<td></td>
<td></td>
<td>1</td>
<td>0.38 (0.18–0.81)</td>
</tr>
<tr>
<td>Medium</td>
<td>152 (51.8)</td>
<td>12 (7.9)</td>
<td>7.323</td>
<td>0.025</td>
<td>0.32 (0.04–2.6)</td>
<td>1.06 (0.11–10.12)</td>
</tr>
<tr>
<td>Good</td>
<td>15 (5.1)</td>
<td>1 (6.7)</td>
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</tbody>
</table>

BCS, body condition scoring; $\chi^2$, chi square; CI, confidence interval; OR, odds ratio; PM, post mortem.

* Statistically significant.

3.4. Molecular characteristics of the isolates

*Mycobacterium* genus typing was carried out on the 22 culture isolates; 16 were identified as member of non-tuberculosis mycobacteria (NTM) and only three were members of *M. tuberculosis* complex (Fig. 1).

The three *M. tuberculosis* complex members were further characterized by spoligotyping (Fig. 2) and they were confirmed to be *M. tuberculosis* strains. Spoligotyping of the three *M. tuberculosis* isolates revealed that one of the three isolates with the octal value of 70337740001771 was SIT 21. The remaining two strains with octal values of 773357776763671 and 773357777763661 were not found in the SITVIT database and hence they were isolated for the first time. The two *M. tuberculosis* strains namely SIT 21 and one of the new strains with octal value of 773357776763671 were isolated from lung lesion, and lesions of lung and mediastinal lymph nodes, respectively from two different cases slaughtered in Jigiga abattoir. While the remaining other new strain with octal value 773357777763661 was isolated from lung lesion in Dire Dawa abattoir. Identification of any epidemiological link between these dromedaries was not possible.

4. Discussion

In the present study, tuberculosis-compatible lesions were investigated in dromedaries slaughtered in eastern Ethiopia using post mortem examination. Prevalence of tuberculosis-compatible lesion was estimated in these dromedaries, and mycobacterial isolation and characterization were performed from gross lesions. The prevalence estimated by the present study was higher than that reported from Dire Dawa and Akaki abattoirs in Ethiopia (Mamo et al., 2009, 2011) on the basis of detailed post-mortem examination while it was lower than the prevalence reported in Kenya on the basis of intradermal tuberculin testing of dromedaries (Wernery et al., 2002). Furthermore, when compared with a similar study in cattle, this prevalence was higher than that reported previously (Berg et al., 2009). However, abattoir-based prevalence of tuberculosis in cattle is mainly reported from highlands and little or no data are available from pastoral regions of the country (Shitaye et al., 2007).

All of the study dromedaries originated from the pastoral areas and the pastoralists depend on the milk of their
dromedaries for subsistence. However, milk is not boiled before consumption for cultural reasons and thus it is consumed raw. Therefore, the result of the present study could suggest public health risk of milk as source of mycobacterial infection. Moreover, as lesions were found most frequently in the lungs and associated lymph nodes, inhalation could be the route of transmission of mycobacteria between dromedaries and their owners. Previous studies have also reported large proportion of lesions in the lungs and associated lymph nodes in dromedary (Mamo et al., 2009) and in cattle (Crone, 1994; Whipple et al., 1996). Such findings could suggest inhalation as the principal route of infection. The occurrence of lesions was significantly associated with female dromedaries as compared to males. Previous studies have also indicated similar results in dromedaries (Mamo et al., 2009) and in cattle (Miliano-Suuzo et al., 2000; Teklu et al., 2004). The high prevalence of the disease in female animals could be due to their longer productive life and other stressful factors (such as pregnancy, parturition, lactation, etc.) associated with female animals (Radostitis et al., 1994; Miliano-Suuzo et al., 2000; Teklu et al., 2004).

The isolation of NTM from most of dromedary having tuberculosis-compatible nodules with granulomatous and caseous lesions in lymph nodes and lung agrees with the previous reports by Mamo et al. (2009) in Ethiopia, Elmossalami et al. (1971) in Egypt and Strauss (1995) in Germany. Similarly, NTM were found to be the predominant isolates from cattle with tuberculosis lesions in different regions of Ethiopia (Berg et al., 2009; Tschopp et al., 2010; Ameni et al., 2011). These studies in general signify the major role of NTM as a cause of tuberculosis-compatible pathological lesions in Ethiopian livestock kept under extensive production system. The isolation of NTM from tubercle lesions underlines their roles in causing lesions; further investigations are required to identify the specific species, the source of infections, transmission route, and the pathogenicity. Moreover, the role and public health significance of NTM in human population of the region should be investigated.

The identification of M. tuberculosis strains from dromedary tissue samples could suggest the possibility of transmission from human to dromedary and its zoonotic risk. Humans suffering from active TB are the most probable source of M. tuberculosis in dromedaries and the infection could spread through sputum, and rarely urine or faeces. Similarly, previous studies have reported the isolation of M. tuberculosis from lesions in cattle from developing countries. Infection rates of 6.2% and 7.4% have been reported in Algeria and Sudan, respectively (Boulahbal et al., 1978; Sulimenan and Hamid, 2002), and a recent slaughterhouse study from Ethiopia indicated that around 7–27% of isolates were M. tuberculosis (Berg et al., 2009; Ameni et al., 2011). Although livestock, wildlife and pastoralists share intensively the same habitat (Tschopp et al., 2010), interestingly there was no M. bovis positive culture isolates obtained from dromedaries tissue in this study. This may suggest that either M. bovis growth may be impeded by over growing of NTMs or by insufficient incubation period (10 weeks were used in this study). According to Corner et al. (2011), incubation period of less than 12 weeks could lead to a significant number of M. bovis infected samples being misclassified.

5. Conclusions

The majority of the isolates from most of the suspicious lesions were NTM which could suggest the pathogenic role of some NTM in dromedary. Moreover, the isolation of M. tuberculosis from tuberculosis lesions of dromedaries could suggest the zoonotic risk represented by consuming the meat.
Conflict of interest

The authors declare no conflict of interest.

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References


