Detection of *Mycobacterium bovis* in cattle lungs from two abattoirs in Western and North Central provinces of Sri Lanka

MTLK Jayasumana¹, DMUNK Dunuwila¹, PGAS Palkumbura¹, R Mudalige², IVP Dharmawardana², RRMKK Wijesundera¹ and HRN Jinadasa*¹

¹ Department of Veterinary Pathobiology, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya.  
² Colombo Municipal Council, Colombo 07.

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*Corresponding author (rnjinadasa@vet.pdn.ac.lk; https://orcid.org/0000-0001-8538-782X)

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Abstract: *Mycobacterium bovis* causes bovine tuberculosis (BTB) primarily in cattle. All mammals are susceptible to this zoonotic disease and it had been reported from the Central and North Western provinces of Sri Lanka. Abattoir monitoring is usually used for BTB surveillance in endemic countries. However, proper ante-mortem inspection of cattle and proper meat inspection is practiced only at a few abattoirs in Sri Lanka. The objective of this study was to conduct a preliminary assessment of BTB incidence among cattle used for beef production at two abattoirs in Western (WP) and North Central (NCP) provinces in Sri Lanka. Randomly collected 115 lung samples (WP = 45; NCP = 70) were tested using direct acid-fast staining, culture on Lowenstein Jensen medium, histopathology and PCR. *Mycobacterium tuberculosis* complex was detected in 7.0 % of the samples by PCR conducted using DNA extracted directly from samples. Only 5.2 % of the samples (4.45 % from WP and 5.7 % from NCP) were positive for *M. bovis* specific PCR. Only one (0.87 %) PCR positive sample from NCP had a granuloma on histopathological observation, suggesting relatively low incidence of BTB among the cattle processed at these abattoirs. *Mycobacterium bovis* isolates were not recovered and acid-fast bacilli were not observed in direct smears. Initiating proper meat inspection at all abattoirs in the country along with increased BTB surveillance capacity of the national veterinary service are required to mitigate this risk. Further studies are essential to determine the exact prevalence of BTB in Sri Lanka and to identify any wildlife reservoirs of BTB in the country.

Keywords: Abattoirs, bovine tuberculosis, cattle, *Mycobacterium bovis*.

INTRODUCTION

Bovine tuberculosis (BTB) is a notifiable livestock disease caused by *Mycobacterium bovis* (OIE, 2008). Cattle are the primary host for *M. bovis*, but all mammals are susceptible to the disease. Therefore, it is an important zoonotic disease and a potential occupational hazard in the livestock industry (Quinn *et al.*, 2015). However, person-to-person transmission is rare among immunocompetent individuals (Sunder *et al.*, 2009; WHO, 2017). The prevalence of BTB is higher in developing countries compared to most developed countries due to inefficient control programmes in developing countries (De la Rua-Domenech *et al.*, 2006; Une & Mori, 2007). National BTB control programmes have been established in many countries with special emphasis on cattle industry (Westrell *et al.*, 2009; Max *et al.*, 2011; Allen *et al.*, 2018). These control programmes generally consist of prevention, surveillance and eradication strategies. The prevention of BTB addresses the herd hygiene and specific biosecurity practices minimizing the pathogen exposure. Routine surveillance methods for *M. bovis* include ante-mortem inspection of cattle mainly including tuberculin skin test and meat inspection at abattoirs (Schiller *et al.*, 2010). Whole-herd depopulation could be used to eradicate *M. bovis* from domestic skin test positive herds and restocking is allowed after a waiting period as the organism may persist in soil for up to 12 months.
depending on the environmental conditions (OIE, 2008; Fine et al., 2011; Good & Duignan, 2011; Barbier et al., 2016). However, the ‘test and slaughter’ policy is widely practiced as an alternative strategy for whole-herd depopulation owing to the financial and animal welfare constraints. Test and slaughter is the accepted method of BTB eradication by the World Organization for Animal Health (Office International des Epizooties-OIE) at present (OIE, 2008; Good & Duignan, 2011).

Abattoir monitoring for BTB is a key surveillance technique in endemic countries and considered as the base for granting and maintaining official BTB-free status for a country (Collins, 2006; De la Rua-Domenech et al., 2006; Radunz, 2006; OIE, 2008). Having an effective routine meat inspection service as a part of BTB surveillance programme also safeguards the public health since BTB is one of the foodborne zoonoses. According to the standard procedures, all cattle entering the food chain should be subjected to ante-mortem examination and proper post-mortem meat inspection should be conducted on dressed carcasses (Amanfu, 2006). In reference to BTB, the carcass is recommended as fit for human consumption when the animal is clinically healthy in ante-mortem inspection and shows no tuberculosis lesions in post-mortem examination (Annon, 1893). However, most developing countries with endemic tuberculosis may not practice fully effective meat inspection protocols due to the limitations in financial allocations, trained personnel and efficient data recording systems in order to trace back the infected animal sources (Ayele et al., 2004; Michel et al., 2010). Ideally all meat inspections need to be conducted under the supervision of a licensed veterinarian specifically trained for the purpose (Haddad et al., 2004). Even though the carcasses from healthy cattle without visible tuberculous lesions can be certified as fit for human consumption, M. bovis DNA can be detected in infected animal tissue well before the appearance of gross lesions (Swift et al., 2016). Therefore, inclusion of molecular detection methods may increase the sensitivity of abattoir monitoring for BTB (Proano-Perez et al., 2011; Sa’idu et al., 2015b; Silva et al., 2018b).

According to the beef production statistics in Sri Lanka, around 150,000 cattle are being processed annually at licensed abattoirs island-wide (Department of Census and Statistics, 2019). The total beef production in Sri Lanka is approximately 30 MT/year (DAPH, 2016). Most of the cattle (approximately 45 %) are processed in abattoirs in the Eastern province followed by Northern and North Western provinces (approximately 15 % each) according to the average production volumes of last five years, (Department of Census and Statistics, 2019). The procedure for inspecting meat produced for human consumption is regulated by the butcher’s ordinance in Sri Lanka (Annon, 1893). According to the provisions in the Butcher’s Ordinance, the legal power for meat inspection is vested with respective local authorities (Annon, 1893). However, many local authorities in Sri Lanka do not have sufficient resources to retain the services of a specially trained veterinarian or any other specifically trained staff for proper meat inspection on a regular basis. These lapses in food safety particularly in major municipalities are attributable not only to the financial limitations, but also to the sensitive religious and cultural reservations on beef production in Sri Lanka (Alahakoon et al., 2016). Although the sale of beef is not legally banned in any locality in Sri Lanka, many major municipalities have withdrawn the support to operate abattoirs due to these religious and cultural issues. The few existing municipal abattoirs do not process live cattle, and only receive dressed carcasses from wholesale fresh meat dealers for inspection and distribution. This prevailing practice regrettably allows beef produced at unauthorized establishments to enter the local market (Alahakoon et al., 2016).

Bovine tuberculosis had been suspected in Sri Lanka for several years and confirmed since 2012 (Kumara et al., 2014; DAPH, 2016; Kumara, 2017). Several cases have been reported from the Central province and relatively high herd prevalence of positive reactors were detected in twenty herds from the same province (Kumara, 2017). One tuberculosis positive carcass was detected at post-mortem examination in Kandy municipal abattoir and the affected animal had originated from the North Western province (Kumara et al., 2015). No data is currently available on the prevalence of BTB in any province other than the Central and North Western provinces in Sri Lanka. Therefore, the objective of this study was to undertake a preliminary assessment of the incidence of BTB in lungs of cattle carcasses at two abattoirs located in the Western province (WP) and North Central province (NCP) in Sri Lanka using direct acid-fast staining, microbiological culture, histopathology and PCR.

**METHODOLOGY**

**Selection of abattoirs**

Two abattoirs located in the WP (Colombo district) and NCP (Anuradhapura district) were selected for sampling. Approximately 15 % of total beef produced in Sri Lanka are processed at abattoirs located in WP and NCP. Each
province has an approximately equal processing capacity (Department of Census and Statistics, 2019). Selection of abattoirs was based on convenience and daily processing capacity. Further, previous BTB surveillance data are not available for WP and NCP. The abattoir in WP receives carcases of cattle originating from the entire country and live cattle are no longer processed at this abattoir. The abattoir in NCP receives live cattle for processing mainly from NCP. Ante-mortem veterinary inspection of cattle is not practiced at both of these abattoirs, but routine meat inspection by a trained veterinarian is practiced at the abattoir in WP.

Sample collection

An expected prevalence of 8 % was assumed for the calculation of sample size based on previous studies on BTB incidence in abattoirs in other endemic countries (Echeverria et al., 2014; Srinivasan et al., 2018). Thrusfield formula at 95 % confidence interval and 5 % desired absolute precision was used (Thrusfield, 2005).

\[ n = \frac{Z^2 \times P_{exp}(1 - P_{exp})}{d^2} \]

\[ n = \text{required sample size}, \ Z = \text{confidence interval (CI at 95 %), } P_{exp} = \text{expected prevalence of BTB in test unit, } d = \text{desired absolute precision (5 %)} \]

Accordingly, 115 lung samples were collected from the abattoirs (WP; n = 45 and NCP; n = 70) over a period of 2 months (from September to November 2019). The samples were collected immediately after the dressed carcases were brought to the abattoir (WP) or immediately after slaughter (NCP). Samples from cranial lung lobes were separately collected into sterile containers, stored in ice and transported on the same day to the laboratory. Approximately the same anatomic location was sampled from all lungs. Each lung sample was divided into three sections and impression smears were prepared for Ziehl-Neelsen (ZN) staining. The sections for culture were processed immediately. The sections for DNA extraction were frozen at -20 °C while the sections for histopathology were fixed in 10 % neutral buffered formalin at 1:10 (sample/ formalin) ratio and stored at room temperature until further processing.

Ziehl-Neelsen (ZN) staining

Direct impression smears from lung samples were used immediately for ZN staining. The slides were stained manually according to the standard protocols (Varello et al., 2008). All slides were examined by scanning the entire area at 1000x magnification. Samples were considered positive when at least one acid-fast bacillus was detected in at least one high-power field (Varello et al., 2008). Smears were also prepared from bacterial isolates and were also stained manually according to the standard protocols (Varello et al., 2008).

Culture procedure for M. bovis and M. tuberculosis complex (MTBC)

Samples were cultured on standard Lowenstein Jensen (LJ) medium with standard NaOH decontamination (Kent, 1985; Tripathi et al., 2014). Briefly, 1 g of sample was decontaminated by adding an equal volume of 4 % NaOH and leaving at room temperature for 10 min. Then it was centrifuged at 3000 g for 5 min and supernatant was discarded. The sediment was re-suspended in distilled water at 1:1 ratio. Thereafter, it was centrifuged at 3000 g for 5 min and supernatant was discarded. Afterwards, 500 µL of normal saline was added to the sediment and left for another 5 min and supernatant was poured off (Kent, 1985; Tripathi et al., 2014). Finally, the decontaminated lung samples were cultured in duplicate on LJ medium (Oxoid, UK) slants prepared with and without sodium pyruvate and incubated with closed caps at 37 °C. Culture bottles were observed for growth daily for first week and thereafter at weekly intervals for 8 wk. Slants with sodium pyruvate enhances the growth of M. bovis while the slants without sodium pyruvate supports the growth of other pathogenic members of MTBC (Wagari, 2016).

Histopathology

Formalin-fixed samples were processed and wax-embedded according to the standard protocols. Then the wax-embedded tissues were sectioned at 2 µm thickness and stained with hematoxylin and eosin (Souza et al., 2013). The slides were examined under light microscope at 1000x magnification.

DNA extraction and PCR

The DNA was extracted from lung tissue samples and all acid-fast bacilli (AFB) isolates using Reli Prep™ gDNA Tissue Miniprep kit (Promega, USA) after proteinase K digestion according to the manufacturer’s instructions. The PCR amplification of extracted DNA was performed with mycobacterial regions of difference 4 and 9 (RD4 and RD9) flanking primers while positive samples were confirmed with RD4 and RD9 internal primers according to published validated protocols (Brosch et al., 2002; Yahyaoui-Azami et al., 2017; Jayasumana et al., 2018a). The combined RD4 and RD9 flanking and internal PCR
assay allows the simultaneous diagnosis of *M. bovis* and other pathogenic members of MTBC (Brosch *et al.*, 2002; Yahyaoui-Azami *et al.*, 2017).

Primer sequences:
- RD4 Flanking F - CTCGTCGAAGGCCACTAAG
- RD4 Flanking R - AAGGCCAACAGATTCACGCAT
- RD4 Internal F - CAAGGGGTATGAGGTTCACG
- RD4 Internal R - CCGGTATGCTGATTGAACA
- RD9 Flanking F - GTGTAAGGTCAGCCCATC
- RD9 Flanking R - GCCCAACAGCTCGACATC
- RD9 Internal F - CGATGCTCAACACCACACTGC
- RD9 Internal R - CTGGACCTCGATGACCTC

The reaction mixture for all PCR assays contained 10 µL of 2x PCR master mix (Promega, USA), 0.5 µL of each forward and reverse primers (10 pmol/µL), 7 µL of nuclease free water and 2 µL of DNA template in a volume of 20 µL. All PCR reactions were performed in a Veriti® thermal cycler (Thermo Fisher Scientific, USA). The cycling conditions for all PCR reactions were an initial denaturation at 95 °C for 15 min followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 61 °C for 1 min and primer extension at 72 °C for 1 min with a final extension at 72 °C for 10 min (Brosch *et al.*, 2002; Warren *et al.*, 2006; Yahyaoui-Azami *et al.*, 2017). The amplified products were analysed by electrophoresis on a 1.5 % agarose gel stained with ethidium bromide.

**RESULTS AND DISCUSSION**

This preliminary assessment was undertaken to investigate the incidence of BTB among cattle processed at two abattoirs located in the Western and North Central provinces of Sri Lanka. Randomly collected samples from cranial lung lobes were used in this study. Different organs including lungs, lymph nodes, intestines, liver, spleen and heart have been used in previous abattoir surveys for BTB (Müller *et al.*, 2008; Cardoso *et al.*, 2009; Proaño-Pérez *et al.*, 2011; Gizaw *et al.*, 2017; Yahyaoui-Azami *et al.*, 2017; Jayasumana *et al.*, 2018a). The amplified products were analysed by electrophoresis on a 1.5 % agarose gel stained with ethidium bromide.

*M. tuberculosis* complex was detected in 7 % of the samples (8/115) during the initial screening PCR assays using RD9 flanking primers (Figure 1C). Among the eight samples that were PCR positive for MTBC, *M. bovis* was detected in six (5.2 %, 6/115) samples (Figure 2). Out of the six *M. bovis* positive samples, two originated from the abattoir in WP, while the remaining four samples originated from the abattoir in NCP, representing approximately equal incidence (4.45 % in WP and 5.7 % in NCP) of *M. bovis* infection in the two abattoirs surveyed in the current study (Figure 1D). All PCR results were confirmed by performing at least two independent assays. The detection rate of MTBC other than *M. bovis* by PCR in the current study was relatively very low [1.7 % (2/115)] compared to similar previous studies. The detection rates of MTBC other than *M. bovis* by PCR were 10 –13 % in previous abattoir surveys of BTB (Araújo *et al.*, 2005; Silva *et al.*, 2018b).

Direct acid-fast staining, microbiological culture, histopathology and PCR were used for this investigation. Acid-fast bacilli were not observed in any of the impression smears prepared directly from the lung tissue samples during the current survey. *Mycobacterium bovis* or MTBC cultures were also not recovered from any of the samples after 8 weeks of incubation. The *M. bovis* isolation rates and AFB detection rates in direct impression smears from cattle lungs without gross BTB lesions are generally very low (Proaño-Pérez *et al.*, 2011; Quinn *et al.*, 2015). However, three rapid growing AFB isolates (2.6 %, 3/115) were recovered within one week of culture (Figure 1A). All three AFB cultures were confirmed as non-tuberculosis mycobacteria (NTM) on the basis of rapid growth and absence of positive bands with RD9 flanking PCR (Brosch *et al.*, 2002; Warren *et al.*, 2006). Isolation rates of NTM in abattoir surveys for BTB in previous studies were 2–3 % (Araújo *et al.*, 2005). Therefore, the NTM isolation rate observed in the current study was comparable with previous studies. The NTM cultures were not characterised further. Additionally, two samples had fungal contaminants. The observed fungal contamination rate [1.7 % (2/115)] was relatively low. Fungal contamination rates up to 8 % have been observed during abattoir surveys for BTB in previous studies using the same NaOH decontamination and LJ medium culture method (Chatterjee *et al.*, 2013).
A granuloma was observed in histopathological examination in one of the PCR-positive samples, which was obtained from the NCP (Figures 1B, Figures 3A, 3B). Concentric layers of fibroblasts with lymphocytic and macrophage aggregations were observed in the granuloma in addition to compression of the adjacent lung parenchyma (Figure 3A). Furthermore, an area of caseous necrosis with mild calcification was observed in the centre of the granuloma (Figure 3B). Another PCR positive sample originating from NCP showed...
atypical pneumonia with destruction of alveoli, moderate consolidation and aggregation of leukocytes (Figure 3C, 3D). Rest of the PCR-positive samples and all of the PCR-negative lung samples showed mild emphysema and varying degree of congestion (Figure 3E, 3F).

Figure 3: Representative photomicrographs of hematoxylin and eosin-stained lung sections that were PCR-positive (A-D) and PCR-negative for \textit{M. bovis} (E and F). A granuloma (arrows) compressing adjacent lung parenchyma is observed (A). Caseous necrosis (arrows) with mild calcification is observed in the centre of the granuloma (B). Atypical pneumonia with destruction of alveoli is observed (C). Aggregations of lymphocytes (arrows) and macrophages (arrow heads) are observed in the lungs with atypical pneumonia (D). Mild emphysema (arrows) and congestion (arrow heads) is observed in PCR-negative lung tissues (E and F). Bar = 100 mm

The incidence of \textit{M. bovis} infection observed among cattle processed at the two abattoirs in WP and NCP in Sri Lanka during the current study was relatively low (5.2 %). Similar previous studies conducted in several endemic countries have frequently reported > 10 % BTB incidence (Proano-Perez et al., 2011; Sa’idu et al., 2015b; Silva et al., 2018b). However, abattoirs in Sri Lanka including these two abattoirs do not maintain complete cattle movement history records. Therefore, the observed incidence of \textit{M. bovis} infection does not necessarily indicate the disease burden in WP or NCP. Bovine tuberculosis has been previously detected and confirmed in a cattle carcass processed at the Kandy municipal abattoir. The affected animal had originated from the North Western province (Kumara et al., 2015). Based on the Mycobacterial Interspersed Repetitive Unit – Variable Number Tandem Repeat (MIRU-VNTR) typing, this \textit{M. bovis} strain was genetically distinct from several other \textit{M. bovis} strains from BTB cases in Central Province (Jayasumana et al., 2018b).

Good agreement between bacteriological culture, AFB in impression smears and PCR results were not observed in the current study. This may be partly attributable to contamination of lung samples as carcasses are not handled separately at the abattoirs. Comparatively similar results were observed in an abattoir survey of BTB conducted among apparently healthy cattle in Palestinian Territories (Ereqat et al., 2013). A total of 2.9 % (6/208) lung samples without gross BTB lesions were positive for \textit{M. bovis} specific PCR while only one sample was positive for AFB (Ereqat et al., 2013). No bacterial growths were observed from the cultured lung, liver and kidney tissues after 8 weeks of incubation using the same NaOH decontamination and LJ medium similar to the ones used in the present study (Ereqat et al., 2013). Similarly, in another study that sampled cattle with gross BTB lesions, only 56 % (28/50) of lymph node samples having caseous tuberculous lesions had positive cultures while granuloma formation was observed in 64 % of the samples (Silva et al., 2018a). Only 12 % of the samples was positive for AFB. \textit{Mycobacterium bovis} was detected by PCR only in 20 % of the samples (Silva et al., 2018a). Contrary to these results, several other studies that sampled cattle with gross BTB lesions have reported good agreement between culture and PCR (Cardoso et al., 2009; Proano-Perez et al., 2011; Gizaw et al., 2017; Yahyaoui-Azami et al., 2017; Silva et al., 2018b). Meat inspection protocols may vary in different jurisdictions. Therefore, the sensitivity of detecting gross lesions of BTB at post-mortem examination may vary according to the protocol employed for meat inspection, anatomical sites examined and the stage of infection since early stage of the disease may result in lower detection rates (Corner, 1994).

Although culture is considered the gold standard test for diagnosing \textit{M. bovis}, identification rates are low compared to molecular diagnostic methods (Ayele et al., 2004). This could be due to several factors including the presence of very low number of live bacteria in tissues, subclinical infection or early stage of disease.
and destruction of live bacteria during sample transport and decontamination process or freeze-thawing cycles (Palomino & Portaels, 1998; Proaño-Pérez et al., 2011). Routine NaOH decontamination protocol and culturing on LJ medium slants were used in the current study due to budgetary limitations. Samples were not frozen before culture. Nearly 100% isolation rates were previously achieved under the same culture conditions from local cattle lung samples with visible BTB lesions (Kumara et al., 2014; Kumara et al., 2015). Stonebrink-Leslie medium have been shown to have better M. bovis isolation rates than LJ medium (Proaño-Pérez et al., 2011; Silva et al., 2018a). Up to 50% isolation rates were observed on Stonebrink-Leslie medium while no bacterial growths were observed on LJ medium (Proaño-Pérez et al., 2011; Silva et al., 2018a).

Approximately 60% of all human pathogens and nearly 75% of all recently emerging diseases in humans are zoonotic (FAO, 2019). Although M. tuberculosis is the main causative agent for human tuberculosis, approximately 3% of human tuberculosis cases are due to M. bovis infections (Cosivi et al., 1998). Therefore, BTB has been listed as one of the seven neglected zoonotic diseases in the world by the WHO (Ayele et al., 2004). It is also listed as one of the important zoonotic diseases in Latin America, Africa and Asia (De la Rua-Domenech, 2006; Smith et al., 2006). Zoonotic M. bovis-induced tuberculosis in humans have not been reported in Sri Lanka (Müller et al., 2013; NPTCCD, 2016). This may be due to underreporting as all AFB positive patients are reported as having ‘bacteriologically confirmed TB’ in Sri Lanka without any further investigations (NPTCCD, 2016). However, two confirmed cases of tuberculosis in Sri Lankan elephants were caused by M. tuberculosis strains that are similar to East-African-Indian (EAI) lineage commonly circulating among humans in the country according to MIRU-VNTR typing (Perera et al., 2014; Jayasumana et al., 2018b).

Infected cattle and carcasses may pose a risk to the workers in abattoirs, by exposing them to M. bovis while handling infected animals or products from infected animals (Tibebu et al., 2014). Handling and consumption of uncooked or partially cooked meat from infected cattle may be a source of infection of M. bovis to consumers (LoBue et al., 2003; Thoen et al., 2006; Sa’idu et al., 2015a). However, consumption of unpasteurised milk or dairy products is considered as the primary risk factor for M. bovis infection in humans (Leite et al., 2003; De la Rua-Domenech, 2006; Kinde et al., 2007). According to the limited information available, M. bovis has not been detected in raw milk in Sri Lanka and all milk products currently available in Sri Lanka are either pasteurised or sterilised (Jayasumana et al., 2018a). Therefore, the zoonotic risk of M. bovis remains low in Sri Lanka.

The cattle processed at the abattoir in NCP were largely originated from the herds managed under extensive cattle management system in the dry zone of Sri Lanka. These cattle herds roam freely and always interact with wild animals at the boarders of the forests and even within the national parks. Therefore, existence of M. bovis positive animals in these herds pose a potential risk for introducing M. bovis to wildlife or may be an indication of carrying the infection from a wildlife reservoir to domestic cattle herds. Since occurrence of M. bovis in wildlife is not yet reported in Sri Lanka, the source of infection of these infected cattle is unknown. Control programmes in Europe and North America have shown that M. bovis can be controlled as long as it is restricted to livestock (Fitzgerald & Kaneene, 2013; Miller & Sweeney, 2013). Eradication is nearly impossible once it has spread into wildlife (Cantas & Suer, 2014). Classic examples of well-established stable wildlife reservoirs of M. bovis are European badger in the United Kingdom (Cheeseman et al., 1989), elk in Canada (Wobeser, 2009) and white-tailed deer in the USA (O’Brien et al., 2006).

Detection of M. bovis even at a low incidence from two abattoirs under less than optimal laboratory conditions suggest that apparently healthy cattle may be a potential source of M. bovis infection in Sri Lanka that may pose a risk to public health. Therefore, appropriate measures including regularised meat inspection at all abattoirs are required together with increased BTB surveillance capacity of the national veterinary service to mitigate this risk. Further studies are essential to determine the exact prevalence of BTB in Sri Lanka and to identify any wildlife reservoirs of BTB in the country.

**CONCLUSIONS**

Relatively low incidence of M. bovis infection (5.2%) was observed among apparently healthy cattle processed at two abattoirs in the Western and North Central provinces in Sri Lanka. Only one PCR positive sample had a granuloma. Detecting M. bovis among extensively managed cattle herds in the dry zone is a potential challenge for controlling BTB in the country. Therefore, increased BTB surveillance and strengthening the testing capacity of the national veterinary service is essential to mitigate this risk.
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