Mycobacterial infections in carcasses of ruminants slaughtered at the two slaughterhouses of Kassala, Sudan

Yassir A. Shuaib[1,2,3*] Stefan Niemann[1]
Eltahir A. G. Khalil[4] Ulrich Schaible[1] Lothar H. Wieler[3,5]
Mohammed A. Bakheit[6] Saad E. Mohamed-Noor[2]
Mohamed A. Abdalla[2] Elvira Richter[1,7]

Summary

Tuberculosis (TB) is a chronic bacterial disease of humans and animals. It is characterized by the progressive development of specific granulomatous lesions in affected organs. Human TB is endemic in EasternSudan. However, knowledge on the epidemiology of TB in ruminants is scarce. In a six-month study from June to November 2014, a total of 2304 carcasses of cattle, sheep, goats and camels slaughtered at the East and West Gaash slaughterhouses of Kassala were inspected to investigate TB prevalence. Only 0.1% (n = 2) of the carcasses had suspicious TB lesions. These lesions were solely found in carcasses of sheep, in the liver, lungs, and peritoneal cavity. The samples collected from the lesions were investigated for the presence of mycobacteria, which were found in one of the two carcasses. The grown bacteria were subjected to a line probe assay (GenoType Mycobacterium CM), and to 16S rDNA and ITS gene sequencing, and whole genome sequencing (WGS). However, none of these methods identified this isolate as a valid *Mycobacterium* species. Nevertheless, 16S sequence allocated this isolate to slow growing mycobacteria. Neither *Mycobacterium bovis* nor *M. caprae* nor *M. tuberculosis* were found in the collected granulomatous lesions. In conclusion, the overall prevalence of TB-suggestive lesions in ruminants in Kassala was very low. Extended studies combining the use of a tuberculin skin test and slaughterhouse-based investigations should be adopted for TB surveillance in ruminants in Sudan.

1. Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany.

2. College of Veterinary Medicine, Sudan University of Science and Technology, Khartoum, Sudan.

3. Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin, Germany.

4. Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan.

5. Robert Koch Institute, Berlin, Germany.

6. Faculty of Veterinary Medicine, University of Khartoum, Khartoum, Sudan.

7. Labor Limbach, Heidelberg, Germany.

* Corresponding author
Tel.: +49 453 71 88 27 50; Email: vet.aboamar@gmail.com

■ INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease of both humans and animals. It is characterized by progressive development of granulomatous lesions in affected tissues and organs (Shitaye et al., 2006; Theon et al., 2006). TB is caused by slow growing non-pigmented bacilli that belong to the *Mycobacterium tuberculosis* complex (MTBC). *Mycobacterium bovis*, *M. caprae*, and *M. tuberculosis* are responsible for causing TB in cattle, goats, and humans respectively and occasionally in other animal species (Malone et al., 2003; Smith et al., 2006; Radostits et al., 2007; Marianelli et al., 2010; Mamo et al., 2011). Moreover, different species of non-tuberculous mycobacteria are able to cause infections in different species of animals (Radostits et al., 2007; Asil et al., 2013; Aljameel et al., 2014).

Worldwide prevalence of TB in cattle and other ruminants varies greatly by region and country. The disease has been effectively controlled in many countries but it is still prevalent in many others (Schiller et al., 2010). In Africa, around 85% of cattle and 82% of human populations live in areas where bovine TB is poorly controlled. In these areas, zoonotic TB caused by *M. bovis* is common (Ayele et al., 2004; Smith et al., 2006; Kulchavenya, 2014). Human TB is endemic in Eastern Sudan, however, knowledge on the prevalence of TB in ruminants is scarce and its epidemiology is not very well understood. This is attributed to the lack of systemic surveys and monitoring programs (Asil et al., 2013). Inadequate animal health infrastructures, traditional extensive grazing system for rearing animals, and a lesser interest in TB are other significant reasons (Asil et al., 2013; Ehsan and Nganwa, 2014).

Several diagnostic techniques can be used to investigate TB prevalence in livestock and to understand better its epidemiology and dynamics (Ramos et al., 2015). Tuberculin skin test and postmortem inspection remain the most important and economically affordable techniques, especially in resource-limited countries (Schiller et al., 2010; Ramos et al., 2015). Recently, few studies have investigated the prevalence of TB-suggestive lesions in cattle carcasses in the central and western parts of Sudan (Osman, 2007; Manal et al., 2010; Asil et al., 2013; Ehsan and Nganwa, 2014; Aljameel et al., 2014). The aim of the present study was to provide a better understanding of the epidemiology of TB and other mycobacterial infections in carcasses of ruminants slaughtered in Kassala in Eastern Sudan, as well as to identify the *Mycobacterium* species causing the infections.

■ MATERIALS AND METHODS

Study area

Kassala State is located in the eastern part of Sudan at latitude 14°–17° N, and longitude 34°–37° E (Figure 1). It is delimited by a border with Eritrea (235 kilometers) in the east, and by national borders with the Red Sea State in the north, Khartoum State and the River Nile State in the west, and Al-Gadarif State in the southwest. It covers an area of 42,282 square kilometers. It falls within the Sahelian climate zone of Africa. Soils are dark, heavy, deep cracking Vertisol. Rainfall is concentrated in a single relatively short autumn season from June to September and reaches about 680 millimeters per year. Temperature ranges from a mean minimum of 17°C in January to a mean maximum of 47°C in April and May (Sulieman and Buchroithner, 2006). During the rainfall season, Kassala State is rich in fodder, grazing areas, by-products of sorghum and sesame. Moreover, the State has abundant amounts of water. Gaash, Siteet
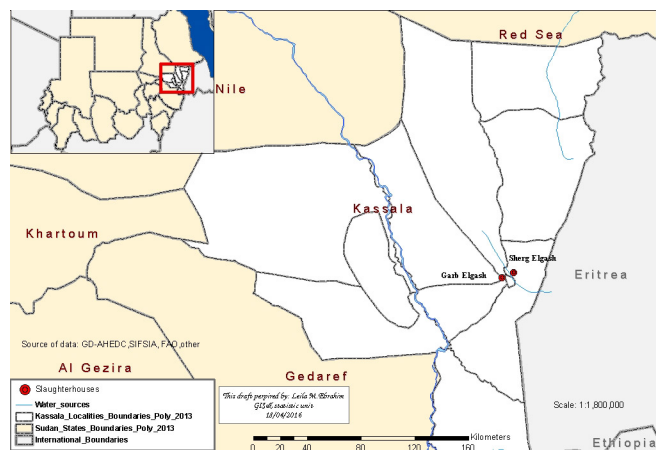

Figure 1: *Location of the study area in Eastern Sudan.*

and Atbrah seasonal rivers, and Halfa Al-Gadeeydah canal are full of water during the rainfall season. Port Sudan, the main port for exportation of livestock and livestock products, is located 575 kilometers to the northeast of Kassala. Mixed crop-livestock, nomadic and semi-nomadic production systems predominate in the State. Animals are bred in Kassala for domestic consumption and for exportation to international markets (Omar et al., 2009). The State has an estimated livestock population of about 4.5 million head (MARF, 2008).

Study design, sample size and sampling

A cross-sectional study was conducted from June to November 2014 in Kassala State. This State was selected as it is one of the animal production sites in Sudan. The main town of the State has two slaughterhouses: East and West Gaash. On average, around 7500 head of animals are slaughtered per month in these slaughterhouses, among which about 2500 cattle, 4000 sheep, 950 goats, and 50 camels. The two slaughterhouses were conveniently included in this survey.

The sample size (n) was calculated based on the standard formula of Thrusfield (2007). The parameters of the formula were as follows: 95% confidence level, ± 5% desired level of precision, an assumed expected prevalence of 50% as the prevalence of TB in ruminants was unknown in Kassala. We used:

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

where n was the required sample size, $(1.96)^2$ the constant, P^{exp} the expected prevalence rate of 50%, and d the desired precision level of ± 5%.

The required sample size n was determined to be 384 animals from each of the two slaughterhouses. This number was inflated threefold to account for the effect of randomness and representativeness (Thrusfield, 2007). Thus, total n was 1152 animals from each slaughterhouse.

Convenient stratification according to species and systematic random sampling were used to select animals (Thrusfield, 2007). Selected animals were identified using permanent markers, kept in a separate place and released for slaughter one after another. The sampling interval was obtained by dividing the total number of slaughtered animals on that day by the estimated daily sample size (Thrusfield, 2007). Four animals were selected each study day from each of the four species. Each of the two slaughterhouses was visited 72 times (three visits per week). Therefore, the total number of animals slaughtered on a particular day from a particular species was divided by four and every n^{th} animal was selected after random selection of the first animal. Accordingly, n comprised a total of 710 cattle, 729 sheep, 700 goats, and 165 camels. Fewer than four camels were slaughtered per day in each slaughterhouse. To account for this, the required n per day was achieved by selecting more animals from other species.

Postmortem inspection

Postmortem inspection was performed as described by Corner et al. (1990), and Corner (1994). This included visual inspection, palpation and *in situ* slicing of lymph nodes, organs of the thoracic and abdominal cavities, and the head. Additionally, lymph nodes of the fore and hind limbs and the inguinal region were also inspected.

Laboratory procedures

Samples that were collected from the suspected granulomatous lesions were frozen at -20°C until shipping to the National Reference Laboratory (NRL) for mycobacteria, Research Center Borstel (FZB), Germany. At NRL, samples were minced, then homogenized and

decontaminated using sodium hydroxide, and the mucolytic agent N-acetyl-L-cysteine (NALC-NaOH) according DIN's guidelines (2011). Smears were prepared from the decontaminated and resuspended specimens according to Kinyoun's method in conjunction with an automated staining system (ZN Aerospray TB Slide Stainer/Cytocentrifuge, Wescor, Logan, USA). Moreover, cultures were made by inoculation into a mycobacteria-growth indication tube (MGIT) liquid medium (Bactec MGIT 960 system) (Becton-Dickinson, Heidelberg, Germany) and onto solid Löwenstein-Jensen and Stonebrink media (own production). Cultures were incubated at 37°C and 31°C for six (MGIT) or eight (solid media) weeks, respectively.

For molecular characterization of the isolate of the positive cultures, DNA was extracted with Tris-EDTA (TE) boiling/sonication extraction technique or the cetyltrimethylammonium bromide (CTAB) method, respectively. Commercially available line probe assay (LPA) (GenoType *Mycobacterium* CM, HAIN Lifescience GmbH, Nehren, Germany) and 16S rDNA and ITS gene sequencing were conducted from TE buffer prepared samples. In addition, whole genome sequencing (WGS) was carried out with Illumina MiSeq sequencer, Nextera XT library preparation kits, and MiSeq reagent kits according to the manufacturer's instructions (Illumina, San Diego, CA, USA). Classification of the reads was done by Kraken and reads were mapped to the *M. tuberculosis* H37Rv genome and *M. intracellulare* MOTT-64 with the exact alignment program Saruman.

Prediction of the complete sequence of 16S gene by WGS

To predict the complete sequence of 16S gene, *de novo* genome assembly was carried out. Reads were trimmed with Trimmomatic version 0.35 for Q30 in a sliding window of 20 bp and nextera adapter contamination discarding all reads shorter than 100 bp (Bolger et al., 2014). Trimmed paired and unpaired reads were assembled with SPAdes version 3.6.2 using the built-in read error correction based on k-mer frequencies and the 'careful' option (Bankevich et al., 2012; Caverly et al., 2016). The 16S gene sequence was predicted by the RNAmmer Prediction Server from the assembled contigs which were filtered for containing ribosomal sequences (Lagesen et al., 2007).

■ RESULTS

In total 40,089 animals were slaughtered in the two surveyed slaughterhouses during the study period. More than three quarters (81.0%) were slaughtered at East Gaash slaughterhouse and 19.0% at West Gaash. The male to female ratio was 1:1 for cattle, goats and camel, whereas it was 1:2 for sheep. Only animals older than one year in the case of cattle, sheep and goats, and older than five years in the case of camels had been slaughtered. The animals were mostly of indigenous breeds, e.g. Gaash cattle, and Garrage, Dubassy and Gaash sheep, Baladi, Garrage and Nubi goats, and Bushari and Anaafy camels. A few were exogenous from Eritrea and Ethiopia, e.g. Karour and Barka cattle, and Arrit and Horro sheep.

The overall prevalence of granulomatous lesions in all inspected carcasses was 0.1% (2/2304). The two carcasses that had suggestive TB lesions were carcasses of sheep. No TB-suggestive lesions were detected in carcasses of cattle (0/710), goats (0/700), and camels (0/165). The detected granulomatous lesions were typical caseous necrosis, with a whitish or yellowish color. They were of various sizes congregating together, or scattered and enclosed in light congested grey fibrous tissue. They were found in the liver and lungs, and in the peritoneal cavity covering the whole abdomen and viscera (Figure 2).

Cultures revealed growth of mycobacteria from samples collected from one carcass. Samples collected from the other carcass were

extensively contaminated with bacteria and yeast, and no mycobacterial growth could be found. Furthermore, MTBC isolates including *M. bovis*, *M. caprae*, and *M. tuberculosis* could not be grown from any of the samples.

LPA revealed a banding pattern (bands 1, 2, 3, and 10) that was not specific for any of the mycobacterium species identified by the assay (Figure 3). Moreover, 16S rDNA and ITS sequences of the isolated mycobacteria did not align with any known validly described *Mycobacterium* species in NCBI Blast database.

The fully sequenced genome had 4.8 mbp. Metagenomics classification by Kraken showed that 71.6% of the reads were unclassified and the closest relative was *Mycobacterium sinense* JDM601. Moreover, Kraken showed that only 0.2% and 0.1% of the sequenced genome was identical to the genome of *M. tuberculosis* H37Rv and *M. intracellulare* MOTT-64, respectively.

The complete sequence of 16S allocated this isolate to slow growing mycobacteria with the closest similarity to members of the *M. terrae* group. The alignment of the sequence of 16S in the NCBI Blast database is shown in Figure 4.

Figure 2: *Granulomatous lesions in the liver and lungs (left), and peritoneal cavity (right) of two carcasses of slaughtered sheep in Eastern Sudan.*
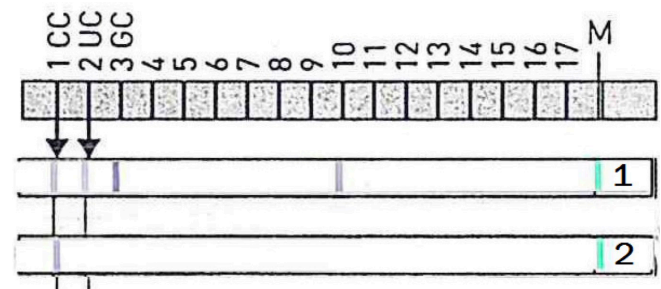
Figure 3: *LPA (GenoType* Mycobacterium *CM) showing the unique banding pattern of the unknown* Mycobacterium *species isolated from the granulomatous lesions collected from a carcass of a slaughtered sheep in Eastern Sudan; 1: isolate; 2: negative control.*

■ DISCUSSION

TB and other mycobacterial infections in animals are economically significant. The consequential production losses from these infections include lower milk production, loss of body weight, and condemnation of infected organs and carcasses. Animal reproduction and breeding as well as livestock trade at national and international levels are directly and adversely affected by mycobacterial infections (Radostits et al., 2007; Ehsan and Nganwa, 2014; Ramos et al., 2015). TB and other mycobacterial infections are quite prevalent in dairy

```
Query      1       AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAAC   60
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1398157       AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAAC  1398216
Query     61       GGAAAGGCCCCTTCGGGGGTGCTCGAGTGGCGAACGGGTGAGTAACACGTGGGTGATCTG  120
                   |||||||||| ||||||||| ||||||||||||||||||||||||||||||||||||||||
Sbjct  1398217       GGAAAGGCCCTTTCGGGGGTACTCGAGTGGCGAACGGGTGAGTAACACGTGGGTGATCTG  1398276
Query    121       CCCTGCACTCTGGGATAAGCTTGGGAAACTGGGTCTAATACCGGATAGGACCATGGGATG  180
                   ||||||||||||||||||||  |||||||||||||||||||||||||||||||||||||||
Sbjct  1398277       CCCTGCACTCTGGGATAAGCCTGGGAAACTGGGTCTAATACCGGATAGGACCATGGGATG  1398336
Query    181       CATGTTCTGTGGTGGAAAGCTTTTGCGGTGTGGGATGGGCCCGCGGCCTATCAGCTTGTT  240
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1398337       CATGTTCTGTGGTGGAAAGCTTTTGCGGTGTGGGATGGGCCCGCGGCCTATCAGCTTGTT  1398396
Query    241       GGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGTGTCCGGCCA  300
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1398397       GGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGTGTCCGGCCA  1398456
Query    301       CACTGGGACTGAGATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA  360
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1398457       CACTGGGACTGAGATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA  1398516
Query    361       ATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGGGGGATGACGGCCTTCGGGTTGTAAAC  420
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1398517       ATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGGGGGATGACGGCCTTCGGGTTGTAAAC  1398576
Query    421       CTCTTTCAGTATCGGCGAAGCTCCCGAAttttttG-GGGGTGACGGGTAGGTACAGAAGA  479
                   ||||||||||||||||||||||||      |     |||||||||||||||||||||||||
Sbjct  1398577       CTCTTTCAGTATCGGCGAAGCTCC--GTGGTTTTCTGCGGGGTGACGGGTAGGTACAGAAGA  1398635
Query    480       AGCACCGGCCAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTGTCCGG  539
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1398636       AGCACCGGCCAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTGTCCGG  1398695
Query    540       AATTACTGGGCGTAAAGAGCTCGTAGGTGGTTTGTCGCGTTGTCCGTGAAAACTCACAGC  599
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1398696       AATTACTGGGCGTAAAGAGCTCGTAGGTGGTTTGTCGCGTTGTCCGTGAAAACTCACAGC  1398755
Query    600       TTAACTGTGGGCGTGCGGGCGATACGGGCAGACTGGAGTACTGTAGGGGAGACTGGAATT  659
                   | |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1398756       TCAACTGTGGGCGTGCGGGCGATACGGGCAGACTGGAGTACTGTAGGGGAGACTGGAATT  1398815
Query    660       CCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCT  719
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1398816       CCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCT  1398875
Query    720       CTGGGCAGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCT  779
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1398876       CTGGGCAGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCT  1398935
Query    780       GGTAGTCCACGCCGTAAACGGTGGGTACTAGGTGTGGGTTTCCTTCCTTTAGGGATCCGT  839
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1398936       GGTAGTCCACGCCGTAAACGGTGGGTACTAGGTGTGGGTTTCCTTCCTTTAGGGATCCGT  1398995
Query    840       GCCGTAGCTAACGCATTAAGTACCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAA  899
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1398996       GCCGTAGCTAACGCATTAAGTACCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAA  1399055
Query    900       AGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGATTAATTCGATGCAACGCGA  959
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1399056       AGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGATTAATTCGATGCAACGCGA  1399115
Query    960       AGAACCTTACCTGGGTTTGACATGCACAGGACGCCGGTAGAGATATCCGGTTCCCTTGTGG  1019
                   ||||||||||||||||||||||||||||||| |||| ||||||| |||||  ||||||||||
Sbjct  1399116       AGAACCTTACCTGGGTTTGACATGCACAGGACGACTGCAGAGATGTGGTTTCCCTTGTGG  1399175
Query   1020       CCTGTGTGCAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTC  1079
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1399176       CCTGTGTGCAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTC  1399235
Query   1080       CCGCAACGAGCGCAACCCTTGTCTCATGTTGCCAGCACGTTATGGTGGGGACTCGTGAGA  1139
                   |||||||||||||||||||||||||||||||||||||||| |||||||||||||||||||
Sbjct  1399236       CCGCAACGAGCGCAACCCTTGTCTCATGTTGCCAGCACGTGATGGTGGGGACTCGTGAGA  1399295
Query   1140       GACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATG  1199
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1399296       GACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATG  1399355
Query   1200       TCCAGGGCTTCACACATGCTACAATGGCCGGTACAAAGGGCTGCGATGCCGTGAGGTTTA  1259
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1399356       TCCAGGGCTTCACACATGCTACAATGGCCGGTACAAAGGGCTGCGATGCCGTGAGGTTTA  1399415
Query   1260       GCGAATCCTTTAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGT  1319
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1399416       GCGAATCCTTTAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGT  1399475
Query   1320       CGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTA  1379
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1399476       CGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTA  1399535
Query   1380       CACACCGCCCGTCACGTCATGAAAGTCGGTAACACCCGAAGCCGGTGGCCTAACCCTTGG  1439
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1399536       CACACCGCCCGTCACGTCATGAAAGTCGGTAACACCCGAAGCCGGTGGCCTAACCCTTGG  1399595
Query   1440       GAGGGAGCCGTCGAAGGTGGGATCGGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTAC  1499
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1399596       GAGGGAGCCGTCGAAGGTGGGATCGGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTAC  1399655
```

Figure 4: *Alignment of the 16S rRNA gene sequence of the isolate (Query) and* Mycobacterium sinense *strain JDM601 (Sbjct) sampled from the carcass of a slaughtered sheep in Eastern Sudan.*

and meat animals in countries where control and management measures are not in place (Cousins, 2001; Humblet et al., 2009).

This study revealed a very low prevalence of granulomatous lesions (0.1%) in carcasses of cattle, sheep, goats, and camels slaughtered at the two slaughterhouses of Kassala in Eastern Sudan. Overall in Sudan, the prevalence of granulomatous lesions suggestive of TB in slaughtered ruminant carcasses has been reported to range from 0.01% to 8.0% in different parts of the country. Lesions have been found in organs or lymph nodes, or generalized in the whole carcass (Manal et al., 2010; Asil et al., 2013; Aljameel et al., 2014; Ehsan and Nganwa, 2014). With the exception of one study that reported a small number of dairy cattle (11/569) that were positive by the tuberculin skin test in Eastern Sudan (Ayman et al., 2014), prevalence of TB in dairy and fattening farms has not been investigated in Sudan. Moreover, in neighboring and other African countries, prevalence of granulomatous lesions in slaughtered food animal carcasses has been up to 16% (Mohamed et al., 2009; Biffa et al., 2010; Manal et al., 2010; Kassa et al., 2012; Youssef and Ahmed, 2014; Okoro et al., 2014; Worku and Abreham, 2016; Nalapa et al., 2017).

Different *Mycobacterium* species have been isolated from granulomatous lesions collected from carcasses in Sudan and elsewhere. The main identified bacteria are *M. bovis*, *M. farcinogenes*, *M. tuberculosis*, *M. avium*, and *Nocardia* species. Unidentified rapidly and slowly growing mycobacterium species have also been detected (Sulieman and Hamid, 2002; Oloya et al., 2007; Osman, 2007; Manal et al., 2010; Asil et al., 2013; Aljameel et al., 2014; Nalapa et al., 2017). Environmental risk factors, malnutrition, presence of other diseases, and heavy infestation with endoparasites are important factors that increase the susceptibility of animals to TB (Radostits et al., 2007; Ehsan and Nganwa, 2014). Environmental risk factors have not been investigated in this study; therefore, any solid conclusion regarding their effect in the low observed prevalence of granulomatous lesions could not be made. On the other hand, emaciated animals that are likely to have TB-suggestive lesions in their organs had been excluded during antemortem examination and were not slaughtered for human consumption, in accordance with public health and animal welfare regulations. Nonetheless, some of these excluded emaciated animals might be illegally slaughtered and prepared for human consumption. This practice is common in Sudan and is locally called 'Keery slaughtering'. TB-suggestive lesions might be more prevalent in carcasses of ruminants that are illegally slaughtered for human consumption.

Sensitivity of postmortem inspection for detection of TB-suggestive lesions in animal carcasses is between 28% and 64% (Biffa et al., 2010; Schiller et al., 2010; Okoro et al., 2014; Ramos et al., 2015; Worku and Abreham, 2016). However, this technique is cost-effective for passive surveillance and TB monitoring in animals. Besides, it is affordable in resource-limited countries (Schiller et al., 2010). The very low prevalence of TB-suggestive lesions in animal carcasses reported in this study could be attributed to the limited sensitivity of postmortem inspection. Furthermore, this study was conducted over a period of six months. Such period is short, especially in case of seasonal variations in the prevalence of animal TB. The sensitivity of the postmortem inspection technique is improved when animals are tested using the tuberculin skin test before slaughtering. Granulomatous lesions are likely to be detected in internal organs of animals that are positive by the tuberculin skin test. Moreover, extended slaughterhouse-based surveys should explore whether there are seasonal variations in the prevalence of granulomatous lesions suggestive of TB or not.

In this study, neither *M. bovis* nor *M. caprae* nor *M. tuberculosis* have been isolated from the detected granulomatous lesions. It suggests that zoonotic transmission of these mycobacteria from animals to humans via the consumption of contaminated meat is unlikely, although it cannot be completely ruled out, especially when the meat is prepared through unsupervised Keery slaughtering. A very slight potential risk of zoonotic transmission of unknown species of mycobacteria to humans via consumption of contaminated meat exists. It is not likely that local and international trade in livestock from Eastern Sudan is going to be restricted or hindered because of animal TB. Nevertheless, occurrence of this disease in animals that are reared on farms and in animals that are grazing in pastures should be monitored.

There is no control program directed against TB in animals in Kassala. Conversely, none of the carcasses inspected at the two slaughterhouses of Kassala had been infected with any of the MTBC bacteria. Radostits et al. (2007) indicate that the overall incidence and prevalence of TB are low in indigenous animals such as zebu type cattle and this might be due to natural resistance. Furthermore, these authors specify that low overall incidences of TB have also been observed in countries where cattle are at pasture all year round. These might probably explain the findings of this study. In Sudan, most of the slaughtered ruminants are indigenous breeds, and animals are at pasture for the whole year in search of fodder and water.

One of the strengths of this study was the investigation of a large number of different animal species at the two slaughterhouses of Kassala. Therefore, the findings might be generalized to the same animal species slaughtered and prepared for human consumption in other slaughterhouses in Eastern Sudan. Nonetheless, the study had limitations. No dairy farms have been investigated, hence, TB prevalence could

not be compared between animals raised for meat and animals raised for milk. Additionally, the study was conducted in a short period. Perhaps TB prevalence was underestimated because of seasonal variations. Furthermore, only postmortem inspection was used as a mean to investigate TB, but granulomatous lesions are small during early stages of the disease and might not be visible with naked eyes.

■ CONCLUSION

TB was not detected in carcasses of slaughtered ruminants in Kassala. This indicates that TB incidence in animals is very low in Kassala, and zoonotic transmission of MTBC bacteria is unlikely. One of the carcasses that was found with typical granulomatous lesions was infected with an unknown *Mycobacterium* species. This isolate could be pathogenic to humans. Slaughterhouse-based surveys combined with pre-slaughtering testing using the tuberculin skin test would help to explore TB epidemiology in animals in Sudan.

REFERENCES

Aljameel M.A., Abdel Wahab M.B., Fayza A.O., El Tigani A.E., Abdellatif M.M., 2014. Occurrence of bovine tuberculosis at Nyala abattoirs in South Darfur State, Sudan. *Rev. Elev. Med. Vet. Pays Trop.,* **67** (2): 61-65, doi: 10.19182/remvt.10185

Asil T.A., El Sanousi S.M., Gameel A., El Beir H., Fathelrahman M., Terab N.M., Muaz M.A., et al., 2013. Bovine tuberculosis in South Darfur State, Sudan: an abattoir study based on microscopy and molecular detection methods. *Trop. Anim. Health Prod.,* **45** (2): 469-72, doi: 10.1007/s11250-012-0241-y

Ayele W.Y., Neill S.D., Zinsstag J., Weiss M.G., Pavlik I., 2004. Bovine tuberculosis: an old disease but a new threat to Africa. *Int. J. Tuberc. Lung Dis.,* **8** (8): 924-937

Ayman E.A., Salih D.A., Gumaa M.M., Omer M.M., Abbas F., Ahmed A.M., 2014. Prevalence of bovine tuberculosis in Kassala State. *Inter. J. Adv. Sci. Techn. Res.,* **4** (1): 238-249

Bankevich A., Nurk S., Antipov D., Gurevich A.A., Dvorkin M., Kulikov A.S., Lesin V.M., et al., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Com. Biol.,* **19** (5): 455-77, doi: 10.1089/cmb.2012.0021

Biffa D., Bogale A., Skjerve E., 2010. Diagnostic efficiency of abattoir meat inspection service in Ethiopia to detect carcasses infected with *Mycobacterium bovis*: Implications for public health. *BMC Public Health,* **10**: 1-12, doi: 10.1186/1471-2458-10-462

Bolger A.M., Lohse M., Usadel B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics,* **30** (15): 2114-20, doi: 10.1093/bioinformatics/btu170

Caverly L.J., Spilker T., LiPuma J.J., 2016. Complete genome sequences of 17 rapidly growing nontuberculous mycobacterial strains. *Genome Announc.,* **4** (5), doi: 10.1128/genomeA.01009-16

Corner L.A., 1994. Postmortem diagnosis of *Mycobacterium bovis* infection in cattle. *Vet. Microbiol.,* **40** (1-2): 53-63, doi: 10.1016/0378-1135(94)90046-9

Corner L., Melville L., McCubbin K., Small K.J., McCormick B.S., Wood P.R., Rothel J.S., 1990. Efficiency of inspection procedures for the detection of tuberculous lesions. *Aust. Vet. J.,* **67** (11): 389-92, doi: 10.1111/j.1751-0813.1990.tb03020.x

Cousins D.V., 2001. *Mycobacterium bovis* infection and control in domestic livestock. *Rev. Sci. Tech. Off. Int. Epizoot.,* **20** (1): 71-85, doi: 10.20506/rst.20.1.1263

DIN, 2011. Medical microbiology - Diagnosis of tuberculosis. Part 3: Detection of mycobacteria by culture methods. Deutsches Institut für Normung, Berlin, Germany

Ehsan A., Nganwa D., 2014. Factors contributing to the transmission of bovine tuberculosis caused by *Mycobacterium bovis* and its control status in Sudan. In: Zoonotic tuberculosis: *Mycobacterium bovis* and other pathogenic mycobacteria, 3rd Edn (Eds Charles O.T., James H.S., John B.K.). John Wiley, USA

Humblet M.F., Boschiroli M.L., Saegerman C., 2009. Classification of worldwide bovine tuberculosis risk factors in cattle: a stratified approach. *Vet. Res.,* **40** (5): 50, doi: 10.1051/vetres/2009033

Kassa G.M., Abebe F., Worku Y., Legesse M., Medhin G., Bjune G., Ameni G., 2012. Tuberculosis in goats and sheep in Afar pastoral region of Ethiopia and isolation of *Mycobacterium tuberculosis* from Goat. *Vet. Med. Int.*: 869146, doi: 10.1155/2012/869146

Kulchavenya E., 2014. Extrapulmonary tuberculosis are statistical reports accurate. *Ther. Adv. Infect. Dis.,* **2** (2): 61-70, doi: 10.1177/2049936114528173

Lagesen K., Hallin P., Rodland E.A., Staerfeldt H.H., Rognes T., Ussery D.W., 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res.,* **35** (9): 3100-8, doi: 10.1093/nar/gkm160

Malone F.E., Wilson E.C., Pollock J.M., Skuce R.A., 2003. Investigations into an outbreak of tuberculosis in a flock of sheep in contact with tuberculous cattle. *Zoonoses Public Health,* **50** (10): 500-504, doi: 10.1046/j.1439-0450.2003.00714.x

Mamo G., Bayleyegn G., Sisay Tessema T., Legesse M., Medhin G., Bjune G., Abebe F., et al., 2011. Pathology of camel tuberculosis and molecular characterization of its causative agents in pastoral regions of Ethiopia. *PLoS One,* **6** (1): e15862, doi: 10.1371/journal.pone.0015862

Manal H.S., Zakia A.M., El Eragi A.M., Hamaad H., 2010. Bovine tuberculosis at Omdurman Central Abattoir, Khartoum State and Wau slaughterhouses (Bahr El-Ghazal) State, Sudan. *Sudan J. Vet. Res.,* **25**: 1-8

MARF, 2008. Number of animals in the Sudan. Department of Information and Statistics, Ministry of Animal Resources and Fisheries, Khartoum, Sudan

Marianelli C., Cifani N., Capucchio M.T., Fiasconaro M., Russo M., La Mancusa F., Pasquali P., et al., 2010. A case of generalized bovine tuberculosis in a sheep. *J. Vet. Diagn. Invest.,* **22** (3): 445–448, doi: 10.1177/104063871002200319

Mohamed A.M., Abou El-Ella G.A., Nasr E.A., 2009. Phenotypic and molecular typing of tuberculous and nontuberculous *Mycobacterium* species from slaughtered pigs in Egypt. *J. Vet. Diagn. Invest.,* **21** (1): 48-52, doi: 10.1177/104063870902100107

Nalapa D.P., Muwonge A., Kankya C., Olea-Popelka F., 2017. Prevalence of tuberculous lesion in cattle slaughtered in Mubende district, Uganda. *BMC Vet. Res.,* **13** (1): 73, doi: 10.1186/s12917-017-0991-x

Okoro O.J., Anosa G.N., Oboegbulem S.I., Nwanta J.A., Ezenduka E.V., 2014. Comparative assessment of postmortem inspection and immunochromatographic techniques for the detection of bovine tuberculosis in slaughter cattle in Nigeria. *Trop. Anim. Health Prod.,* **46** (5): 831-6, doi: 10.1007/s11250-014-0573-x

Oloya J., Kazwala R., Lund A., Opuda-Asibo J., Demelash B., Skjerve E., Johansen T.B., et al., 2007. Characterisation of mycobacteria isolated from slaughter cattle in pastoral regions of Uganda. *BMC Microbiol.,* **7**: 95, doi: 10.1186/1471-2180-7-95

Omar H.E., Mohammad A.J., Babiker I.B., 2009. Constraints in the market chains for export of Sudanese sheep and sheep meat to the Middle East. CGSpace/ CGIAR, https://cgspace.cgiar.org/discover

Osman A.B., 2007. Caseation in lymph nodes of slaughtered cattle with a special reference to bovine tuberculosis. *Vet. Med. J. Giza,* **55**: 1101-1109

Radostits O.M., Gay C.C., Blood D.C., Hinchelift K.W., 2007. Diseases associated with bacteria - IV – *Mycobacterium* spp. In: Veterinary medicine: A text book of disease of cattle, sheep, pig, goat and horses, 10th Edn. Harcourt, London, UK, 1007-1044

Ramos D.F., Silva P.E.A., Dellagostin O.A., 2015. Diagnosis of bovine tuberculosis: review of main techniques. *Braz. J. Biol.,* **75** (4): 830-837, doi: 10.1590/1519-6984.23613

Schiller I., Oesch B., Vordermeier H.M., Palmer M.V., Harris B.N., Orloski K.A., Buddle B.M., et al., 2010. Bovine tuberculosis: a review of current and emerging diagnostic techniques in view of their relevance for disease control and eradication. *Transbound. Emerg. Dis.,* **57** (4): 205-20, doi: 10.1111/j.1865-1682.2010.01148.x

Shitaye J.E., Getahun B., Alemayehu T., Skoric M., 2006. A prevalence study of bovine tuberculosis by using abattoir meat inspection and tuberculin skin testing data histopathological and IS 6110 PCR examination of tissues with tuberculous lesions in cattle in Ethiopia. *Vet. Med. Czech.,* **51** (11): 512-522

Smith N.H., Gordon S.V., de la Rua-Domenech R., Clifton-Hadley R.S., Hewinson R.G., 2006. Bottlenecks and broomsticks: the molecular evolution of *Mycobacterium bovis*. *Nat. Rev. Microbiol.,* **4** (9): 670-81, doi: 10.1038/nrmicro1472

Sulieman H.M., Buchroithner M.F., 2006. Assessment of natural vegetation clearing and re-growth in Southern Gadarif (Sudan) using change vector analysis based on remote sensing and field data. In: Proc. ISPRS Commission VII mid-term Symp., Remote sensing: From pixels to processes, Enschede, Netherlands, 8-11 May 2006

Sulieman M.S., Hamid M.E., 2002. Identification of acid-fast bacteria from caseous lesions in cattle in Sudan. *J. Vet. Med. B,* **49** (9): 415-418, doi: 10.1046/j.1439-0450.2002.00565.x

Theon C.O., Huchzermeyer H., Himies E.M., 2006. *Mycobacterium bovis* infection in animals and humans, 2dn Edn. Blackwell Publishing Professional, IA, USA, 63-72, doi: 10.1002/9780470344538

Thrusfield M., 2007. Veterinary epidemiology, 3rd Edn. Wiley-Blackwell Science, London, UK, 274-282

Worku A., Abreham S., 2016. Cross-sectional study and comparison of different diagnostic methods of bovine tuberculosis in Gondar Elfora abattoir, Ethiopia. *Mycobact. Dis.,* **6** (3), doi: 10.4172/2161-1068.1000218

Youssef A.I., Ahmed A.M., 2014. Bovine tuberculosis survey based on meat inspection and microscopic examination in central city abattoir in Ismailia, Egypt, and its hazards to the abattoir workers. *Inter. Food Res. J.,* **21** (2): 577-582

Résumé

Shuaib Y.A., Niemann S., Khalil E.A.G., Schaible U., Wieler L.H., Bakheit M.A., Mohamed-Noor S.E., Abdalla M.A., Richter E. Infections mycobactériennes dans les carcasses de ruminants abattus dans les deux abattoirs de Kassala au Soudan

La tuberculose (TB) est une maladie bactérienne chronique chez les humains et les animaux. Elle se caractérise par le développement progressif de lésions granulomateuses spécifiques dans les organes affectés. La tuberculose humaine est endémique dans l'est du Soudan. Cependant, les connaissances sur l'épidémiologie de la tuberculose chez les ruminants sont rares. Dans le cadre d'une étude de six mois menée de juin à novembre 2014, 2304 carcasses de bovins, d'ovins, de caprins et de chameaux abattus dans les abattoirs de Gaash Est et Ouest de Kassala ont été inspectées afin d'enquêter sur la prévalence de la tuberculose. Seulement 0,1 % (n = 2) des carcasses présentait des lésions suspectes de tuberculose. Ces lésions n'ont été retrouvées que dans les carcasses de moutons, dans le foie, les poumons et la cavité péritonéale. Les échantillons prélevés sur les lésions ont été analysés pour détecter la présence de mycobactéries. Celles-ci ont été retrouvées dans l'une des deux carcasses. Cet isolat a été soumis à un test par sonde linéaire (GenoType Mycobacterium CM) et à un séquençage de l'ADNr 16S et du gène ITS, ainsi qu'à un séquençage du génome entier (WGS). Cependant, aucune de ces méthodes n'a permis d'identifier cette souche comme une espèce de *Mycobacterium* valide. Néanmoins, la séquence 16S a attribué cet isolat à des mycobactéries à croissance lente. Ni *Mycobacterium bovis*, ni *M. caprae* ni *M. tuberculosis* n'ont été retrouvés dans les lésions granulomateuses collectées. En conclusion, la prévalence globale des lésions évocatrices de tuberculose chez les ruminants à Kassala était très faible. Des études approfondies combinant l'utilisation de tests cutanés à la tuberculine et des enquêtes menées dans les abattoirs devraient être conduites pour la surveillance de la tuberculose chez les ruminants au Soudan.

Mots-clés : ruminant, *Mycobacterium*, tuberculose, abattoir, Soudan

Resumen

Shuaib Y.A., Niemann S., Khalil E.A.G., Schaible U., Wieler L.H., Bakheit M.A., Mohamed-Noor S.E., Abdalla M.A., Richter E. Infecciones micobacterianas en carcasas de rumiantes sacrificados en dos mataderos de Kassala, Sudan

La tuberculosis (TB) es una enfermedad bacteriana crónica de humanos y animales. Se caracteriza por un desarrollo progresivo de lesionas granulomatosas específicas en los órganos afectados. La TB humana es endémica en el est de Sudan. Sin embargo, los conocimientos de la epidemiología de TB en rumiantes son raros. En un estudio de seis meses, de junio a noviembre 2014, 2304 carcasas de ganado, ovejas, cabras y camellos sacrificados en los mataderos de Gaash Este y Oeste en Kassala, fueron inspeccionadas para investigar la prevalencia de TB. Solamente 0,1% (n = 2) de las carcasas presentaron lesiones sospechosas de TB. Estas lesiones solo se encontraron en carcasas de ovejas, en hígado, pulmones y cavidad peritoneal. Las muestras colectadas en estas lesiones fueron investigadas para la presencia de micobacteria. Se encontraron en una de las dos carcasas. Este aislamiento se sometió a un ensayo de sonda lineal (GenoType Mycobacterium CM), y a secuencias genéticas ITS y 16S rDNA, así como secuencia genómica completa (WGS). Sin embargo, ninguno de estos métodos identificó esta cepa como una especie válida de *Mycobacterium*. No se encontró ni *Mycobacterium bovis* ni *M. caprae* ni *M. tuberculosis* en las lesiones granulomatosas colectadas. En conclusión, la prevalencia general de lesiones sugestivas de TB en rumiantes en Kassala fue muy baja. Estudios extensivos, combinando el uso del test de tuberculina dérmica y una investigación centrada en los mataderos, debe ser adoptarse para la supervisión de TB en los rumiantes de Sudan.

Palabras clave: rumiante, *Mycobacterium*, tuberculosis, matadero, Sudan