

8 Virology Announcement

Near-complete genome sequences of multiple genotype 1 African swine fever virus isolates from 2016 to 2018 in Cameroon

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ABSTRACT African swine fever virus has been endemic in Cameroon since 1982. Here, we announce the sequences of Cameroon/2016/C1, Cameroon/2016/C5, Cameroon/2017/C-A2, Cameroon/2018/C02, and Cameroon/2018/CF3, five genotype 1 African swine fever virus genomes collected from domestic pigs between 2016 and 2018.

KEYWORDS ASFV, ASF, Cameroon, African swine fever, African swine fever virus

A frican swine fever virus (ASFV) of the genus *Asfivirus* and Asfarviridae family has been endemic in Cameroon since its first outbreak of African swine fever in 1982 (1). Historically, ASFV has been classified into 24 genotypes based on the partial sequencing of the B646L (p72) gene (2). Derivatives of the current pandemic strain (ASFV Georgia 2007/01) belong to genotype 2 and have been circulating in Asia (3), Europe (4), and the Dominican Republic (5). Yet, as of early 2020, only ASFV isolates belonging to genotype 1 have been observed in Cameroon (6, 7). However, in 2020, Nigeria, which shares a border to the west of Cameroon, reported an outbreak caused by a unique genotype 2 strain, highlighting the need for continued ASFV surveillance programs in the area (5, 8–10).

ASFV isolates were passed once in primary swine macrophage cultures produced from blood, as previously described (11). Viral DNA was extracted from infected macrophage cultures using the MagMax Pathogen RNA/DNA Kit (Applied Biosystems) as previously described (12). For sequencing, CpG-methylated (host) DNA was depleted using a NEBNext Microbiome Enrichment Kit E2612L (New England Biolabs, MA, USA), followed by library construction using a KAPA LTP Library Preparation Kit 796188001 (Roche Diagnostics, IN, USA) and sequencing on a NextSeq 500 instrument (Illumina, CA, USA) (13). Sequence analysis was performed using CLC Genomics Workbench v23 software (CLCBio, Waltham, MA, USA). Reads were trimmed for ambiguous nucleotides (max = 2), quality (Quality limit = 0.05), minimum length (50 nt), and nucleotide composition (20 and 5 nts removed from the 5' and 3' ends, respectively) resulting in 744,540 (Cameroon/2016 /C1), 1,234,348 (Cameroon/2016 /C5), 1,588,982 (Cameroon/2017 /C-A2), 49,740,870 (Cameroon/2018 /C02), and 28,694,650 (Cameroon/2018/ CF3) as described in Table 1; paired-end reads ranging in size from 50 to 126 nt were assembled using the default parameters of CLC Genomic Workbench's de novo assembly and resulted in one scaffold per isolate. Ns were inserted in scaffold regions and represent an approximate size gap. Reads were mapped back to the scaffolds, and unmapped regions on the 5' and 3' ends of the genome were trimmed. From the read map, the consensus sequence was extracted resulting in a genome length of 183,210 (Cameroon/2016 /C1), 183,381 (Cameroon/2016 /C5), 183,395 (Cameroon/2017 /C-A2), 183,390 (Cameroon/2018 /C02), and 183,406 (Cameroon/2018/CF3) nucleotides with a 13×, 10×, 81×, 13×, and 189 × coverage, respectively. All genomes had a GC content of 38.5%. Whole-genome alignments, performed using CLC Genomics Workbench, revealed that the genomes were 99.93% (Cameroon/2017 /C-A2 vs Cameroon/2016 /C1)

Editor Jelle Matthijnssens, Katholieke Universiteit Leuven, Leuven, Belgium

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The authors declare no conflict of interest.

See the funding table on p. 2.

Received 3 November 2023 Accepted 20 February 2024 Published 13 March 2024

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GenBank accession	Sample	Collection date	Location	Genotype	Closest RefSeq match	Sequencing reads
namber						Tedds
OQ971722	Cameroon/2018/C-F3	2018	Cameroon: Lokoundj	1	Benin 97/1 (NC_044956.1)	SRR25629145
OQ971723	Cameroon/2016/C1	2016	Cameroon: Yaound III	1	Benin 97/1 (NC_044956.1)	SRR25629144
OQ971724	Cameroon/2016/C5	2016	Cameroon: Mfou	1	Benin 97/1 (NC_044956.1)	SRR25629142
OQ971725	Cameroon/2017/C-A2	2017	Cameroon: Mezam	1	Benin 97/1 (NC_044956.1)	SRR25629141
OQ971726	Cameroon/2018/C02	2018	Cameroon: Bertoua I	1	Benin 97/1 (NC_044956.1)	SRR25629143

TABLE 1	ASEV is	solates se	equenced	and	collection	dates	and	locations i	n C	ameroon
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to 99.99% (Cameroon/2018 /C-F3 vs Cameroon/2016 /C-A2) similar. BlastN against the NCBI database revealed Benin 97/1 (NC_044956.1) to be the closest match, ranging from 99.51% (Cameroon/2016 /C5) to 99.56% (Cameroon/2016 /C1) similarity based on whole-genome nucleotide alignment. Annotations were transferred from Benin 97/1 using the Genome Annotation Transfer Utility (14) using the default parameters resulting in 152 (Cameroon/2016 /C1), 151 (Cameroon/2016 /C5), 155 (Cameroon/2017 /C-A2), 151 (Cameroon/2018 /C02), and 155 (Cameroon/2018/CF3) predicted genes (15).

ACKNOWLEDGMENTS

This work was supported by the Wellcome Trust (grant number 105684/Z/14/Z), the Medical Research Council (grant numbers MC_UU_12014/3 and MC_UU_12014/12), and the African Union Commission (grant number AURG-II- 1–196-2016).

The funders did not play any role in the study design, data collection and interpretation, or the decision to submit the work for publication.

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FUNDING

Funder	Grant(s)	Author(s)
Wellcome Trust (WT)	105684/z/14/z	Charles Massembe
UKRI Medical Research Council (MRC)	MC_UU_12014/3, MC_UU_12014/12	Charles Massembe

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DATA AVAILABILITY

The genome sequences for isolates Cameroon/2018/CF3, Cameroon/2016 /C1, Cameroon/2016 /C5, Cameroon/2017 /C-A2, and Cameroon/2018 /C02 have been deposited in NCBI GenBank under the accession no. OQ971722.1, OQ971723.1, OQ971724.1, OQ971725.1, and OQ971726.1, respectively. Raw sequence data can be found in the GenBank SRA under BioProject accession no. PRJNA1005237.

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