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Genetic Heterogeneity of African Swine Fever Virus within the Sylvatic Cycle in Central Mozambique

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To determine if African swine fever (ASF) is maintained in a sylvatic cycle in Mozambican wildlife areas, we investigated the presence of soft tick reservoirs of ASFV and their infection rates in the Gorongosa National Park (GNP) and its surrounding villages. The GNP is one of the largest national parks located in the centre of the country. A total of 1,658 soft ticks were recovered from warthog burrows and pigpens at the wildlife/livestock interface and viral DNA was detected by nested PCR in 19% of *Ornithodoros porcinus porcinus* and 15% of *O. p. domesticus*. Isolation of the virus was successful in approximately 50% of the PCR-positive samples with nineteen haemadsorbing virus isolates recovered. These were genotyped using a combination of partial gene sequencing (p72, p30 and p54) and analysis of the central variable region (CVR) of the B602L gene. Eleven isolates clustered in genotype II and were homologous to contemporary isolates from southern Africa, the Indian Ocean and Eastern Europe. Three isolates grouped within genotype V were similar to previous isolates from Mozambique and Malawi. The remaining five isolates constituted a new, previously unidentified genotype, designated as genotype XXIV. The isolates classified within genotypes II and XXIV clustered into three major subtypes based on their p30 and p54 sequences. Based on analysis of the CVR gene, the viruses were classified into eight subgroups. This work corroborates for the first time the wildlife origin of Genotype II and confirms that a large diversity of ASFV strains maintained in wildlife areas can act as a permanent source of virus for the domestic pig value chain in Mozambique and beyond its boundaries. Their genetic similarity to ASFV strains currently spreading across Eastern Europe suggests that the African sylvatic cycle has a high potential to disseminate different ASFV into new geographic areas and justifies the need to continue studying the epidemiology and ecology of ASF sylvatic hosts in sub-Saharan Africa.

Keywords: African swine fever, genetic characterisation, Mozambique, *Ornithodoros moubata*, sylvatic cycle

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