A NOVEL IFNγ RELEASE ASSAY FOR THE DETECTION OF *MYCOBACTERIUM BOVIS* INFECTION IN AFRICAN BUFFALOES (*SYNCERUS CAFFER*)

Netanya Bernitz[1], *Dr Sven Parsons*[1], *Prof Paul van Helden*[1], *Prof Michele Miller*[1]

[1] *DST/NRF Centre of Excellence for Biomedical Tuberculosis Research/SAMRC Centre for TB Research/Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa*

African buffaloes *(Syncerus caffer)* are maintenance hosts of *Mycobacterium bovis*, the cause of bovine tuberculosis (bTB), and pose an infection risk for cattle and other wildlife species. Bovine tuberculosis (bTB) is a chronic disease and its early diagnosis relies on the detection of mycobacteria-specific cell mediated immune (CMI) responses. In South Africa, the Tuberculin Skin Test (TST) is the accepted test for *M. bovis* diagnosis in buffaloes, however it poses logistical challenges for testing wild animals and its interpretation differs between operators. The QuantiFERON (QFT) stimulation platform, regularly used with human interferon gamma (IFNγ) release assays (IGRA), is highly specific and sensitive and has been advocated for use in wildlife. Our group developed a modified QFT assay using QFT tubes together with an in-house ruminant-specific IFNγ enzyme-linked immunosorbent assay (ELISA). We further demonstrated that the sensitivity of the assay could be improved with an increased incubation time. Based on these findings, the manufacturers of the QFT system (Qiagen) developed the Cattletype® IGRA using QFT tubes and a bovine-specific IFNγ ELISA.

The aim of this pilot study was to determine:

1) the test performance of this novel IGRA compared to current tests in detecting *M. bovis* infection in buffaloes,
2) the effect of a lengthened incubation time on the sensitivity of the assay
3) the reproducibility of the assay and
4) the assay specificity.

In 2016, buffaloes in the Hluhluwe iMfolozi and Madikwe Game Reserves were tested using the TST, Bovigam®, and the Qiagen IGRA, and test agreement calculated. TST and/or Bovigam® test-positive buffaloes were culled and post-mortem examinations performed. *M. bovis* infection was confirmed using mycobacterial culture and genetic speciation. A lengthened 40-hour incubation time was compared to the standard 20-hour assay incubation time. The reproducibility of the assay was calculated by determining the intra- and inter-assay variability. Additionally, 21 *M. bovis*-free buffaloes were tested to calculate the assay specificity. The test agreements between the TST, Bovigam®, and Qiagen Cattletype® IGRA in both populations ranged between 0.638 and 0.858 (Cohens κ coefficient). Increased sensitivity was achieved by a lengthened incubation time and implementing a buffalo-specific cutoff. The Qiagen IGRA demonstrated high reproducibility and 100% sensitivity. The test performance of the novel IGRA using QFT tubes is as good as currently available diagnostic tests. Furthermore, a lengthened antigen incubation time increased the sensitivity of the assay. Improved sensitivity was also achieved by calculating a buffalo-specific cutoff value. The novel IGRA was shown to be highly reproducible and highly specific. The data from this pilot study suggests that the Qiagen IGRA is a promising assay to diagnose bTB in buffaloes. However, further research is needed to confirm our findings in larger populations.