

Historical Outbreaks of Simian Hemorrhagic Fever in Captive Macaques Were Caused by Distinct Arteriviruses

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Simian hemorrhagic fever (SHF) is lethal for macaques. Based on clinical presentation and serological diagnosis, all reported SHF outbreaks were thought to be caused by different strains of the same virus, simian hemorrhagic fever virus (SHFV; *Arteriviridae*). Here we show that the SHF outbreaks in Sukhumi in 1964 and in Alamogordo in 1989 were caused not by SHFV but by two novel divergent arteriviruses. Our results indicate that multiple divergent simian arteriviruses can cause SHF.

In late August 1964, a viral hemorrhagic fever epizootic occurred among captive macaques at the Institute of Experimental Pathology and Therapy in Sukhumi, Georgia, Soviet Union. All 64 infected animals succumbed. The disease was named simian hemorrhagic fever (SHF), as the etiological virus was not related to any other virus known at the time (1–3). In November 1964, an epizootic with a highly similar clinical presentation and case fatality rate occurred among macaques at the National Institutes of Health (NIH) Primate Quarantine Unit in Bethesda, MD, killing a total of 223 animals. Outbreak investigations revealed that the macaques affected at both facilities had been imported from the same primate supplier in India, suggesting that the two outbreaks were related (4).

A list of additional SHF outbreaks, the majority of which had connections to Indian suppliers of nonhuman primates, is provided in Table 1. The diagnosis of SHF during these additional outbreaks was based on the typical clinical and pathological presentation of sick macaques (4–6) and on serological assays such as indirect fluorescence assay (IFA) or complement fixation (CF) (7–10). Several observations suggested that different strains of SHFV may have caused the various outbreaks. For example, central nervous system (CNS) manifestations were much more commonly observed among macaques during the 1964 Sukhumi outbreak than during the 1964 Bethesda and 1967 Sukhumi outbreaks (11). The presence or absence of CNS involvement could be reproduced in macaques experimentally infected with clinical material from the different outbreaks (11). CF, IFA, and enzyme-linked immunosorbent assay studies performed with virus antigen or nonhuman primate sera from various outbreaks often yielded contradictory results, either confirming or refuting direct relationships between the etiological viruses (7–10, 12, 13). Unfortunately, virus isolation in cell culture was reported for only a few outbreaks, and with the exception of 1964 Bethesda isolate NIH LVR42-0/M6941, all of the isolates and most of the associated materials have been accidentally or deliberately destroyed (Table 1).

The origin of SHFV is unclear, although various African non-

human primates, such as patas monkeys (*Erythrocebus patas*) (14, 15), Guinea baboons (*Papio papio*) (14), and olive baboons (*Papio anubis*) (16), have been implicated as possible carriers. This hypothesis is strengthened by the recent discovery in apparently healthy African nonhuman primates of several novel and highly divergent simian arteriviruses, all of which are more closely related to SHFV and each other than to the other known arteriviruses, i.e., equine arteritis virus, lactate dehydrogenase-elevating virus, porcine reproductive and respiratory syndrome virus, wobbly possum disease virus, and a yet-to-be-described arterivirus from a forest giant pouched rat. These simian arteriviruses include Kibale red colobus virus 1 (KRCV-1) and KRCV-2 found in the Ugandan red colobus (*Procolobus [Piliocolobus] rufomitratus tephrosceles*) (17, 18) and Kibale red-tailed guenon virus 1 (KRTGV-1) and KRTGV-2 found in red-tailed monkeys (*Cercopithecus ascanius*) (19) in Uganda's Kibale National Park, Mikumi yellow baboon virus 1 (MYBV-1) found in yellow baboons (*Papio cynocephalus*) in Tanzania's Mikumi National Park (20), and a yet-to-be-described virus from DeBrazza's monkeys (*Cercopithecus neglectus*) in the Democratic Republic of Congo. Another novel simian arterivirus, southwest baboon virus 1 (SWBV-1),

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TABLE 1 History of SHF outbreaks

Yr of outbreak	Location of outbreak	Nonhuman primates affected (species)	Basis of SHF diagnosis	Isolate	Reference(s)
1964	Institute of Experimental Pathology and Therapy, Sukhumi, Georgia, Soviet Union	Assam macaques, ^a rhesus monkeys, ^b southern pig-tailed macaques, ^c stump-tailed macaques ^d	Clinical presentation, serology, virus isolation, exptl reproduction	Sukhumi-64 (destroyed?)	1–3, 30, 31
1964	NIH Primate Quarantine Unit, Bethesda, MD	Rhesus monkeys	Clinical presentation, serology, virus isolation, exptl reproduction	NIH LVR42-0/M6941, ^g NIH LVR543/654 (destroyed), LVR M8930 (destroyed)	4, 5, 18, 32
1965	National Center for Primate Biology, Davis, CA	?	Retrospective diagnosis based on clinical presentation	None	8
1966–1967	Shamrock Farm Ltd., Small Dole, West Sussex, United Kingdom	Crab-eating macaques, ^e rhesus monkeys	Clinical presentation	None	10, 33
1967	Institute of Experimental Pathology and Therapy, Sukhumi, Georgia, Soviet Union	Rhesus monkeys	Clinical presentation	None	15, 34
1967	National Center for Primate Biology, Davis, CA	Rhesus monkeys	Serology, exptl reproduction	None	8, 10
1968	Institute of Experimental Pathology and Therapy, Sukhumi, Georgia, Soviet Union	Rhesus monkeys	Clinical presentation	None	15, 34
1968	Shamrock Farm Ltd. Small Dole, West Sussex, United Kingdom	Rhesus monkeys	Clinical presentation, exptl reproduction	None	9, 10, 35
1968	Austria	Rhesus monkeys	Clinical presentation	None	36
1969	Shamrock Farm Ltd., Small Dole, West Sussex, United Kingdom	Crab-eating macaques, rhesus monkeys	Clinical presentation, serology, virus isolation, exptl reproduction	Unnamed isolate (destroyed)	9, 10, 33, 37
1970	Institute of Experimental Pathology and Therapy, Sukhumi, Georgia, Soviet Union	Rhesus monkeys	Clinical presentation	None	15, 34
1972	NIH Corbel Facility, Rockville, MD	Rhesus monkeys	Clinical presentation, serology, virus isolation	P-180 (destroyed), P-248 (destroyed), P-741 (destroyed)	12, 14, 38, 39
1974	Institute of Experimental Pathology and Therapy, Sukhumi, Georgia, Soviet Union	Patas monkeys, ^f southern pig-tailed macaques	Clinical Presentation	None	15, 34, 40
1989	Primate Research Institute of New Mexico State University, Alamogordo, NM	Crab-eating macaques	Clinical presentation, serology, electron microscopy, virus isolation	I-621 (destroyed), Ph0080 (destroyed)	41, 42
1989	Undisclosed facility, Miami, FL	?	?	?	43
1989	Hazelton Research Products, Reston, VA	Crab-eating macaques	Clinical presentation, serology, virus isolation	4 unnamed isolates (destroyed?)	44
1996	Hazelton Research Products, Alice, TX	Crab-eating macaques	Clinical presentation, virus isolation	1 unnamed isolate (destroyed?)	45

^a *M. assamensis*.^b *M. mulatta*.^c *M. nemestrina*.^d *M. arctoides*.^e *M. fascicularis*.^f *E. patas*.^g Still available from the American Type Culture Collection; RefSeq accession no. [NC_003092](#), GenBank accession no. [AF180391](#) and [KM373784](#) to [KM371110](#).

was found in healthy captive olive baboons at the Southwest National Primate Research Center in San Antonio, TX (20).

We attempted to recover archived materials such as frozen or fixed tissues from past SHF outbreaks to identify and characterize the viruses responsible by next-generation sequencing (NGS). Thus far, we have been successful in two instances. We obtained a

vial containing lyophilized brain tissue (10% homogenate) of a rhesus monkey (*Macaca mulatta*) that succumbed during the 1964 Sukhumi epizootic from the Russian State Collection of Viruses at the D. I. Ivanovsky Institute of Virology in Moscow, Russia. This vial was one of several provided to the collection by two of us (Z.V.S. and B.A.L.) in 1969. In the second instance, we obtained

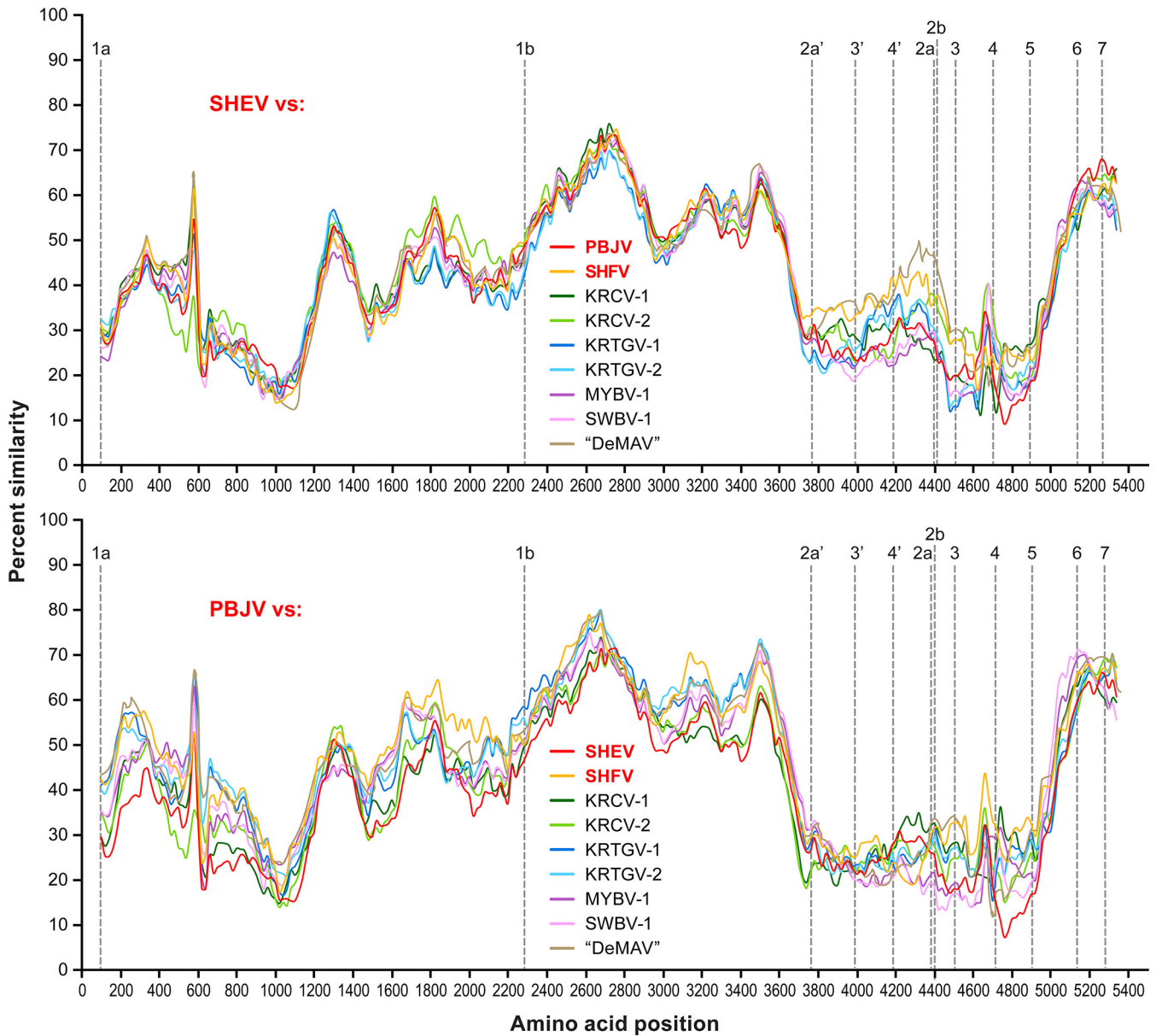


FIG 1 Genomic and amino acid sequence comparisons of SHEV, PBJV, and other arteriviruses. Sliding-window similarity plots of percent amino acid identity of SHEV and PBJV compared to the coding genomes of other simian arteriviruses, i.e., SHFV, KRCV-1, KRCV-2, KRTGV-1, KRTGV-2, MYBV-1, SWBV-1, and “DeBrazza’s monkey arterivirus” (“DeMAV”). Simian arteriviruses associated with SHF outbreaks are shown in red. Open reading frame 1a (ORF1a), ORF1b, ORF2a’, ORF3’, ORF4’, ORF2a, ORF2b, and ORF3 to ORF7 were individually aligned with other simian arteriviruses by a codon-based version of the multiple alignment with fast Fourier transform (MAFFT) algorithm (24) implemented in Translator X (25). Individual ORF alignments were then concatenated, and sliding-window plots of inferred amino acid sequence similarity were created with SimPlot v3.5.1 (window size, 200 amino acids; step size, 25 amino acids) (26). Dashed vertical lines represent the start positions of the ORFs. RefSeq/GenBank accession numbers of the sequences used for the analysis: SHEV, [KM677927](#); PBJV, [KR139839](#); SHFV, [NC_003092](#); KRCV-1, [HQ845737](#); KRCV-2, [KC787631.1](#); KRTGV-1, [JX473847](#); KRTGV-2, [JX473849](#); MYBV-1, [NC_025112.1](#); SWBV-1, [NC_025113.1](#); “DeMAV,” [NC_026509](#).

three vials containing serum of three rhesus monkeys (samples F1167, I621, and F628) experimentally infected with material from crab-eating macaques (*Macaca fascicularis*) that had succumbed during the 1989 Alamogordo epizootic from the United States Army Medical Research Institute of Infectious Diseases at Fort Detrick, Frederick, MD.

To determine the sequences of the Sukhumi-64, F1167, I621, and F628 viruses, we purified their total RNAs with TRIzol (Invitrogen) and the QIAamp MinElute virus spin kit (Qiagen) and

removed host rRNA and tRNA with the GeneRead rRNA depletion kit (Qiagen). For the Sukhumi-64 virus, a DNA library for NGS was prepared from 10 ng of depleted RNA with the NEBnext Ultra RNA Library Prep kit (New England BioLabs). For the F1167, I621, and F628 viruses, a randomly primed double-stranded cDNA was synthesized as previously described (21) and NGS libraries were prepared with the Nextera XT kit (Illumina). Sequencing was performed on an Illumina MiSeq instrument with MiSeq Reagent kits V2 (300PE; Illumina Inc.). Low-quality

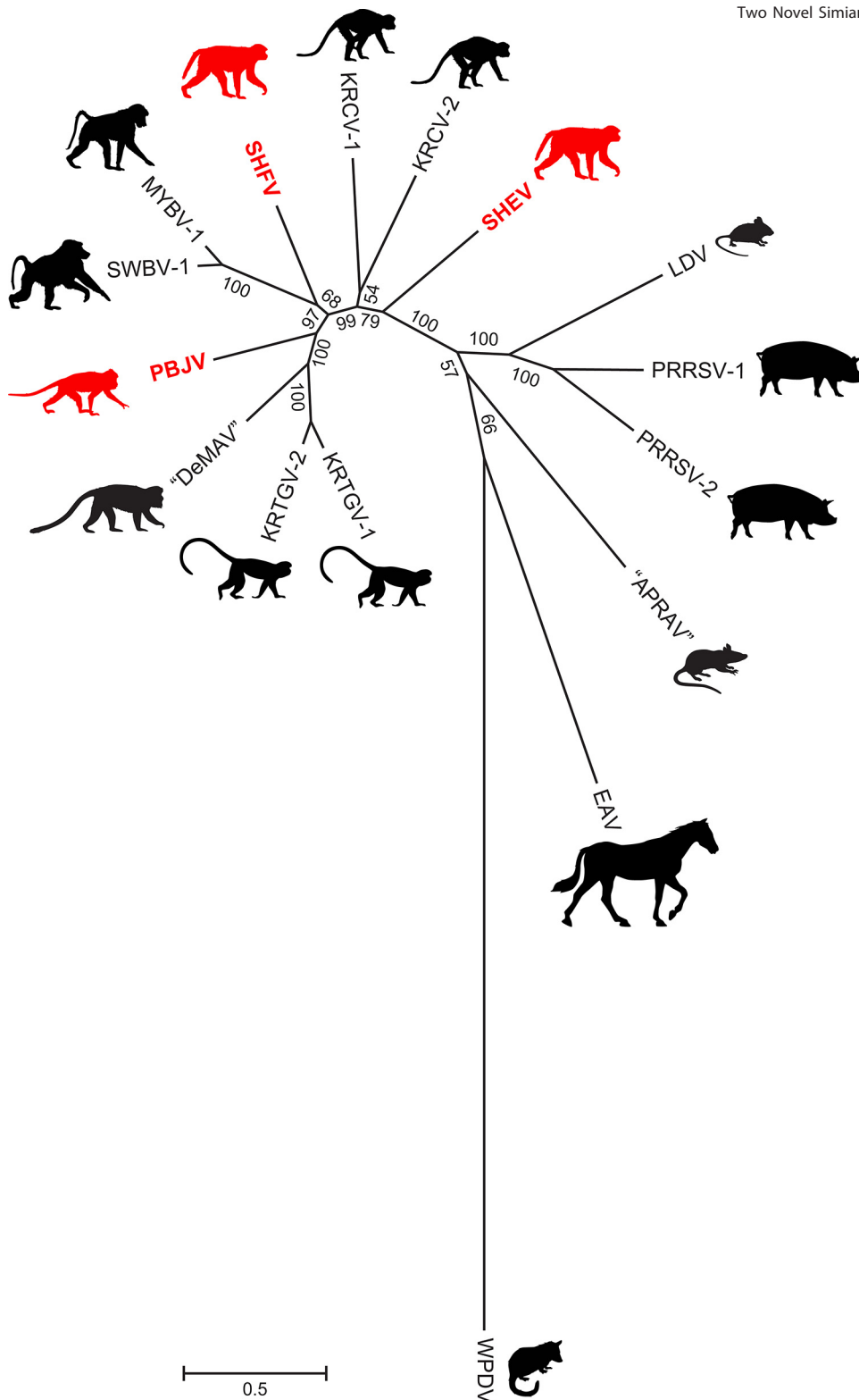


FIG 2 Arterivirus phylogeny based on ORF1b nucleotide sequences. Sequences were aligned by a codon-based version of the MAFFT algorithm with regions of poor alignment removed by the Gblocks method implemented in the computer program Translator X (25). The tree was constructed by Phylogenetic Analysis Using Parsimony (PAUP*) version 4.0b10 (27) from a final alignment of 3,840 positions by the maximum-likelihood method with a best-fit substitution model of the form GTR+I+ Γ (general time-reversible model with invariable sites parameter and gamma distribution accounting for rate variation among sites) and parameters identified through jModeltest (28, 29). Numbers beside branches represent bootstrap values (based on 1,000 bootstrap replicates of the data), and branch lengths are proportional to the numbers of nucleotide substitutions per site (scale bar). Silhouettes indicate hosts (see the text), with nonhuman primates associated with SHF outbreaks shown in red, as the natural hosts of those three viruses remain unknown. The virus name abbreviations and GenBank accession numbers shown are identical to those in Fig. 1 plus equine arteritis virus (EAV; accession no. [NC_002532](https://www.ncbi.nlm.nih.gov/nuccore/NC_002532)), lactate dehydrogenase-elevating virus (LDV; accession no. [NC_001639](https://www.ncbi.nlm.nih.gov/nuccore/NC_001639)), porcine reproductive and respiratory syndrome viruses 1 (PRRSV-1; accession no. [M96262.2](https://www.ncbi.nlm.nih.gov/nuccore/M96262.2)), PRRSV-2 (accession no. [NC_001961](https://www.ncbi.nlm.nih.gov/nuccore/NC_001961)), wobbly possum disease virus (WPDV; accession no. [JN116253](https://www.ncbi.nlm.nih.gov/nuccore/JN116253)), and “African pouched rat arterivirus” (“APRAV”; accession no. [NC_026439.1](https://www.ncbi.nlm.nih.gov/nuccore/NC_026439.1)).

(<Q30) and short (<100 bp) reads were removed, and the remaining reads were assembled *de novo* with CLC Genomics Workbench 7 (CLC bio). Sequence gaps in the genome were filled by Sanger sequencing on an Applied Biosystems 3130 genetic analyzer. The genomic 3' and 5' termini of the Sukhumi-64 virus were determined with the Mint RACE kit (Evrogen). Viral genomes were annotated in CLC Genomics Workbench 7.1. A query against the NCBI GenBank database (22) revealed two novel simian arteriviruses, here named simian hemorrhagic encephalitis virus (SHEV) and Pebjah virus (PBJV), from the samples obtained in Sukhumi, Russia, and Alamogordo, NM, respectively. The genomic organization of SHEV and PBJV is highly similar to that of all known simian arteriviruses. However, the SHEV and PBJV genomes differ from each other by >52.9% in pairwise analyses. KRCV-1 and KRCV-2 are the closest relatives of SHEV (45.4 and 45.6% identity) and PBJV (46.5 and 46.6% identity). In a pairwise sequence comparison with a modified basic local alignment search tool (BLAST) algorithm (23), the SHEV and PBJV genomes differed from each other by 54% and from KRCV-2 and SHFV, the most closely related simian arteriviruses, by ~54 and ~48%, respectively. The resulting histograms, visualizing the distribution of the number of arterivirus pairs at each identity percentage, can be found at <http://www.ncbi.nlm.nih.gov/sutils/pasc/viridty.cgi?cmdresult=main&id=377>. These stark differences are confirmed by amino acid similarity plots (Fig. 1). PBJV genomes from the three macaques that succumbed during the Alamogordo epizootic were highly similar to each other (99.89 to 99.96% identity on the genome level), suggesting that the outbreak was caused by a single etiological agent. Finally, phylogenetic analysis (Fig. 2) indicates that SHEV and PBJV indeed cluster with the simian arterivirus clade, although both viruses are distinct and divergent from their congeners.

Our results indicate that on at least two occasions, SHF in captive macaques has, in all likelihood, been caused by viruses distinct from SHFV and distinct from each other (SHEV and PBJV). We conclude that SHF is caused not by a single etiological agent but rather by diverse members of the simian clade of the family *Arteriviridae*. Our findings raise the possibility that other recently discovered simian arteriviruses (17–20) may also be capable of infecting macaques and causing SHF.

Nucleotide sequence accession numbers. The genomic sequences obtained in this study were submitted to GenBank and assigned accession no. [KM677927](https://www.ncbi.nlm.nih.gov/nuccore/KM677927) (Sukhumi-64), [KR139840](https://www.ncbi.nlm.nih.gov/nuccore/KR139840) (F1167), [KR139839](https://www.ncbi.nlm.nih.gov/nuccore/KR139839) (I621), and [KR139838](https://www.ncbi.nlm.nih.gov/nuccore/KR139838) (F628).

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