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Application of the gamma-interferon assay to determine the prevalence of bovine tuberculosis in slaughter livestock at abattoirs in Gauteng, South Africa

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Abstract

Background: Bovine tuberculosis (bTB) is a zoonotic disease with great economic impact estimated at billions of dollars annually worldwide. Meat inspection represents a long-standing form of disease surveillance that serves both food safety and animal health. The objective of this study was to determine the prevalence of bTB in livestock at abattoirs using a cell-mediated immune (CMI) assay, the gamma interferon (IFN-γ) assay. This cross-sectional study was conducted at selected abattoirs (low-throughput, high-throughput and rural/informal) in Gauteng province, where animals were also subjected to routine meat inspection.

Results: A total of 410 fresh blood samples were collected from slaughter livestock (369 cattle and 41 sheep) from 15 abattoirs, and analysed using Bovigam® test kit with bovine, avian and Fortuitum purified protein derivatives (PPD) as blood stimulating antigens. The estimated prevalence of bTB in cattle was 4.4% (95% CI: 2.4%–7.3%). The prevalence of bTB in cattle varied between abattoirs ($p = .005$), ranging from 0% to 23%; however, there were no significant differences among genders, breeds, municipality, districts, origins of animals (feedlot, auction or farm) or throughput of abattoirs. The prevalence of avian reactors was 6.0% (95% CI: 3.6%–9.2%) in cattle, varying between abattoirs ($p = .004$) and ranging from 0% to 20.7%. None of the sheep with valid test results was positive for bTB and none was avian reactors (95% CI: 0%–15%).

Conclusion: The detection of bTB reactor cattle in our study clearly shows the limitation of disease surveillance using a meat inspection approach, as all the 410 slaughter animals sampled had passed visual abattoir inspection and been classified as bTB-free. Our findings therefore emphasize the risk of zoonotic transmission of bTB to abattoir workers and potential food safety hazard to consumers. Furthermore, our study highlights the potential for the use of the IFN-γ assay to reduce this risk.

KEYWORDS

abattoirs, bovine tuberculosis, gamma interferon assay, cattle, zoonosis

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Impact of the study

- The main aim of this study was to determine the prevalence of bovine tuberculosis (bTB) in slaughter livestock at abattoirs in Gauteng province of South Africa using the modified interferon gamma (IFN-γ) assay.
- Our study highlighted the inadequacy of meat inspection alone to detect bTB in cattle slaughtered for human consumption; hence imperative to apply additional methods, such as the IFN-γ assay to establish the bTB infection status in slaughter cattle at abattoirs.
- This approach will likely reduce the risk of TB posed to abattoir workers and consumers of meat from infected cattle.

1 | INTRODUCTION

Mycobacterial species that are responsible for tuberculosis infections in both humans and animals belong to the *Mycobacterium tuberculosis* complex (MTBC). The complex comprises organisms such as *Mycobacterium tuberculosis*, *Mycobacterium bovis* (Karlson & Lessel, 1970), *M. bovis* Bacillus Calmette–Guerin (BCG), *Mycobacterium africanum*, *Mycobacterium microti*, *Mycobacterium canettii*, *Mycobacterium pinipeddii* (Cousins et al., 2003) and M*ycobacterium caprae* (Aranaz et al., 1999). Additionally, species such as *Mycobacterium orygis* (Van Ingen et al., 2012), *Mycobacterium mungi* (Alexander et al., 2010) and *Mycobacterium suricattae* (Parsons et al., 2013) have been identified recently. The MTBC complex has high levels of genetic similarity, up to 99.9%, amongst its members (Rogal et al., 1990) and is responsible for infecting different host species. Bovine tuberculosis (bTB), which is mainly caused by *M. bovis*, is considered as one of the largest veterinary health problems and a public health concern (Michel et al., 2010). Organizations such as World Organisation for Animal Health (OIE), World Health Organization (WHO) and Food and Agriculture Organization (FAO) have classified bTB as a neglected zoonotic disease, especially in developing countries. Approximately 50 million cattle are infected annually worldwide, resulting in economic losses of approximately $3 billion (Waters et al., 2012). Due to the scourge of the disease, WHO declared a global emergency in 1993 (Grange & Zumla, 2002).

Bovine tuberculosis has a wide host range consisting of wild animals, domestic animals and humans and the hosts are categorized into two groups, i.e. the maintenance and the spill-over or dead-end hosts (Ayele et al., 2004). In South Africa, bTB is endemic and *M. bovis* infection has been reported in cattle, pigs and 21 different wildlife species (Hlokwe et al.,2014, 2019; Michel, 2008). A recent study by Hlokwe and colleagues (2014) demonstrated that bTB had not only increased in spatial distribution in South Africa but that the number of wildlife that can be infected by the disease has also increased in comparison to a decade ago (Hlokwe et al., 2019). The prevalence of bTB in commercial cattle herds in South Africa was reduced to 0.4% in 1995 due to implementation of the test and slaughter programme which was introduced in 1969 (Michel, 2008). In recent years, sporadic outbreaks in different regions have been reported (Hlokwe et al., 2014; Rodwell et al., 2001; Sichewo et al., 2019).

Early and accurate diagnosis of bTB is important to limit the spread of the disease because of its chronic nature (Churbanov & Milligan, 2012). Globally, abattoirs are used for passive and active surveillance of diseases of both economic and public health significance. Information generated from abattoir surveillance could provide an early warning system for impending epidemics or failures of intervention programmes (Alton et al., 2015).

The Bovigam® test (Prionics AG) is a blood-based cell-mediated immune assay that has been validated, included in the OIE register as a diagnostic kit for bTB and is commonly applied as an ancillary test for the ante-mortem diagnosis of bTB in cattle, goats, sheep (Munoz-Mendoza et al., 2016) and buffaloes (Michel et al., 2011). The test is used to detect latent bTB by measuring the amount of IFN-γ released by white blood cells upon infection and may be modified by inclusion of Fortuitum purified protein derivative (PPD) as an additional antigen to increase its specificity (unpublished data). The current study was conducted at selected abattoirs in the Gauteng province, South Africa and the main objective was to determine the prevalence of livestock bTB in slaughter livestock in these abattoirs using the modified IFN-γ assay. The results should indicate the status of bTB in livestock farms from where the animals originated.

2 | MATERIALS AND METHODS

2.1 | Type of study, sample size determination and animal species

This was a cross-sectional study conducted at selected abattoirs (2 low throughput and 13 high throughput) in Gauteng province of South Africa. The required sample size of 410 was determined using a formula (Thrusfield et al., 2013) in order to estimate a prevalence of 1% with a 1% precision. At each selected abattoir, slaughter cattle and/or sheep were sampled on a single day using a systematic random sampling method. A total of 410 samples were collected comprising 369 adult cattle (Bonsmara, $n = 277$; Jersey, $n = 39$; Nguni, $n = 51$; Brahman, $n = 1$; Holstein, =1) and 41 sheep (Dorper) of any gender for serological testing for livestock bTB using the IFN-γ assay.

2.2 | Pre- and post-slaughter inspection

The professional meat inspectors assigned to each abattoir conducted pre- and post-slaughter inspection according to a standard

procedure, which included detection of clinical TB lesions. Pre-slaughter inspection was conducted by checking for abnormalities in respiration, behaviour, etc., of the animal that was being inspected. A post-slaughter inspection was performed as follows: the internal and external surfaces of the carcass were examined for the detection of any lesions. For the head inspection, lymph nodes such as retropharyngeal lymph node, parotid and submaxillary were incised and examined. In cattle, the oesophagus was separated and examined. For inspection of the viscera in sheep, the bronchi were opened, and in cattle, the larynx, trachea and bronchi were opened and examined. The heart was incised from the base to the apex and examined. The hepatic system and the liver were also excised and examined. Other organs such as the stomach, spleen including its lymph nodes and the gastrointestinal tract were also examined. Any part of the carcass that showed abnormalities was condemned. Information regarding animal species, gender, breed, district, municipality, origin of animals and abattoir name and throughput was recorded in a spreadsheet.

2.3 | Blood collection

During the slaughter of animals, blood samples were collected into sterile heparinized tubes, transported to the laboratory at room temperature and processed within 8 hr of collection. All samples were processed according to established standard laboratory protocols used at the Tuberculosis laboratory of the Agricultural Research Council-Onderstepoort Veterinary Research (ARC-OVR), Onderstepoort, South Africa.

2.4 | Stimulation of whole blood samples and detection of gamma interferon

In the first phase of the test, fresh blood samples were stimulated with the purified protein derivatives (PPD). For each animal, blood in the heparin tube was carefully mixed and 1.5 ml aliquoted into 5 individual wells of a 24-well plate. Blood samples were sensitized with 30 μl bovine PPD (600 IU/ml); 60 μl avian PPD (1,000 IU/ml) (Prionics AG); 25 μl PPD-Fortuitum (0.5 mg/ml) in individual wells. As an internal positive control, 11 μl Pokeweed mitogen (5 μg/ml) was aliquoted into the next well of the plate. Unstimulated whole blood from each animal served as negative controls for the assay. The tuberculin and blood were carefully mixed by gentle hand agitation and incubated at 37°C for 20–24 hr. After incubation, the samples were centrifuged at 3,000 rpm for 10 min and 150 μl of the plasma transferred into appropriately identified tubes with corresponding labels. Plasma samples were stored at −20°C until tested. The plasma samples were assayed for the presence of IFN-γ using a commercially purchased Bovigam® 1G-test kit (Prionics AG), following the manufacturer's instructions. The production of IFN-γ by the lymphocytes was detected using a monoclonal antibody-based sandwich enzyme immunoassay (EIA). Optical densities were measured on a BioTek ELx800 Plate reader (BioTek Instruments Inc.) at 450 nm. Results were interpreted as previously described (Michel et al., 2011).

2.5 | Statistical analysis

Univariate associations of breed, gender, district, municipality, abattoir, origin of animals, abattoir type and animal species with the prevalence of bTB, of avian reactors, and of overall *Mycobacterium* spp. exposure, were assessed using cross-tabulation and the Fisher's exact test. Where possible, variables significant in the univariate analysis were included in multivariable logistic or exact logistic regression models to adjust for confounding. Data were analysed using Stata 15 (StataCorp); and $p < .05$ was regarded as statistically significant.

3 | RESULTS

Of the 369 cattle sampled, valid IFN-γ results (i.e. test samples passed quality control checks) were obtained in 318 (86.2%) of the cattle. The estimated prevalence of cattle positive for bTB was 4.4% (95% CI: 2.4%–7.3%; Table 1). Of the eight variables analysed, seven (animal species, gender, breed, district, municipality, origin of animals and abattoir throughput) were not associated with the estimated prevalence of bTB. However, the prevalence varied significantly between abattoirs (p =.005), ranging between 0% and 23.1% (Table 1). The estimated prevalence of avian reactors was 5.9% (95% CI: 3.6%–9.2%; Table 2), also varying significantly between abattoirs (p = .004), ranging from 0% to 20.7%. The prevalence of avian reactors in cattle was not significantly different to that of bTB. The estimated prevalence of cattle tested reacting to *Mycobacterium* spp. (combined bTB and avian reactors) was 10% (95% CI: 7.0%–14%; Table 3). In the univariate analysis, the prevalence varied by gender of animal (3.0% in females and 11.9% in males) and by breed (5.4% in Jersey, 13% in Bonsmara, 0% in other breeds), but these differences were not significant after adjusting for confounding using exact logistic regression. Of the 41 sheep sampled, valid IFN-γ results were obtained in 22 (54%) of the animals and none were positive for bTB nor were there any avian reactors (95% CI: 0%–15%) (Table 1-3). No nodular bTB lesions were detected in any of the slaughtered livestock during post mortem examination.

4 | DISCUSSION

Detection of bTB and avian reactors in our study is an indication that IFN-γ was released from T lymphocytes of cattle infected with a member of the MTBC or *Mycobacterium avium* species respectively. While MTBC species are the causative agents of bTB, *M. avium* species are non-tuberculous mycobacteria that do not cause bTB in cattle. The IFN-γ assay has long been used for the detection of bTB in cattle (Gormley et al., 2006) and buffalo (Michel et al., 2011). One of

TABLE 1 Overall estimated prevalence of bovine tuberculosis (bTB) in cattle in Gauteng abattoirs

Variable	Level	*n*	Prevalence (%)	95% CI	*p*-value
Species	Bovine	318	4.4	2.4–7.3	.612
	Ovine	22	0	0–15.4	
Sex	Male	252	5.2	2.8–8.7	.315
	Female	66	1.5	0.04–8.2	
Breed	Bonsmara	231	5.6	3.0–9.4	.27
	Nguni	48	0	0–7.4	
	Jersey	37	2.7	0.07–14.2	
	Brahman	1	0	0–97.5	
	Holstein	1	0	0–97.5	
District	Tshwane	138	7.2	3.5–12.9	.213
	Sedibeng	94	8.5	0.26–5.8	
	Metsweding	4	0	0–60.2	
	West Rand	29	3.4	0.08–17.8	
	Ekurhuleni	53	3.8	0.5–13.0	
Municipality	City of Tshwane	129	6.9	3.2–12.8	.387
	Ekurhuleni Metro	53	3.8	0.5–13.0	
	Emfuleni	37	0	0–94.8	
	Kungwini	13	7.7	0.19–36	
	Lesedi	57	1.8	0.04–9.3	
	Mogale City	29	3.4	0.08–17.8	
Abattoirs	Abattoir A	8	0	0–36.9	.005
	Abattoir B	26	23.1	8.9–43.6	
	Abattoir C	21	4.8	0.12–23.8	
	Abattoir D	29	3.4	0.08–17.8	
	Abattoir E	28	3.6	0.09–18.3	
	Abattoir F	19	0	0–17.6	
	Abattoir G	13	7.7	0.19–36.0	
	Abattoir H	25	4	0.1–20.4	
	Abattoir I	28	3.6	0.09–18.3	
	Abattoir J	29	0	0.0–11.9	
	Abattoir K	31	0	0.0–11.2	
	Abattoir L	20	0	0.0–16.8	
	Abattoir M	24	0	0.0–14.2	
	Abattoir *N*	17	0	0.0–19.5	
	Abattoir O	19	10.5	1.3–33.1	
Origin of animals	Auctions	43	2.3	0.05–12.3	.702
	Farm/Feedlot	275	4.7	2.5–7.9	
Abattoir type	HT-Multi	297	4.4	2.4–7.4	1
	LT-Multi	21	4.8	0.1–23.8	
Total	318	4.4	2.4–7.3		

its advantages is that it is able to detect early stages of infection as compared to post mortem examination of lesions that are as a result of late stages of infection.

The IFN-γ assay is recognized by authorities such as the European Union and in countries such as New Zealand as an approved test for the diagnosis of bTB (Ozturk et al., 2010) and it has been used in many countries for surveillance and in bTB eradication control programs as an ancillary test or a confirmatory test. A study conducted by Ozturk and colleagues (2010) in Turkey revealed comparable sensitivity and specificity between the intradermal test and the IFN-γ assay. The study showed that the IFN-γ assay had a sensitivity of 90% and specificity of 97% and they recommended that it could

TABLE 2 Overall estimated prevalence of avian reactors in cattle in Gauteng abattoirs

Variable	Level	*n*	Prevalence (%)	95% CI	*p*-value
Species	Bovine	318	5.9	3.6–9.2	.623
	Ovine	22	0	0–15.4	
Sex	Male	252	7.1	4.3–11.0	.14
	Female	66	1.5	0.04–8.2	
Breed	Bonsmara	231	7.8	4.7–12.0	.178
	Nguni	48	0	0–7.3	
	Jersey	37	2.7	0.07–14.2	
	Brahman	1	0	0–97.5	
	Holstein	1	0	0–975	
District	Tshwane	138	3,6	1.2–8.3	.169
	Sedibeng	94	8,5	3.7–16.0	
	Metsweding	4	0	0–60.2	
	West Rand	29	13.8	3.9–31.7	
	Ekurhuleni	53	3.8	0.5–13.0	
Municipality	City of Tshwane	129	3.9	1.2–8.8	.096
	Ekurhuleni Metro	53	3.8	0.5–13	
	Emfuleni	37	2.7	0.07–14.1	
	Kungwini	13	0	0–24.7	
	Lesedi	57	12.3	5.1–23.7	
	Mogale City	29	13.8	3.8–31.7	
Abattoirs	Abattoir A	8	12.5	0.31–52.6	.004
	Abattoir B	26	0	0–13.2	
	Abattoir C	21	19	5.4–41.9	
	Abattoir D	29	13.8	3.9–31.6	
	Abattoir E	28	3.6	0.09–18.3	
	Abattoir F	19	0	0–17.6	
	Abattoir G	13	0	0–24.7	
	Abattoir H	25	4	0.1–20.4	
	Abattoir I	28	3.6	0.09–18.3	
	Abattoir J	29	20.7	7.9–39.7	
	Abattoir K	31	0	0.0–11.2	
	Abattoir L	20	0	0.0–16.8	
	Abattoir M	24	0	0.0–14.2	
	Abattoir *N*	17	5.9	0.2–28.6	
	Abattoir O	19	0	0.0–17.6	
	Farm/feed lot	275	6.9	4.2–10.6	
Origin animals	Auctions	43	0	0.0–8.2	0.088
	Farm/feed lot	275	6.9	4.2–10.6	
Abattoir type	HT-Multi	297	6	3.6–9.4	1
	LT-Multi	21	4.8	0.1–23.8	
Total		318	5.9	3.6–9.1	

be used as an alternative to the intradermal tuberculin test (Ozturk et al., 2010). In South Africa, the IFN-γ assay was modified (by inclusion of Fortuitum PPD as an additional antigen) to counter the observed false positive test results caused by cross reaction with environmental mycobacteria in cattle, providing a high specificity of over 99% and sensitivity of 86% in cattle (unpublished data). This modified Bovigam® test has since been applied in both cattle and buffaloes in South Africa (Hlokwe et al., 2016; Sichewo et al., 2019).

In the current study, we applied the IFN-γ assay in slaughter livestock and estimated the prevalence of bTB in cattle to be 4.4%.

TABLE 3 Overall estimated prevalence of cattle reacting to any Mycobacterium spp

Variable	Level	*n*	Prevalence (%)	95% CI	*p*-value
Species	Bovine	318	10	7.0–14.0	.246
	Ovine	22	0	0–15.4	
Sex	Male	252	11.9	8.2–16.6	.037
	Female	66	3	0.36–10.5	
Breed	Bonsmara	231	13	8.9–18	.023
	Nguni	48	0	0–7.4	
	Jersey	37	5.4	0.7–18.2	
	Brahman	1	0	0–97.5	
	Holstein	1	0	0–975	
District	Tshwane	138	7.2	3.5–12.9	.681
	Sedibeng	94	8.5	0.26–5.8	
	Metsweding	4	0	0–60.2	
	West Rand	29	3.4	0.08–17.8	
	Ekurhuleni	53	7.5	2.1–18.2	
Municipality	City of Tshwane	129	10.1	5.5–11.6	.357
	Ekurhuleni Metro	53	7.5	2.1–18.2	
	Emfuleni	37	2.7	0.06–14.2	
	Kungwini	13	7.7	0.19–36.0	
	Lesedi	57	14	6.25–25.8	
	Mogale City	29	17.2	5.8–35.8	
Abattoirs	Abattoir A	8	12.5	0.31–52.6	.063
	Abattoir B	26	23.1	8.9–43.6	
	Abattoir C	21	19	5.4–41.9	
	Abattoir D	29	17.2	5.8–35.8	
	Abattoir E	28	7.1	0.9–26.0	
	Abattoir F	19	0	0–17.6	
	Abattoir G	13	7.7	0.19–36.0	
	Abattoir H	25	8	0.1–26.0	
	Abattoir I	28	7.1	0.09–23.5	
	Abattoir J	29	20.7	7.9–39.7	
	Abattoir K	31	0	0.0–11.2	
	Abattoir L	20	0	0.0–16.8	
	Abattoir M	24	0	0.0–14.2	
	Abattoir *N*	17	5.8	0.1–28.7	
	Abattoir O	19	10.5	1.3–33.1	
Origin animals	Auctions	43	2.3	0.05–12.3	.098
	Farm/feed lot	275	11.3	7.8–15.6	
Abattoir type	HT-Multi	297	10.1	6.9–14.1	1
	LT-Multi	21	9.5	1.2–30.3	
Total		318	10	7.0–13.0	

Variables such as species, breed, abattoir throughput and source of animals were not associated with the prevalence, although it varied significantly between abattoirs, suggesting geographic variation in the prevalence of bTB in and around Gauteng.

No MTBC infection in sheep was detected, although our sample size was small; moreover, a high percentage (46%) of invalid test results were obtained in sheep. Bovine tuberculosis in sheep has never been reported in South Africa and the true disease status is

currently unknown. Additionally, the sensitivity and specificity of the IFN-γ assay has also not been evaluated in sheep. Although the infection in sheep is generally uncommon worldwide, in countries such as Spain, several outbreaks of the disease epidemiologically linked to cattle have been reported (Munoz-Mendoza et al., 2016). A recent report from Ethiopia (Gelalcha et al., 2019) indicated that sheep serve as spill-over hosts as they become infected only if there is a source of infection and sheep-to-sheep transmission is highly unlikely.

Meat inspection is a long-standing form of disease surveillance for both food safety and animal health. For diseases that produce slowly progressive but evident lesions, such as bTB, slaughterhouse inspection is considered an effective surveillance tool (Gormley et al., 2014). The detection of positive bTB reactors in our study has, however, clearly shown the limitations of this method of disease surveillance, as the carcasses of all the 410 slaughter animals sampled had passed visual meat inspection and been classified as TB-free. It is, however, possible that visible lesions had not yet formed, as they represent late stage of infection. It is known that abattoir workers are faced with a high risk of exposure of bTB since they might be unknowingly inspecting an infected carcass in closed spaces, which may lead to direct inhalation of contaminated droplets. Additionally, exposure might occur as a result of occupational injuries since they are working with knives (de la Rua-Domenech, 2005; Vayr et al., 2018). Hence, the potential zoonotic risk of transmission to abattoir workers as well as food safety hazard to consumers cannot be ignored. The risk might however, be lessened in meat consumers provided that the meat is well cooked.

The IFN-γ assay is a rapid test, with results available within 48 hr following blood collection and it is cost effective. Although it may not be feasible to test every animal destined for slaughter, a structured screening mechanism may be developed for testing animals originating from the same farm to determine their disease status. Studies have demonstrated that the use IFN-γ assay in combination with other TB tests leads to more accurate screening for bTB in cattle (Ahir et al., 2016; Neeraja et al., 2014).

5 | CONCLUSION

This study demonstrated the presence of bTB in animals classified as TB-free by routine meat inspection. Our study further highlighted the inadequacy of meat inspection alone to detect bTB in cattle slaughtered for human consumption. It is therefore imperative to apply additional methods, such as the IFN-γ assay, to establish the bTB infection status in slaughter cattle at abattoirs. This approach will likely reduce the risk of TB posed to abattoir workers and consumers of meat from infected cattle.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. This study was approved by the Animal Ethics Committees for the Agricultural Research Council-Onderstepoort Veterinary Research (AEC12.16) and the University of Pretoria (V104-17). Permission to undertake the study was also granted by the Department of Agriculture, Land Reforms and Rural Development (DALRRD) through section 20 approval in terms of the animal diseases Act number 35 of 1984.

PEER REVIEW

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