International Journal of Infectious Diseases 61 (2017) 27–37

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Prevalence of tuberculous lymphadenitis in slaughtered cattle in Eastern Cape, South Africa

Nolwazi L. Bhembe[a,b,*], Ishmael F. Jaja[c], Uchechukwu U. Nwodo[a,b], Anthony I. Okoh[a,b], Ezekiel Green[d]

[a] SAMRC Microbial Water Quality Monitoring Centre, University of Fort Hare, PBX 1314, Alice, 5700, South Africa
[b] Applied and Environmental Microbiology Research Group, Department of Biochemistry and Microbiology, University of Fort Hare, PBX 1314, Alice, 5700, South Africa
[c] Department of Livestock and Pasture Science, University of Fort Hare, PBX 1314, Alice, 5700, South Africa
[d] Department of Biotechnology and Food Technology, Faculty of Science, University of Johannesburg, Doornfontein, 20280, South Africa

ARTICLE INFO

Article history:
Received 2 March 2017
Received in revised form 29 April 2017
Accepted 9 May 2017
Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Amplification
Bovine tuberculosis
Fingerprinting
Multiplex PCR

SUMMARY

Objective: To detect the prevalence of *Mycobacterium tuberculosis* complex (MTBC) in the lymph nodes of slaughtered cattle collected from selected abattoirs in Eastern Cape Province of South Africa.
Methods: A total of 376 lymph nodes were collected from slaughtered cattle over a period of 12 months. Certain characteristics (sex, age, body condition score, and breed) were observed to be associated with MTBC among slaughtered cattle. Collected samples were cultured and tested for acid-fast bacilli (AFB). DNA was isolated, purified, and quantified using a spectrophotometer. Quantified DNA was confirmed to be MTBC by multiplex PCR targeting two genes (IS*6110* and *mpb64*).
Results: Of the 376 collected lymph nodes, 182 were positive when tested by Ziehl–Neelsen stain and 162 were confirmed positive for MTBC by PCR. MTBC was isolated from lymph nodes with nodular lesions (72.8%, 118/162) and inflamed lymph nodes (27.1%, 44/162). All detected MTBC isolates were positive for region of deletion 1 (RD1). No isolate was detected to have *Mycobacterium bovis* bacille Calmette–Guérin (BCG). However, 3.1% had *M. bovis* and 96.9% had *M. tuberculosis*.
Conclusions: The presence of live *Mycobacterium* strains in slaughtered cattle poses a health risk to beef consumers and abattoir workers.

Introduction

The World Health Organization (WHO) Global Tuberculosis Report published in 2016 (World Health Organization, 2016) states that South Africa and five other countries (India, Indonesia, China, Pakistan, and Nigeria) accounted for 60% of total global tuberculosis (TB) cases in 2015. Approximately 10.4 million new TB cases were detected in 2015 and about 1.8 million people died globally (World Health Organization, 2016). TB, a disease caused by infection with members of the *Mycobacterium tuberculosis* complex (MTBC), remains a problem worldwide in an extensive array of domestic and wild animals (Alexander et al., 2010). Detecting animals infected with MTBC is a challenge, as infected animals (wild and domestic) may not show clinical signs – it may take years for an animal to develop symptoms of TB following infection (Innes, 2002).

Mycobacterium bovis, a member of the MTBC, causes bovine TB (BTB), a prominent disease in cattle (Du et al., 2011). *M. bovis* is an important pathogen in cattle and humans, with implications for medicine and public health (Cosivi et al., 1998). As well as infecting animals, *M. bovis* can infect humans via direct contact with infected animals during slaughter or hunting (Anon, 2011) and through the consumption of contaminated, unpasteurized milk and dairy products (de la Rua-Domenech, 2006; Silaigwana et al., 2012). The transmission of *M. bovis* from animals to humans through the air is considered uncommon; however, *M. bovis* can be spread by infected cattle through saliva, urine, or droppings, and also through discharging lesions (Griffin and Buchan, 1994). As yet, the consumption of meat has not been recognized as one of the means of MTBC transmission to humans. Nevertheless,

* Corresponding author at: PBX 1314 Department of Biochemistry and Microbiology, University of Fort Hare, Alice 5700, South Africa. Tel: +27-632 441 675.
E-mail addresses: bhembelwez27@gmail.com, 200900322@ufh.ac.za (N.L. Bhembe), ijaja@ufh.ac.za (I.F. Jaja), UNwodo@ufh.ac.za (U.U. Nwodo), AOkoh@ufh.ac.za (A.I. Okoh), egreen@uj.ac.za (E. Green).

http://dx.doi.org/10.1016/j.ijid.2017.05.005

farmers, farm workers, and veterinarians are at great risk of contracting the infection from infected animals.

BTB has not received much attention in South Africa; however, the isolation of *M. bovis* strains from buffaloes has been reported from most provinces (Michel et al., 2008; Hlokwe et al., 2011). This isolation of *M. bovis* led to the introduction of the national bovine TB control and eradication scheme in 1969, which facilitated a decline in BTB (Michel et al., 2008).

South Africa is made up of nine provinces: Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, Northern Cape, North West, and Western Cape. Eastern Cape Province is described as the poorest province in South Africa (Katiyatiya et al., 2014). This province is well known for livestock production in the country, and the economy of the province depends largely on agriculture (Anon, 2013). Farms in this province provide large volumes of raw milk to urban and rural areas at low cost, and this is consumed unpasteurized by many people to supplement their diet (Silaigwana et al., 2012). The consumption of unpasteurized milk represents a public health problem due to the potential of MTBC transmission to humans. This public health situation is exacerbated by the poor sensitivity of the regular visual-only ante-mortem and post-mortem meat inspections performed to detect TB nodular lesions in carcasses, subsequent to which contaminated meat may be approved for human consumption (Demelash et al., 2009).

The diagnosis of TB can be achieved through the use of conventional laboratory methods such as culture and smear microscopy (Parsons et al., 2011). These methods are economical, but their sensitivity is low. The culture method, regarded as the gold standard, takes a long time to show growth of the organisms (6 to 8 weeks) (Parsons et al., 2002), and the analysis is unreliable and prone to errors, i.e. dissimilarity in colony morphology (Ameni et al., 2010). Although microscopy has limitations, it is still used in some developing countries (Al-Saqur et al., 2009). The tuberculin skin test (TST), which also has limitations in terms of a lack of sensitivity and specificity, is still used in Eastern Cape Province for the diagnosis of TB (Arend et al., 2008).

Recently, better performing and more affordable molecular techniques have been introduced, such as PCR. This is a nucleic acid-based amplification (NAA) method that amplifies the targeted region reliably for the diagnosis of non-pulmonary TB (Sharma et al., 2012). Several studies have reported the detection of MTBC through the amplification of one gene (*mpb64* or IS*6110*) (Kano et al., 2001; Abe and Hara, 2002). A single gene target may result in false-negative reported results, as some of the targeted genes may be absent in some MTBC strains (Sharma et al., 2012). Multiplex PCR techniques perform better for the detection of MTBC. These techniques, which amplify two or more targeted genes in a reaction (Sharma et al., 2012), have the added advantage of being rapid and accurate – characteristics that are required to help eradicate the TB burden.

Detected isolates can be characterized further using different methods such as spoligotyping and analysis of the region of difference (RD) and *oxy*R. The RD analysis is also a PCR method; this detects the presence of the RD regions (Brosch et al., 2002) and differentiates the members of the MTBC (Brosch et al., 2002). This analysis is useful for the treatment of TB patients and epidemiological studies (Parsons et al., 2002), because it differentiates between the members of the MTBC, thus helping with the decision on the types of drugs to be given to the patient (certain strains are naturally resistant to some drugs; e.g., *M. bovis* is resistant to pyrazinamide). The method is used with *oxy*R analysis for further identification. This method combines PCR and restriction fragment length polymorphism (RFLP) and distinguishes *M. bovis* from the other MTBC members (Niemann et al., 2000).

The aim of this study was to investigate the presence of MTBC in cattle lymph nodes using a multiplex PCR targeting the IS*6110* and *mpb64* genes and to distinguish *M. bovis* and *M. bovis* bacille Calmette–Guérin (BCG) from the other MTBC members through RD1 and *oxy*R analyses.

Materials and methods

Study site

Samples for this study were collected from two different commercial abattoirs in Eastern Cape Province, South Africa: East London and Queenstown, located at 31°54′S 26°53′E and 32.97°S and 27.87°E, respectively. The location of these abattoirs in the different municipalities is shown in Figure 1.

Lymph node sample collection

The study was approved by the University of Fort Hare Research Ethics Committee (UREC), with ethical clearance certificate REC-270710-028-RA Level 01. Following consultations with regional veterinary officers, protocols were established for the collection of suspected material by district meat inspectors for the abattoir prevalence survey. A convenient purposive sampling was adopted, where lymph nodes were selected randomly and enlarged lymph nodes were also collected.

A total of 14 950 carcasses from the two abattoirs were examined for nodular lesions and enlarged lymph nodes over a 12-month period, from July 2014 to June 2015. The distribution of the breeds of these 14 950 cattle was as follows: 239 Angus, 563 Beefmaster, 877 Bonsmara, 948 Brangus, 112 Hereford, 661 Holstein, 383 Jersey, 2709 Nguni, and 5053 Simmentaler; 3405 were of non-descript breeds. A total 376 enlarged lymph nodes or lymph nodes with nodular lesions were obtained from the slaughtered cattle and examined. These were collected from the head (including mandibular, parotid, and retropharyngeal lymph nodes), the respiratory system (bronchial lymph nodes, including the anterior, middle, and posterior mediastinal lymph nodes), and the gastrointestinal tract (mesenteric lymph nodes, including the superficial inguinal lymph nodes).

Information regarding the sex, age, breed, and condition of the carcass of the slaughtered cattle was also recorded. The age of the animal was identified using records from the farmers; where such records did not exist, an age estimation was done by inspection of the dentition (Food Safety and Inspection Service, 2013). This information was grouped into two categories for ease of analysis: cattle between 1 and 2 years of age were termed young, while those >2 years were termed old.

The lymph nodes from each animal were grouped according to body region and subsequently cut into thin sections. These were placed in sterile 50-ml universal tubes containing phosphate-buffered saline (PBS) at pH 7.2 and transported at 4 °C to the Microbial Pathogenicity and Molecular Epidemiology Research Group (MPMERG) laboratory for further processing. At the MPMERG laboratory, samples were kept at 4 °C and processed within 48 h. Some samples were stored at −20 °C and processed 2 days after sample collection, following the method of Berg et al. (2009). It is important to note that not all lymph nodes were inflamed or with nodules. During the sampling period, greater focus was placed on lymph nodes suggestive of infection.

Sample decontamination

The lymph node samples were homogenized using a hand-held blender. They were decontaminated by shaking in an equivalent volume of 4% NaOH for 15 min and concentrated by centrifugation at 3000 × *g* for 15 min. The residue was neutralized with 2NHC