Complete Genome Sequences of Five Rabies Virus Strains Obtained from Domestic Carnivores in Liberia

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**ABSTRACT**

As in other African countries, canine rabies is endemic in Liberia. However, data concerning the genetic diversity of rabies virus isolates circulating in this country remain limited. We report here the complete genome sequences of five rabies viruses obtained from domestic animals. All of them belonged to subgroup H within the Africa 2 clade.

Rabies virus (RABV) is the main etiological agent of rabies, an acute and always fatal form of encephalomyelitis which can potentially affect all mammalian species. This zoonotic virus belongs to the prototype species *Rabies lyssavirus* within the genus *Lyssavirus*, family *Rhabdoviridae* (order *Mononegavirales*) (1). Rabies viruses circulating in dogs are the main cause of human rabies, with an estimated 59,000 deaths worldwide each year, especially in Asia and Africa (2). As in other sub-Saharan countries, canine rabies remains endemic in Liberia (3). However, available data about the genetic diversity of RABV isolates circulating in this country remain limited.

Brain samples collected from four dogs and one cat suspected of rabies were collected from different regions of Liberia in 2017 and 2018, within the framework of a joint effort program to strengthen rabies surveillance in the country (Table 1) (3). All the samples were confirmed positive for rabies by fluorescence antibody test (FAT) (4) and by a modified version of a rapid immunochromatographic diagnostic test (RIDT) (5). For four samples, RNA was extracted locally from brain biopsy specimens (approximately 0.5 cm$^3$ each) using the Direct-zol RNA miniprep kit (Zymo Research) and then purified using Agencourt RNAClean XP beads (Beckman Coulter) at a 1:1.8 ratio. The last sample was extracted at Institut Pasteur using TRIzol reagent (Invitrogen) from an FTA card (Whatman FTA card technology; Sigma-Aldrich) impregnated with ground brain material as previously described (Table 1) (6). The five RNA samples were processed for next-generation sequencing (NGS) as previously described (7–9). Briefly, an rRNA depletion step was first carried out using Terminator 5'-phosphate-dependent exonuclease (Epicentre Biotechnologies). After purification, depleted RNA was reverse-transcribed into cDNA using Superscript III reverse transcriptase (Invitrogen), and double-stranded DNA (dsDNA) was synthesized as already described (7–9). Finally, dsDNA libraries were constructed using the Nextera XT DNA library preparation kit (Illumina) and sequenced using a 2 × 150-nucleotide (nt) paired-end strategy on the NextSeq 500 platform (7–9). NGS data were analyzed using *de novo* assembly and mapping (both using CLC Assembly Cell; Qiagen), with a dedicated workflow built on the Institut Pasteur Galaxy platform (7–10). Contig sequences were assembled to produce the final genome.
**TABLE 1** Description of the genome sequences of the five rabies virus strains obtained from Liberian domestic carnivores

| Virus     | Host | Animal status | Location | Yr of collection | Support | Total no. of reads | No. of mapped reads (%) | Avg coverage (x) | Genome nucleotide length (bp) | GC content (%) | ORF nucleotide length (aa) | GenBank accession no. | SRA accession no. |
|-----------|------|---------------|----------|------------------|---------|-------------------|------------------------|----------------|--------------------------------|----------------|----------------------------|---------------------|-------------------|
| 18005LIB  | Cat  | Owned         | Margibi  | 2017             | Beads   | 4,317,876         | 934,436 (21.6)         | 11,567.36        | 11,922                         | 45             | 1,353 (450) 891 (296) 609 (202) 1,575 (524) 6,384 (2,127) | OK135144          | SRX12176932 |
| 18007LIB  | Dog  | Owned         | Montserrado | 2017          | Beads   | 2,228,096         | 4,593 (0.2)          | 56.95            | 11,923                        | 45             | 1,353 (450) 891 (296) 609 (202) 1,575 (524) 6,384 (2,127) | OK135145          | SRX12176933 |
| 18008LIB  | Dog  | Owned         | Montserrado | 2018          | Beads   | 5,132,556         | 12,554 (0.2)         | 155.23           | 11,885                         | 45             | 1,353 (450) 891 (296) 609 (202) 1,575 (524) 6,384 (2,127) | OK135146          | SRX12176934 |
| 18009LIB  | Dog  | Owned         | Lofa     | NA              | FTA     | 1,916,466         | 25,596 (1.3)         | 316.18           | 11,923                        | 45             | 1,353 (450) 894 (297) 609 (202) 1,575 (524) 6,384 (2,127) | OK135147          | SRX12176935 |
| 18018LIB  | Dog  | NA            | NA       | Beads           | 7,209,068 | 826,598 (1.5) | 10,135.10          | 11,923           | 45                             | 1,353 (450) 894 (297) 609 (202) 1,575 (524) 6,384 (2,127) | OK135148          | SRX12176936 |

*Total RNA was extracted in Liberia from strains 18005LIB, 18007LIB, 18008LIB, and 18018LIB (recovered from brain biopsy specimens [approximately 0.5 cm³] from separate animals) and purified using Agencourt RNAClean XP beads (Beckman Coulter) at a 1:1.8 ratio following the manufacturer’s instructions, with the exception of the last resuspension step in nuclease-free water. The dried beads with RNA were shipped at a cold temperature with ice packs to Institut Pasteur (Paris), where they were resuspended in 30 μL nuclease-free water. Strain 18009LIB was sent to Institut Pasteur using an FTA card (Whatman FTA card technology; Sigma-Aldrich) impregnated with ground brain material and then extracted using TRIzol reagent (Invitrogen).*

*ORF, open reading frame; aa, amino acid.*

*P ORF with premature stop codon (missing the last amino acid C).*

*Strain 18008LIB was also partially sequenced at Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe-FAO Reference Center for Rabies) in Italy, and the sequence was found to be identical to the one obtained by Institut Pasteur Paris (IPP).*

*Genome with incomplete 5' UTR (untranslated region) (leader sequence missing 38 nucleotides).*

*NA, not available.*
The genome sequences presented the five canonical genes encoding the nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and RNA polymerase (L) (Table 1). The leader and trailer sequences were 58 and 70 nucleotides long, respectively. The transcription initiation (TI) signal AACA and the transcription termination (TTP) TGA was observed for all the genes, except for the G gene, which presented the AGA motif for TTP. Three sequences presented a premature stop codon in the P gene. The nucleotide identity between four of the genome sequences was high (99.1%), whereas strain 18018LIB was slightly more divergent (97.5%). Genetic analysis confirmed that they clustered together in group H within the Africa 2 clade (3, 15, 16) (Fig. 1).
ACKNOWLEDGMENTS

We thank the dog owners, the county livestock officers, the health surveillance officers, the members of the OH platform, and the laboratory staff for their great commitment. We are grateful to the sequencing facilities of the Plate-forme de microbiologie mutalisée (P2M) of Institut Pasteur (Paris, France) for technical assistance with NGS sequencing. We also acknowledge Lisa Crump for language editing.

This work was funded through the Global Alliance for Vaccines and Immunisation (GAVI) (VISLACRV19122014, work stream 3), the Wolfermann Nägeli Foundation, the Swiss African Research Cooperation (SARECO), research funds from Stay on Track of the Global Alliance for Vaccines and Immunisation (GAVI) (VISLACRV19122014, work stream 3), the Wolfermann Nägeli Foundation, the Novartis Foundation for Biomedical Research. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

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