

Letter

First complete genomic sequencing of recent canine rabies viruses in Sierra Leone suggests cross-border transmission of Africa 2H subclade

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Dear Editor,

Rabies, caused by rabies virus (RABV), is a neglected tropical disease that remains endemic in nearly every country in Africa, with an estimated 21,500 human rabies deaths occurring annually across the continent (Mbilo et al., 2021). RABVs in Africa are divided into three major genetic clades: Africa 2, Africa 3, and Cosmopolitan clades (Troupin et al., 2016). The Africa 2 clade is widely distributed across sub-Saharan West and central Africa (Fooks and Jackson, 2020; Talbi et al., 2009). Phylogenetic analysis further categorizes Africa 2 clade into eight subclades, denoted A–H (Talbi et al., 2009; Voupawoe et al., 2021). Due to the limited number of RABV sequences available from West Africa, the patterns of rabies transmission across the region remain poorly understood, and the source of the virus could not be traced. In Sierra Leone, rabies is a notifiable disease and one of the six prioritized zoonotic diseases (Mbilo et al., 2021). Dog-mediated rabies causes about 300 human deaths annually (Bucher et al., 2023; Mshelbwala et al., 2024). The first canine rabies case in the country was reported in 1949 by the Teko Central Veterinary Laboratory (Teko CVL, Makeni) (Mbilo et al., 2021; Mshelbwala et al., 2024). Due to limited laboratory capacity, most cases were identified only based on clinical symptoms. The transmission routes and sources of the virus remain unclear due to the lack of epidemiological surveillance data, particularly viral sequencing. To date, only one RABV strain from Sierra Leone (collected in 1997) has been partially sequenced (N and G genes) and deposited in NCBI, which belongs to the Africa 2B subclade (Talbi et al., 2009; Voupawoe et al., 2021). In this study, three domestic dogs exhibiting suspected rabies symptoms were sent to the Sierra Leone Animal Welfare Society

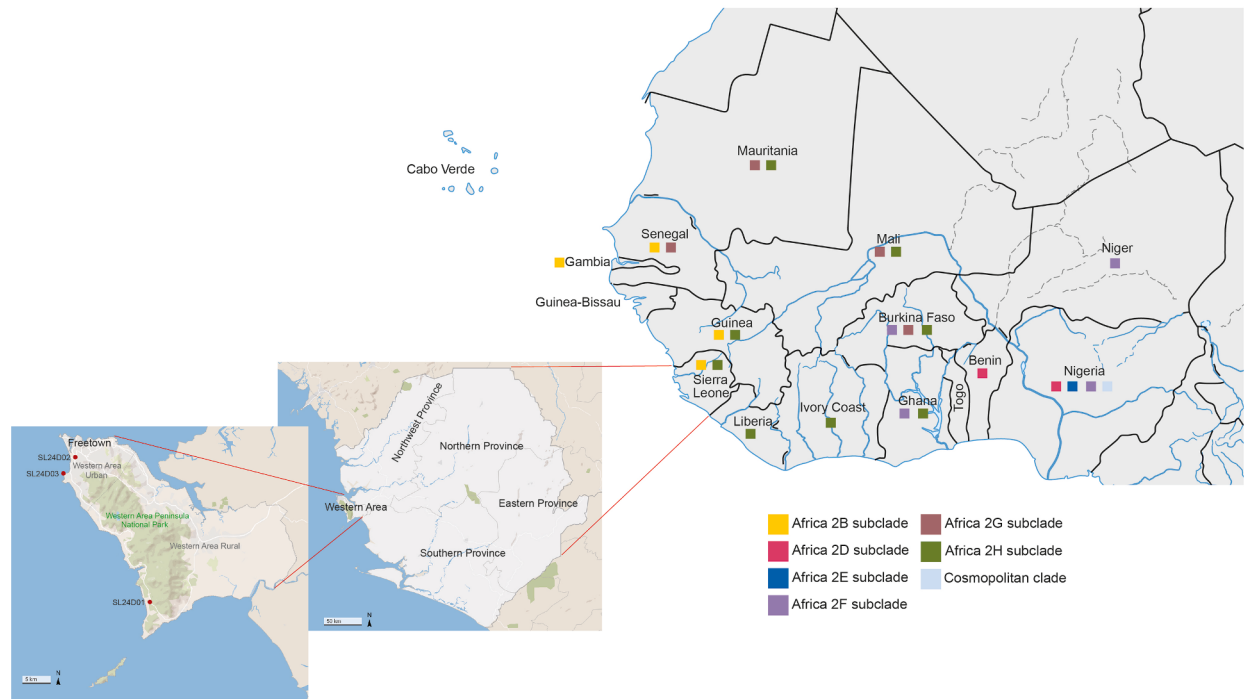
(SLAWS, Freetown) and confirmed positive for rabies by the Tropical Infectious Diseases Prevention and Control Center of Sierra Leone (IDPC, Freetown). In order to trace to the source and understand the genetic and evolutionary characteristics of the rabies virus and its epidemic patterns with neighboring countries, we sequenced three complete genomic sequences of RABVs from Sierra Leone and performed phylogenetic analysis for a better understanding of the molecular epidemiology in Sierra Leone and neighboring countries.

On 18 December 2023, an unleashed free-roaming guard dog, one year old, was attacked by three wild dogs in Casava farm, Bureh, Western Area Rural, Freetown (SL24D01, Fig. 1A and B). One month later, this dog showed clinical symptoms of rabies, including running, salivation, convulsions, dull eyes, and urinary incontinence. During this period, the dog bit a puppy on the inside of its neck as well as a pet cat. On 20 February 2024, a leashed pet dog bit its owner in Ecobank, Wilkinson Rd., Leicester, Western Area Urban, Freetown (SL24D02, Fig. 1A and B). The dog also showed clinical symptoms of rabies, including salivation, dull eyes, and sensitivity to noise. The dog owner received post-exposure prophylaxis (PEP) for rabies. On 20 April 2024, another owned dog exhibited abnormal signs, including salivation, dull eyes, in Turays Drive, Goderich, Western Area Urban, Freetown (SL24D03, Fig. 1A and B). None of these three dogs were vaccinated. Following the onset of suspected rabies symptoms, these three dogs were sent to the SLAWS by their owners for rabies diagnosis. Thereafter, the animal health personnel wore personal protection equipment to decapitate the dogs after they died naturally, and immediately submitted the samples to the IDPC, where the presence of rabies was confirmed positive using

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A



B

Virus name	Host	Animal status	Date of death	Location	Genome length (bp)	Mapped reads	Mean depths	Genome coverage (%)	GC content (%)	ORF nucleotide length (nt) (protein length (aa))					GenBank accession number
										N	P	M	G	L	
SL24D01	Dog	Guard dog (unleashed)	01-24-2024	Casava Farm, Bureh Town	11,922	345,786	8,701.21	99.96	45.38	1,353 (450)	894 (297)	609 (202)	1,575 (524)	6,384 (2,127)	PQ740963
SL24D02	Dog	Pet (unleashed)	02-24-2024	Ecobank, Wilkinson Road	11,921	73,878	1,859.19	99.95	45.31	1,353 (450)	894 (297)	609 (202)	1,575 (524)	6,384 (2,127)	PQ740964
SL24D03	Dog	Pet (leashed)	04-22-2024	Turays Drive, Goderich	12,024	13,8092	3,445.41	100.00	45.34	1,353 (450)	894 (297)	609 (202)	1,575 (524)	6,384 (2,127)	PQ740965

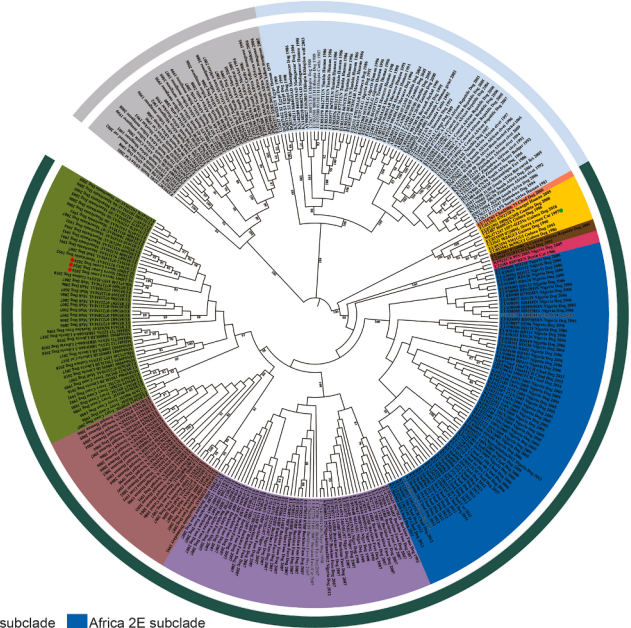
C

Genomic sequences of RABVs



D

N genes of RABVs



- Africa 2 clade
- Africa 3 clade
- Cosmopolitan clade
- Africa 2A subclade
- Africa 2B subclade
- Africa 2C subclade
- Africa 2D subclade
- Africa 2E subclade
- Africa 2F subclade
- Africa 2G subclade
- Africa 2H subclade

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Fig. 1. Rabies virus (RABV) in canines in Sierra Leone. **A** Geographical distribution of confirmed canine rabies cases in West Africa. **B** Genomic characterization, sequencing coverage and assembly statistics of the three 2H subclade RABV strains from domestic dogs in Sierra Leone. **C** Phylogenetic tree of 143 complete genomic sequences of RABVs in Africa (three 2H subclade strains from this study, 140 reference sequences from GenBank, Supplementary Table S1). Complete genomic sequences for groups A and C were unavailable for analysis. **D** Phylogenetic tree based on 300 complete *N* genes of RABVs in Africa (three from this study, 297 reference sequences from GenBank, Supplementary Table S1). The three 2H subclade sequences from this study are marked with red dots, while the reference Africa 2B strain from a previous study in Sierra Leone is marked with green dots. The phylogeny was reconstructed by MEGA-X using the neighbor-joining (NJ) method with 1000 bootstrap replicates. The trees were visualized with iTOL version 7.

both conventional RT-PCR and real-time RT-PCR (WOAH, 2024). These samples were re-tested at Teko CVL for rabies using direct fluorescent antibody test simultaneously. Brain tissue homogenates were loaded onto FTA cards (Whatman, WB120205, England) according to description from L  chenne et al. (2016) (L  chenne et al., 2016), and then shipped to the WOAH Reference Laboratory for Rabies in China to obtain the complete genomic sequences of the RABVs by high-throughput sequencing (Zhang et al., 2025). Briefly, FTA cards were cut into small pieces and incubated overnight at 4  C in 1   PBS (pH 7.4) to facilitate total RNA extraction using TRIzol reagent, following the manufacturer's protocol (Invitrogen, USA). Subsequently, high-throughput RNA sequencing was performed according to the metatranscriptomic tracing (MTT) method described by Jiang et al. (2022). Raw sequencing reads were subjected to quality control: sequencing adaptors and low-quality reads were removed. For each sample, approximately 6 Gb of high-quality, 150-bp paired-end clean reads were generated on an Illumina NovaSeq 6000 platform. Host-derived sequences and rRNA reads were computationally subtracted. The remaining reads were aligned against a curated reference viral protein database to identify viral sequences; candidate RABV reads were then subjected to *de novo* assembly using Diamond (for homology-based seeding) and SPAdes (for contig extension), yielding complete or near-complete RABV genomes. Sequence alignments and phylogenetic analyses were conducted using MegAlign in DNASTar and the neighbor-joining (NJ) method in MEGA-X with 1000 bootstrap replicates (Zhang et al., 2025). Sequencing coverage and assembly statistics were calculated based on the reference strain CVS-11 (GenBank accession: GQ918139.1), as shown in Fig. 1B.

In results, three complete genomic sequences of RABVs (SL24D01, SL24D02 and SL24D03) were obtained and submitted to GenBank and ScienceDB. To determine their phylogenetic position, a total of 140 representative complete genomic sequences and 297 representative complete nucleoprotein (*N*) genes of RABVs from Africa were retrieved from GenBank for use in the phylogenetic reconstruction (Supplementary Table S1). The result revealed that three strains belong to Africa 2H subclade (Fig. 1C and D), which shared 97.2%–99.2% genomic identity with strains from Guinea and 97.0%–98.0% identity with those from Liberia, respectively. Despite a relatively high degree of genetic diversity between the RABVs in Sierra Leone, the amino acid sequence of the nucleoprotein (*N*) was the most highly conserved among all viral proteins (Supplementary Table S2). No certain key function region was found, except the central part of the RABV L (residues 530 and 1177), which are involved in the binding and utilization of ATP (Fooks and Jackson, 2020). While the impact of these mutations on transcriptional activity remains unknown.

The cat strain 07208SL isolated in Sierra Leone in 1997 was clustered within the Africa 2B subclade (Fig. 1D), although its precise geographical origin is not available. The Africa 2 clade is widely distributed across West Africa (Mbilo et al., 2021; Talbi et al., 2009), with the Africa 2B subclade being endemic in Sierra Leone, Guinea, Gambia, and Senegal, while the Africa 2H subclade circulates in Sierra Leone, Guinea, Liberia, Ivory Coast, Ghana, Burkina Faso, Mali, and Mauritania (Fig. 1A). This distribution pattern indicates that RABV circulates in transmission cycles involving frequent cross-border spread.

Genomic surveillance plays a crucial role in the management of contemporary viral diseases; however, it has not yet been routinely implemented to monitor endemic disease transmission and control in low-income

countries. This is primarily due to widespread poverty, limited public health infrastructure, high dog-to-human densities, and suboptimal laboratory-based surveillance (Fooks and Jackson, 2020). By utilizing FTA cards for cross-continental sample transportation, we successfully obtained the first complete genomic sequences of rabies virus from Sierra Leone through high-throughput sequencing. Phylogenetic analysis revealed that Africa 2H subclade are endemic in Sierra Leone and its neighboring countries.

Dogs are widely distributed in Sierra Leone, with an estimated population of 874,255 dogs and a projected annual increase of 0.99% (Bucher et al., 2023). However, dog vaccination coverage is merely 0.8%, significantly below the WHO-recommended 70% threshold required to interrupt RABV transmission (Mshelbwala et al., 2024). The dog-to-human ratio was estimated at 1:14, and notably, 59% of dog owners do not monitor or register their dogs with veterinary authorities in Freetown (Suluku et al., 2012). The survey indicated that 59.7% of dog owners had never heard of rabies, 81.2% believed that RABV cannot be transmitted to other animals through bites from infected animals, 86.7% had not vaccinated their dogs against rabies in Sierra Leone (Mshelbwala et al., 2024). Therefore, developing statutory instruments for responsible dog ownership, conducting effective dog population assessments and large-scale vaccination campaigns through strengthening public awareness and education is necessary to prevent and control dog-mediated rabies in Sierra Leone.

All three cases in this study involved owned dogs that had close contact with humans, presenting a high risk of rabies transmission. Dog-mediated rabies is a serious health problem in Sierra Leone, and rabies control cannot be effectively achieved by Sierra Leone alone. To achieve the 2030 goal of eliminating human rabies transmitted by dogs, multinational cooperation must be strengthened under the One Health framework, ensuring coordinated prevention and control measures across borders.

FOOTNOTES

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Supplementary data to this article can be found online at <https://doi.org/10.1016/j.virs.2026.06.010>.

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