Molecular detection and characterisation of an African swine fever *p72* genotype XXV virus in *Ornithodoros* ticks from the Kruger National Park, South Africa

Magali Jacquier[1], Glen Malherbe[1], T. Alan Harrison[1], Livio Heath[2], Roy Bengis[3], Gavin R. Thomson[2], Sarita Maree[1], Liezl Retief[1] & Armanda D. Bastos[1]

[1] *Department of Zoology and Entomology, University of Pretoria, South Africa*
[2] *Onderstepoort Veterinary Research, Agricultural Research Council, Onderstepoort, South Africa*
[3]*Office of the State Veterinarian, Skukuza, Kruger National Park, South Africa*

Twenty-four genotypes of African swine fever (ASF) virus have been described to date based on C-terminal sequencing of the *p72* gene. In this retrospective study of *Ornithodoros* ticks sampled from 163 warthog burrows throughout the Kruger National Park (KNP), we confirm the presence of a novel genotype (XXV) through *p72* genotyping and multi-locus sequence analysis. Briefly, 78 burrows were sampled in the dry season (austral winter) in 2002 and 85 burrows being sampled in the wet season (austral summer) in 2003, across a 300 km latitudinal gradient in the Kruger National Park (KNP), the largest wildlife reserve in South Africa. Homogenates were prepared for individual ticks and pooled prior to DNA extraction (a maximum of three ticks) and screened using a host-informative duplex PCR assay (Bastos et al. 2009). The individual tick homogenates making up the positive tick pool were subsequently selected for individual extraction and rescreening to identify the positive tick/s. Of the 1079 ticks assessed in this manner, 14 (1.31 %) were shown to be positive for ASF virus genome presence and the *p72* phylogeny recovered three discrete genotypes, viz. XI, XXV and XX. These three genotypes each occurred at an overall prevalence of 0.185 %, 0.278 % and 0.834 % and at a relative prevalence of 14.29%, 21.43% and 64.29%, respectively. Whilst season was shown to have a significant effect on burrow infestation rate (higher in the wet season), tick developmental stage (more nymphs present in the dry season) and the sex of the adult ticks (more males collected during the dry season), the differences in ASF PCR-positivity rates between the dry season (1.08 %) and the wet season (1.37 %) were not significant. Of relevance is that the overall ASF virus prevalence of 1.31 % is comparable to the 1.40 % tick infection rate reported in a prior extensive survey conducted in KNP from 1979-1981 (Thomson et al. 1983). However, none of the genotypes isolated from ticks collected in the 1979-1981 survey (viz. genotypes I, VII, XVII and XXII), were detected in our study. In contrast, genotype XX, the most prevalent genotype in our study, was also identified in a 1996 survey of ticks in southern KNP. Together these results indicate that whilst ASF virus prevalence is sustained at very low levels in *Ornithodoros* ticks from KNP, the levels of ASF virus diversity are extremely high, with multiple genotypes occurring here.

Keywords: multi-locus sequence analysis (MLSA), seasonal variation, warthog burrows, duplex-PCR, phylogeny

References

Bastos, A.D.S., Arnot, L., Jacquier, M.D. & Maree, S. 2009. A host species-informative internal control for molecular assessment of African swine fever virus infection rates in the African sylvatic cycle *Ornithodoros* vector. *Medical and Veterinary Entomology*, 23(4), 399-409.

Thomson, G.R., Gainaru, M., Lewis, A., Biggs, H., Nevill, E., van der Pypekamp, H., Gerber, L., Esterhuysen, J., Bengis, R., Bezuidenhout, D. & Condy, J. 1983. The relationship between African swine fever virus, the warthog and *Ornithodoros* species in southern Africa. In: P.J. Wilkinson (ed.) *African swine fever. Commission of the European Communities, EUR. 8644 EN*, pp 85-100.