

# A CANINE INTERFERON- $\gamma$ RELEASE ASSAY FOR THE DETECTION OF *MYCOBACTERIUM BOVIS* INFECTION IN AFRICAN WILD DOGS (*LYCAON PICTUS*)

*RL Higgitt, SDC Parsons, MA Miller*

*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*

## **Background**

Infectious diseases, including bovine tuberculosis (bTB) caused by *Mycobacterium bovis*, could have a detrimental impact on conservation of the African wild dog (*Lycaon pictus*). However, there are no tests for the diagnosis of bTB in this species. This study aimed to develop a novel Interferon- $\gamma$  Release Assay (IGRA) using commercially available reagents.

## **Methods**

Whole blood was collected from 39 randomly selected *M. bovis*-exposed wild dogs from the KNP and incubated in the "Nil" and "TB Antigen" (TB Ag) tubes of the QuantiFERON<sup>®</sup>-TB Gold (QFT) system, with pokeweed mitogen (PWM) included as a positive control. Following overnight incubation, plasma was harvested for cytokine measurement. Pooled PWM-stimulated samples were used to compare the sensitivities of three commercial IFN- $\gamma$  ELISA kits. The IFN- $\gamma$  concentration in plasma harvested from the Nil and TB Ag tubes was measured using the selected ELISA and compared using a student's t-test. A provisional cut-off was calculated as the mean of the QFT Nil stimulation + 2 standard deviations (mean + 2SD). Samples harvested from the TB Ag tube were categorized as "positive" or "negative" if they were greater than this value.

## **Results**

The R&D kit displayed the greatest sensitivity for detection of IFN- $\gamma$  in PWM-stimulated blood and was used to measure antigen-specific IFN- $\gamma$  release in QFT-processed samples. There was a significant difference in IFN- $\gamma$  release in the QFT Nil and TB Ag tubes and a provisional cut-off of 197 pg/ml was calculated, with 61% of the animals testing positive.

## **Conclusion**

The R&D kit, in conjunction with the QFT system, is useful for the detection of antigen-specific IFN- $\gamma$  in *M. bovis*-exposed wild dogs. This IGRA shows promise as a diagnostic tool for use in African wild dogs; however, further analysis of test performance is needed.