## II. AIM OF WORK

Highly infectious diseases among zoo animals like TB or *herpesvirus* infections in elephants and other species are considered great threats. Particularly, the currently available diagnostics contain problems not only in sensitivity and specificity but are also often not suitable for wildlife populations. Especially the determination of the cellular immune status is rarely possible. Moreover, antibody levels tend to be low in sub-clinically infected animals and are mostly not relevant to determine a protective immunity.

Recently developed *in vitro* diagnostic tests for humans and some mammalian species analyse cytokine profiles based on the detection of key Th1/Th2 cytokines and thus provide valuable information about the nature of the host response to infection. For zoo and wildlife animal species, however, there is a particular lack of immunologic reagents available. To address this problem, IFNγ and IL-4 were selected as hallmark Th1/Th2 immune balance cytokines for establishing quantitative real-time RT-PCRs (QPCRs).

It was intended to clone and sequence the cytokine-specific cDNA from different zoo species such as Somali wild ass (*Equus africanus somalicus*), Grevy's zebra (*Equus grevyi*), Hartmann's mountain zebra (*Equus zebra hartmannae*), Indian one-horned rhinoceros (*Rhinoceros unicornis*), Asian elephant (*Elephas maximus*), European bison (*Bison bonasus*), African buffalo (*Syncerus caffer*) and Nubian giraffe (*Giraffa camelopardalis).* The domestic horse (*Equus caballus*) served as model animal where blood samples, sequences and plasmid templates were readily available.

For establishing first real-time PCR tests, horse-specific primers and probes were designed, and plasmids were used as templates to optimize primers with SYBR-green. Optimized primers were then tested with horse cDNA, before TaqMan probes were added to the QPCRs. Horse PBMC were activated using mitogens and anti-equine CD3 monoclonal antibodies with the assistance of polyclonal anti-human CD28 as co-stimulant. Relative quantification of IFNγ and IL-4 mRNA expression was obtained when 18S rRNA was used as the normalizing housekeeping gene. Finally, *equine herpesvirus-1 (EHV-1)* and tetanus toxoid (TT) were applied as specific recall antigens in a test using horse PBMC.