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Detection of *Mycobacterium bovis* infection in African buffaloes (*Syncerus caffer*) using QuantiFERON®-TB Gold (QFT) tubes and the Qiagen cattletype® IFN-gamma ELISA

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ABSTRACT

African buffaloes (*Syncerus caffer*) are wildlife maintenance hosts of *Mycobacterium bovis*, the cause of bovine tuberculosis. Consequently, *M. bovis* infected buffaloes pose a transmission risk for cattle and other wildlife species. Previously, a modification to the Qiagen QuantiFERON®-TB Gold (QFT) system, using QFT tubes and an in-house bovine interferon-gamma (IFN-γ) ELISA, was evaluated for the detection of *M. bovis* infection in buffaloes. Subsequently, Qiagen has developed a commercially available cattletype® IFN-gamma ELISA for the detection of antigen-specific IFN-γ release in ruminants. The aim of this study was to investigate the use of QFT tubes and the cattletype® IFN-gamma ELISA, in a cattletype IFN-γ release assay (IGRA), to detect *M. bovis* infection in African buffaloes. The test agreements between the cattletype IGRA, single comparative intradermal skin test (SCITT) and Bovigam® 1G IGRA in two *M. bovis*-exposed buffalo populations ($n = 134$ and $n = 92$) were calculated and κ coefficients ranged from 0.65 (95% CI 0.48–0.82) to 0.86 (95% CI 0.72–0.99). Increasing the QFT incubation time in one *M. bovis*-exposed buffalo cohort ($n = 92$), from 20 to 40 h, had no effect on the cattletype IGRA test results. Inter-assay and intra-assay reproducibility determination for the cattletype IGRA produced coefficient of variations (CV) < 9.1% and < 1.7%, respectively. A total of 21/21 known *M. bovis*-unexposed buffaloes tested negative in the cattletype IGRA. Moreover, the cattletype IGRA test result values were significantly greater for 13 *M. bovis* culture-positive buffaloes compared with 14 *M. bovis*-exposed culture-negative ($P < .01$) and 21 *M. bovis*-unexposed ($P < .001$) buffaloes, respectively. These findings suggest that the combination of QFT tubes and the cattletype® IFN-gamma ELISA is a promising new diagnostic assay for the detection of *M. bovis* infection in African buffaloes. However, further research is needed to evaluate the sensitivity and specificity of the assay in larger African buffalo populations.

1. Introduction

Mycobacterium bovis (*M. bovis*) infection causes bovine tuberculosis (bTB), a zoonotic disease affecting a broad range of hosts (Michel et al., 2006). *M. bovis* is endemic in South Africa's two largest wildlife reserves, the Kruger National Park (KNP) and Hluhluwe iMfolozi Park (HiP). Within these reserves, African buffaloes (*Syncerus caffer*) are one of the most important maintenance hosts of *M. bovis* (de Vos et al., 2001). More recently, buffaloes in smaller reserves like Madikwe Game Reserve (MGR) and privately-owned farms in South Africa have been diagnosed with bTB (Hlokwe et al., 2016). *M. bovis*-infected buffaloes do not only pose a transmission risk to other wildlife species,

Abbreviations: ARC, Agricultural Research Council; bTB, bovine tuberculosis; CFP-10, culture filtrate protein 10; CMI, cell mediated immunity; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; ESAT-6, early secretory antigen target 6; HiP, Hluhluwe iMfolozi Park; IFN-γ, interferon gamma; IGRA, interferon gamma release assay; KNP, Kruger National Park; *M. bovis*, *Mycobacterium bovis*; MGR, Madikwe Game Reserve; mQFT, modified QuantiFERON®-TB Gold assay; PPD_a, *Mycobacterium avium* protein purified derivative; PPD_b, *Mycobacterium bovis* protein purified derivative; QFT, QuantiFERON®-TB Gold; RD1, region of difference one; S/P, sample to positive ratio; SCITT, single comparative intradermal tuberculin test; SFT, skin fold thickness

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particularly large carnivores such as African lions (*Panthera leo*) (Olivier et al., 2017), but also to economically important livestock such as domestic cattle populations (de Vos et al., 2001; Musoke et al., 2015). Early detection of *M. bovis* infection in buffaloes is necessary to reduce the risk of transmission to other animals. Furthermore, prompt identification of these animals will reduce the risk of infection in buffaloes through translocation and improve the success of disease management strategies.

The early diagnosis of *M. bovis* infection relies primarily on the measurement of pathogen-specific cell-mediated immune responses (Vordermeier et al., 2000). In South Africa, the only such test approved for buffaloes is the single comparative tuberculin test (SCITT) that detects a delayed-type hypersensitivity response to *M. bovis* purified protein derivative (PPD_b). However, this test has numerous drawbacks: i) the use of PPD_b, a cocktail of *M. bovis* antigens, may result in cross-reactive immune responses to other non-virulent mycobacteria (Michel et al., 2008); ii) repeating the SCITT may lead to the desensitization of infected animals (Coad et al., 2010) and iii) the interpretation of the SCITT response can be subjective and may vary between operators (Pers. Comm. A McCall). Furthermore, administering and reading the test in wildlife poses logistical challenges as animals need to be confined for three days and immobilized twice during this period.

An alternative test, the interferon-gamma (IFN-γ) release assay (IGRA) is an *in vitro* whole blood assay that is more suitable than the SCITT for use in wildlife since it only requires the collection of a single blood sample (Grobler et al., 2002). The Bovigam® 1G IGRA, utilizing both PPD_b and *Mycobacterium avium* PPD (PPD_a) as stimulating antigens, is a commercially available IGRA used for the diagnosis of *M. bovis* infection in domestic cattle (de la Rua-Domenech et al., 2006) and has shown great promise for use in buffaloes (Grobler et al., 2002). The inclusion of PPDs as antigens in this IGRA, as for the SICTT, may also result in a cross-reactive immune response to non-virulent mycobacteria; however, the specificity of IGRAs can be improved by using specific mycobacterial peptides as stimulating antigens (Bass et al., 2013; Vordermeier et al., 2001).

The test performance of a modified QuantiFERON®-TB Gold (QFT) assay (mQFT), using commercially available QFT tubes and an in-house bovine-specific IFN-γ ELISA, has previously been reported for African buffaloes (Parsons et al., 2011). The QFT system uses three specific antigenic peptides, early secretory antigen target 6 (ESAT-6), culture filtrate protein 10 (CFP-10) and TB7.7, encoded in the region of difference one (RD1) that is not present in most nontuberculous mycobacteria and *M. bovis* Bacille Calmette-Guèrin (Vordermeier et al., 2001, Warren et al., 2006). Parsons et al. (2011) showed the relative sensitivity and specificity of the mQFT to be comparable to that of the SCITT. Additionally, the relative sensitivity of the mQFT could be improved by increasing the whole blood antigen incubation time (Goosen et al., 2014).

Qiagen has developed a new commercially available cattletype® IFN-gamma ELISA, suitable to detect antigen-induced IFNγ release, with a specified cutoff for determining positive and negative responses. In this study, we aimed to investigate the use of QFT tubes in conjunction with the cattletype® IFN-gamma ELISA in a novel cattletype IGRA. We aimed to: i) compare the performance of the cattletype IGRA with the SCITT and Bovigam® 1G IGRA; ii) investigate the effect of increased duration of blood incubation on the assay result; iii) determine the reproducibility of the assay and iv) evaluate the assay in *M. bovis*-unexposed buffaloes.

2. Materials and methods

2.1. M. bovis-exposed buffaloes

2.1.1. Hluhluwe-iMfolozi Park (HiP)

During July 2016, 403 randomly selected free-ranging buffaloes were mass captured during the park's 18th annual bTB test-and-cull program. All buffaloes were immobilized, lithium heparin whole blood was collected via jugular venipuncture and the SCITT were performed as described below. All suspect and positive SCITT buffaloes ($n = 22$) were culled by gunshot. In addition to the 22 buffaloes, an additional five SCITT-negative buffaloes were culled based on suspicion of bTB. Of all the SICTT-negative buffaloes ($n = 381$), 70 animals were randomly selected and used together with all culled buffaloes for this study.

2.1.2. Madikwe Game Reserve (MGR)

One hundred and thirty-four buffaloes were opportunistically sampled from the MGR during bTB prevalence testing from May to July 2016. Free-ranging buffaloes were individually captured in MGR and immobilized, after which lithium heparin whole blood was collected via jugular venipuncture and SCITTs performed as described below. Bovigam® 1G IGRAs were performed at the Tuberculosis Laboratory of the Agricultural Research Council (ARC)-Onderstepoort Veterinary Institute in Pretoria, South Africa as described previously (van der Heijden et al., 2017). All suspect and positive SCITT buffaloes and positive Bovigam® 1G IGRA buffaloes ($n = 34$) were culled by gunshot. All buffaloes sampled ($n = 134$) were used in this study.

2.2. M. bovis-unexposed and uninfected buffaloes

Twenty-one buffaloes were opportunistically sampled from *M. bovis*-free farms (based on previous herd history) in South Africa during 2016 and were used as *M. bovis*-negative control animals. Ethical approval for all buffalo work was granted by the Stellenbosch University Animal Care and Use Committee (SU-ACUD15-00065 and SU-ACUD16-00072).

2.3. SCITT

All SCITTs were performed in HiP and MGR as previously described (Parsons et al., 2011). Briefly, an area on either side of the buffalo's neck was shaved and the baseline skin fold thickness (SFT) measured. Intradermal injections of 0.1 ml of PPD_b (3000 IU) and 0.1 ml of PPD_a (2500 IU) were administered on the left and right side of the animal's neck, respectively. After three days, buffaloes were immobilized and their SFT measured. A differential SFT measurement of ≥2 mm between the PPD_b injection site compared with the PPD_a injection site was defined as SCITT-positive. Subjective criteria were used to classify animals with differential inflammatory reactions of between 0 and 2 mm as SICTT-suspect (Parsons et al., 2011).

2.4. Bovigam® 1G IGRA in HiP

Whole blood aliquots (250 µl) from all 403 buffaloes sampled were co-incubated with i) 25 µl PBS (Nil) ii) 25 µl PPD_a (2750 IU/ml) (Onderstepoort Biological Products, Pretoria, South Africa) iii) 25 µl PPD_b (3300 IU/ml) (Onderstepoort Biological Products) and iv) 25 µl PWM, 5 µg/ml final concentration (Sigma-Aldrich, St. Louise, MO, USA) in 2 ml safe-cap microcentrifuge tubes. All samples were inverted three times, incubated at 37 °C for 20 h, centrifuged at 2550 x *g* for 10 min after which the plasma fraction was harvested and stored at −4 °C for later detection of IFN-γ using the Bovigam® 1G ELISA (Prionics AG, Schlieren-Zurich, Switzerland) per manufacturer's protocols. Due to the unavailability of Bovigam® 1G ELISA plates at the time of testing in HiP, the Bovigam® 1G ELISA was conducted retrospectively on samples from selected animals.

2.5. Cattletype IGRA

For all HiP ($n = 403$) and MGR ($n = 134$) buffaloes, 1 ml aliquots of heparinized whole blood were transferred to QFT tubes (Qiagen, Venlo, Limburg, Netherlands) comprising of: i) Nil tube containing saline; ii) TB antigen tube containing antigenic peptides and iii) Mitogen tube, as previously described (Parsons et al., 2011). All tubes were inverted ten times