57) DEVELOPMENT OF AN ELISA FOR THE DETECTION OF INTERFERON-GAMMA AS A DIAGNOSTIC TOOL FOR TUBERCULOSIS IN BLACK RHINOCEROS (*DICEROS BICORNIS*) AND WHITE RHINOCEROS (*CERATOTHERIUM SIMUM*)

Darshana Morar[1], Edwin Tijhaar[2], Anita L. Michel[3], Aurel S. Negrea[2], Jacques Godfroid[1], Koos Jaw Coetzer[1] and Victor PMG Rutten[2]

[1]Department of Veterinary Tropical Diseases, University of Pretoria, Onderstepoort, South Africa 0110; [2]Department of Immunology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584 CL Utrecht, The Netherlands; [3]TB Laboratory, Onderstepoort Veterinary Institute, Onderstepoort, South Africa

Bovine Tuberculosis (BTB) is believed to have entered the Kruger National Park (KNP) in the 1960's and was first diagnosed in July 1990 in an African buffalo (*Syncerus caffer*). Since then, in addition to buffalo, BTB has been found in at least 14 other mammalian species including kudu (*Tragelaphus strepsiceros*), baboon (*Cynocephalus papio*) and lion (*Panthera leo*). This has raised concern about the spillover into other potentially susceptible species like rhinoceros, jeopardising breeding and reallocation projects. Practical and reliable procedures to diagnose BTB in black rhinoceros (*Diceros bicornis*) and white rhinoceros (*Ceratotherium simum*) need to be developed. Skin testing as a diagnostic method for BTB in pachyderms has important practical limitations. More, intrinsic values of the test, i.e. sensitivity and specificity, are unknown. In cattle the bovine Interferon-gamma (IFNγ) assay is used as a routine diagnostic test for BTB. As a first step towards an *in vitro* diagnostic test for BTB in rhinoceros, a capture ELISA for the detection of rhinoceros IFNγ (RhIFNγ) was developed. The RhIFNγ was cloned, sequenced, expressed and purified. Subsequently two hybridoma cell-lines were established producing monoclonal antibodies (MoAbs) specific to recombinant RhIFNγ (rRhIFN-gamma). In parallel polyclonal anti-rRhIFNγ antibodies were produced in chicken eggs. Specific binding of the two MoAbs to rRhIFN-gamma was demonstrated in an indirect ELISA. In the development of a capture ELISA the two MoAbs were independently used for the capture of rRhIFNγ and the chicken antibodies anti-rRhIFNγ in the detection step. Both assays were shown to detect rRhIFNγ. Subsequently both systems were shown to detect native RhIFNγ in tissue culture supernatant, obtained after stimulation of purified rhinoceros lymphocytes with Concavalin A (Con A).

The RhIFNγ ELISA established now will enable further development of a whole blood assay that will be instrumental in diagnosis of BTB in rhinoceros.