




Genome Sequences of Five African Swine Fever Virus Genotype IX Isolates from Domestic Pigs in Uganda

 Charles Masembe,^{a,b} Vattipally B. Sreenu,^b Ana Da Silva Filipe,^b Gavin S. Wilkie,^b Peter Ogweng,^a Francis Johnson Mayega,^a Vincent B. Muwanika,^c Roman Biek,^d Massimo Palmarini,^b Andrew J. Davison^b

^aCollege of Natural Sciences, Makerere University, Kampala, Uganda

^bMRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

^cCollege of Agricultural and Environmental Sciences, Makerere University, Kampala, Uganda

^dInstitute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, United Kingdom

ABSTRACT Complete genome sequences of five African swine fever virus isolates were determined directly from clinical material obtained from domestic pigs in Uganda. Four sequences were essentially identical to each other, and all were closely related to the only known genome sequence of p72 genotype IX.

African swine fever (ASF) is a contagious, highly lethal, hemorrhagic disease of domestic pigs caused by African swine fever virus (ASFV) (1). ASF results in up to 100% mortality. Its epidemiology is complex and adopts different patterns in Africa and Europe (2–6). The lack of effective interventions makes it extremely difficult to prevent or control and results in severe economic losses (7–11). ASFV is the sole member of the genus *Asfivirus*, family *Asfarviridae*, and has a linear, double-stranded DNA genome of 170 to 190 kbp (12, 13). There are currently 24 recognized genotypes (14, 15).

Full genome sequences enable large strides toward developing control measures to use during epidemics. Available ASFV complete genome sequences number only 20, of which 3 are from eastern Africa (Kenya) but none are from Uganda, in which ASFV is also endemic. We present the first genome sequences of strains collected from domestic pigs in Uganda.

Five domestic pig blood samples (strains N10, R7, R8, R25, and R35) were collected during a suspected ASF outbreak in 2015 from two villages in the district of Tororo, Uganda. Total genomic DNA from these samples was extracted with the DNeasy blood and tissue DNA extraction kit (Qiagen) and shown to contain ASFV sequences using a PCR assay for part of gene B646L (p72). Methylated DNA was depleted from approximately 400 ng of DNA with a NEBNext Microbiome DNA enrichment kit (New England Biolabs). The DNA was fragmented to about 450 bp with an S220 focused ultrasonicator (Covaris). Sequencing libraries were prepared with a Kapa DNA library preparation kit (Kapa Biosystems), including ligation of Illumina adapters (New England Biolabs) followed by tagging with single-index primers. The libraries were pooled in equimolar concentrations, denatured, and sequenced on a NextSeq 500 instrument (Illumina) to produce approximately 150 million paired-end reads (2 × 150 nucleotides [nt]) per sample. Trimming was done with Trim Galore 0.4.0 (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) with a minimum Phred quality of 30 and read length of 75 nt. Reads in the quality-trimmed data were mapped to the pig genome sequence (GenBank accession number [GCA_000003025.6](https://www.ncbi.nlm.nih.gov/nuccore/GCA_000003025.6)) with SNAP aligner 1.0beta.18 (16). The remaining reads were assembled *de novo* into almost complete contigs with SPAdes 3.10.1 (17) and finished manually. Assemblies were made with Tanoti 1.3 (<http://www.bioinformatics.cvr.ac.uk/tanoti.php>) or Bowtie 2 2.3.1 (18), and final contig joins and corrections were made after visual inspection of the assemblies viewed with

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Address correspondence to Charles Masembe, cmasembe@cns.mak.ac.ug.

C.M. and V.B.S. contributed equally to this publication.

Tablet 1.14.04.10 (19). Multiple sequence alignments were generated with Decipher 2.0 (20) or GAP 4 4.11.2-r (21).

The sequences for strains N10, R7, R8, R25, and R35 had average coverages of 23, 439, 309, 869, and 1,487 reads per nucleotide, respectively, and sizes of 188,611, 188,628, 188,627, 188,630 and 188,629 bp, respectively, with an average G+C content of 38.5%. The sequences of four strains (except N10) were identical, with a few heterogeneous G · C tracts of more than 7 nucleotides for which mode sizes were incorporated. In addition to size differences in these and other repeated sequences, the sequence of strain N10 differed by 52 substitutions. A BLAST search of this data set against the NCBI nucleotide database showed the most similar GenBank sequence to be Ken06.Bus (GenBank accession number [KM111295](#)), with 99% identity and coverage of 98%. This sequence belongs to ASFV p72 genotype IX (22).

Data availability. The ASFV genome sequences in this communication are already publicly available in GenBank under accession numbers [MH025916](#) to [MH025920](#).

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REFERENCES

- Montgomery RE. 1921. On a form of swine fever occurring in British East Africa (Kenya Colony). *J Comp Pathol* 34:159–191. [https://doi.org/10.1016/S0368-1742\(21\)80031-4](https://doi.org/10.1016/S0368-1742(21)80031-4).
- Anderson EC, Hutchings GH, Mukarati N, Wilkinson PJ. 1998. African swine fever virus infection of the bushpig (*Potamochoerus porcus*) and its significance in the epidemiology of the disease. *Vet Microbiol* 62:1–15. [https://doi.org/10.1016/S0378-1135\(98\)00187-4](https://doi.org/10.1016/S0378-1135(98)00187-4).
- Bastos AD, Penrith M-L, Crucièrè C, Edrich JL, Hutchings G, Roger F, Couacy-Hymann E, Thomson RG. 2003. Genotyping field strains of African swine fever virus by partial p72 gene characterisation. *Arch Virol* 148:693–706. <https://doi.org/10.1007/s00705-002-0946-8>.
- Heuschele WP, Coggins L. 1969. Epizootiology of African swine fever virus in warthogs. *Bull Epizoot Dis Afr* 17:179–183.
- Penrith ML, Vosloo W. 2009. Review of African swine fever: transmission, spread and control. *J S Afr Vet Assoc* 80:58–62.
- Plowright W, Parker J, Pierce MA. 1969. The epizootiology of African swine fever in Africa. *Vet Rec* 85:668–674.
- Chenais E, Boqvist S, Emanuelson U, von Brömssen C, Ouma E, Aliro T, Masembe C, Ståhl K, Sternberg-Lewerin S. 2017. Quantitative assessment of social and economic impact of African swine fever outbreaks in northern Uganda. *Prev Vet Med* 144:134–148. <https://doi.org/10.1016/j.prevetmed.2017.06.002>.
- Chenais E, Sternberg-Lewerin S, Boqvist S, Liu L, LeBlanc N, Aliro T, Masembe C, Ståhl K. 2017. African swine fever outbreak on a medium-sized farm in Uganda: biosecurity breaches and within-farm virus contamination. *Trop Anim Health Prod* 49:337–346. <https://doi.org/10.1007/s11250-016-1197-0>.
- Fasina FO, Lazarus DD, Spencer BT, Makinde AA, Bastos AD. 2012. Cost implications of African swine fever in smallholder farrow-to-finish units: economic benefits of disease prevention through biosecurity. *Transbound Emerg Dis* 59:244–255. <https://doi.org/10.1111/j.1865-1682.2011.01261.x>.
- Lichoti JK, Davies J, Maru Y, Kitala PM, Githigia SM, Okoth E, Bukachi SA, Okuthe S, Bishop RP. 2017. Pig traders' networks on the Kenya-Uganda border highlight potential for mitigation of African swine fever virus transmission and improved ASF disease risk management. *Prev Vet Med* 140:87–96. <https://doi.org/10.1016/j.prevetmed.2017.03.005>.
- Samui KL, Nambota AM, Mweene AS, Onuma M. 1996. African swine fever in Zambia: potential financial and production consequences for the commercial sector. *Jpn J Vet Res* 44:119–124. <https://doi.org/10.14943/jjvr.44.2.119>.
- Dixon LK. 1988. Molecular cloning and restriction enzyme mapping of an African swine fever virus isolate from Malawi. *J Gen Virol* 69:1683–1694. <https://doi.org/10.1099/0022-1317-69-7-1683>.
- Dixon LK, Chapman DAG, Netherton CL, Upton C. 2013. African swine fever virus replication and genomics. *Virus Res* 173:3–14. <https://doi.org/10.1016/j.virusres.2012.10.020>.
- Achenbach JE, Gallardo C, Nieto-Pelegrin E, Rivera-Arroyo B, Degefa-Negi T, Arias M, Jenberie D, Mulisa DD, Gizaw D, Gelaye E, Chibssa TR, Belaye A, Loitsch A, Forsa M, Yami M, Diallo A, Soler A, Lamien CE, Sánchez-Vizcaíno JM. 2017. Identification of a new genotype of African swine fever virus in domestic pigs from Ethiopia. *Transbound Emerg Dis* 64:1393–1404. <https://doi.org/10.1111/tbed.12511>.
- Quembo CJ, Jori F, Vosloo W, Heath L. 2018. Genetic characterization of African swine fever virus isolates from soft ticks at the wildlife/domestic interface in Mozambique and identification of a novel genotype. *Transbound Emerg Dis* 65:420–431. <https://doi.org/10.1111/tbed.12700>.
- Zaharia M, Bolosky WJ, Curtis K, Fox A, Patterson D, Shenker S, Stoica I, Karp MR, Sittler T. 2011. Faster and more accurate sequence alignment with SNAP. *arXiv arXiv:1111.5572* [cs.DS]. <https://arxiv.org/abs/1111.5572>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. *Nat Methods* 9:357–359. <https://doi.org/10.1038/nmeth.1923>.
- Milne I, Stephen G, Bayer M, Cock PJA, Pritchard L, Cardle L, Shaw PD, Marshall D. 2013. Using Tablet for visual exploration of second-generation sequencing data. *Brief Bioinform* 14:193–202. <https://doi.org/10.1093/bib/bbs012>.
- Wright ES. 2015. DECIPHER: harnessing local sequence context to improve protein multiple sequence alignment. *BMC Bioinform* 16:322. <https://doi.org/10.1186/s12859-015-0749-z>.
- Staden R, Beal KF, Bonfield JK. 2000. The Staden package, 1998. *Methods Mol Biol* 132:115–130.
- Bishop RP, Fleischauer C, de Villiers EP, Okoth EA, Arias M, Gallardo C, Upton C. 2015. Comparative analysis of the complete genome sequences of Kenyan African swine fever virus isolates within p72 genotypes IX and X. *Virus Genes* 50:303–309. <https://doi.org/10.1007/s11262-014-1156-7>.