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Two Novel Simian Arteriviruses in Captive and Wild Baboons (*Papio* spp.)

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ABSTRACT

Since the 1960s, simian hemorrhagic fever virus (SHFV; *Nidovirales*, *Arteriviridae*) has caused highly fatal outbreaks of viral hemorrhagic fever in captive Asian macaque colonies. However, the source(s) of these outbreaks and the natural reservoir(s) of this virus remain obscure. Here we report the identification of two novel, highly divergent simian arteriviruses related to SHFV, Mikumi yellow baboon virus 1 (MYBV-1) and Southwest baboon virus 1 (SWBV-1), in wild and captive baboons, respectively, and demonstrate the recent transmission of SWBV-1 among captive baboons. These findings extend our knowledge of the genetic and geographic diversity of the simian arteriviruses, identify baboons as a natural host of these viruses, and provide further evidence that baboons may have played a role in previous outbreaks of simian hemorrhagic fever in macaques, as has long been suspected. This knowledge should aid in the prevention of disease outbreaks in captive macaques and supports the growing body of evidence that suggests that simian arterivirus infections are common in Old World monkeys of many different species throughout Africa.

IMPORTANCE

Historically, the emergence of primate viruses both in humans and in other primate species has caused devastating outbreaks of disease. One strategy for preventing the emergence of novel primate pathogens is to identify microbes with the potential for cross-species transmission in their natural state within reservoir species from which they might emerge. Here, we detail the discovery and characterization of two related simian members of the *Arteriviridae* family that have a history of disease emergence and host switching. Our results expand the phylogenetic and geographic range of the simian arteriviruses and define baboons as a natural host for these viruses. Our findings also identify a potential threat to captive macaque colonies by showing that simian arteriviruses are actively circulating in captive baboons.

Viruses in the family *Arteriviridae* (order *Nidovirales*) infect and cause disease in a wide variety of mammals, including pigs, mice, horses, possums, and primates. These viruses form spherical, enveloped particles that contain a single-stranded, positive-sense RNA genome (1). Human arterivirus infection has never been documented, although viruses within the rapidly growing taxa of the simian arteriviruses possess several features suggestive of a high potential for cross-species transmission and adaptation (2; our unpublished data). Indeed, one particular simian arterivirus, simian hemorrhagic fever virus (SHFV), has caused numerous outbreaks of fatal simian hemorrhagic fever (SHF) in captive Asian macaques (*Macaca assamensis*, *M. arctoides*, *M. fascicularis*, *M. nemestrina*, and *M. mulatta*) (3–9). Results of previous work have shown that captive patas monkeys (*Erythrocebus patas*) can harbor SHFV infection without any signs of disease (6, 10, 11), and contact with captive patas monkeys and/or biological material from these animals has been implicated in multiple outbreaks of SHF in macaques (5, 6). However, not all outbreaks of SHF have been associated with SHFV, and the virus(es) and source(s) responsible for several SHF outbreaks remain unknown (12).

The distribution of simian arteriviruses in wild primates remains poorly characterized. Importantly, simian arterivirus infection has never been demonstrated in a free-living patas monkey, and monkeys from the two species known to harbor simian arteriviruses in nature, the red colobus (*Procolobus rufomitratus tephrosceles*) and the red-tailed guenon (*Cercopithecus ascanius schmidti*), were not associated with past outbreaks of SHF in macaques (2, 13, 14). Grivets (*Chlorocebus aethiops*) and baboons (genus *Papio*) have long been suspected of harboring simian arteriviruses in captivity, but empirical evidence demonstrating infection of these hosts, either in the wild or in captivity, has, to our knowledge, never been presented (6, 15).

Here, we used unbiased deep sequencing to examine simian arterivirus infection in three populations of baboons: 23 wild olive baboons (*Papio anubis*) from Kibale National Park in Uganda, 43 wild yellow baboons (*Papio cynocephalus*) from Mikumi National Park in Tanzania, and 31 captive olive and hybrid olive/yellow

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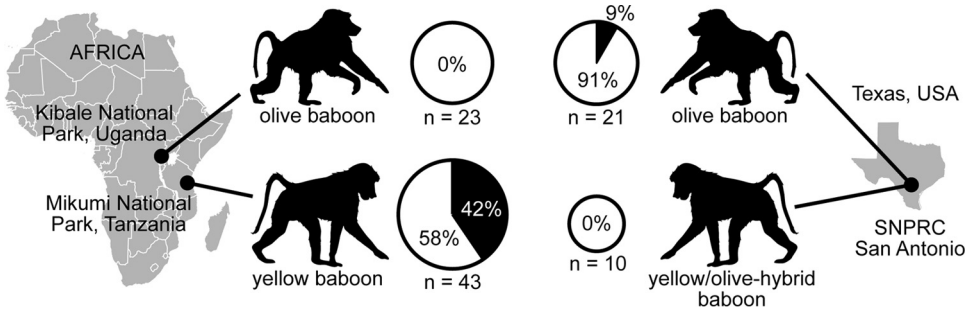

FIG 1 Prevalence of simian arteriviruses in wild and captive baboon populations. Unbiased deep sequencing and qRT-PCR specific for MYBV-1 and SWBV-1 were used to determine the percentage of each baboon species that was viremic (black) or aviremic (white) at the time of sampling. Simian arterivirus prevalences were 18/43 (41.9%, 95% confidence interval [CI] = 28.4% to 56.7%) in Mikumi yellow baboons, 0/23 (0.0%, 95% CI = 0.0% to 16.9%) in Kibale olive baboons, 2/21 (9.5%, 95% CI = 1.5% to 30.1%) in SNPRC olive baboons, and 0/10 (0.0%, 95% CI = 0.0% to 32.1%) in SNPRC hybrid olive/yellow baboons. The prevalence of simian arterivirus infections was significantly higher in Mikumi baboons than in baboons from Kibale or SNPRC (Fisher's exact test, $P < 0.01$ in both cases). Maps and animal silhouettes are not to scale.

baboons (Southwest baboons) from the Southwest National Primate Research Center (SNPRC) in San Antonio, TX. We detected a novel, highly divergent simian arterivirus in 18 wild Mikumi yellow baboons (Mikumi yellow baboon virus 1 [MYBV-1]) and were able to assemble coding complete genomes (16) of MYBV-1 variants in samples from 11 of these baboons. We also identified a second divergent simian arterivirus, Southwest baboon virus 1 (SWBV-1), in two SNPRC olive baboons, whereas no simian arteriviruses could be detected in SNPRC hybrid olive/yellow baboons or wild Kibale olive baboons. Our study reveals that baboons of different species can be infected by divergent simian arteriviruses in the wild and that transmission of these viruses occurs in captivity.

MATERIALS AND METHODS

Sample collection. Blood samples from Kibale olive baboons (*Papio anubis*) were collected from 2010 to 2014 as described previously (13), and collection of the samples was approved by the Uganda Wildlife Authority (permit UWA/TDO/33/02), the Uganda National Council for Science and Technology (permit HS 364), and the University of Wisconsin Animal Care and Use Committee (protocol V01409-0-02-09). Samples from Mikumi yellow baboons (*Papio cynocephalus*) were collected by Jeffrey Rogers and colleagues in 1985 and 1986 using standard methods for field studies of baboons, as described previously (17). All procedures were approved by the appropriate Tanzanian government authorities and by the U.S. institutions involved (Washington University and Yale University). Briefly, most study subjects were humanely trapped and sedated with ketamine. Blood samples were drawn from the femoral vein into evacuated blood collection tubes coated with EDTA anticoagulant. These animals were released as soon as possible after recovery from sedation. A small proportion of study subjects were sedated using a blowgun. These animals were bled in the same manner and were returned to their social group immediately following processing. Only samples from male baboons from Mikumi were available for this study. At SNPRC, blood samples were collected opportunistically from two purebred olive baboons from a group cage when they were sedated for tuberculosis tests. Blood was also collected at a later time to screen for simian arteriviruses in 32 baboons: 21 olive baboons, 10 hybrid olive/yellow baboons, and 1 hybrid olive/hamadryas (*Papio hamadryas*) baboon (SWBV-1 negative; data for this baboon were not included in the analyses). Fifteen baboons were selected from the pedigree 1-genotyped breeding group (males and females, including two trios). The other 15 baboons were from the pedigree 3 group-housed animals in order to include animals of both sexes who were breeding and/or cohoused with the two purebred olive baboons initially bled and found to be infected with SWBV-1. All animals were bled from the femoral vein, and the blood was placed in evacuated blood collection tubes coated with EDTA anticoagulant, which were shipped overnight on gel cold packs. All research involving SNPRC baboons was preapproved by the Texas Biomed Animal Care and Use Committee.

Deep sequencing. Samples were processed for sequencing in a biosafety level 3 laboratory as described previously (13, 14), with slight modifications. Briefly, for each animal, viral RNA was isolated from approximately 200 μl of plasma using a Qiagen QIAamp MinElute virus spin kit (Qiagen, Hilden, Germany), omitting carrier RNA. Samples were then treated with DNase, and cDNA synthesis was primed using random hexamers (double-stranded cDNA synthesis kit; Invitrogen, Carlsbad, CA). Samples were fragmented, and sequencing adaptors were added using a Nextera DNA sample preparation kit (Illumina, San Diego, CA, USA). Deep sequencing was performed on an Illumina MiSeq apparatus. Sequence data were analyzed using CLC Genomics Workbench (version 6.5) software (CLC Bio, Aarhus, Denmark) and Geneious R5 software (Biomatters, Auckland, New Zealand). Low-quality (Phred quality score, <Q30) and short reads (<100 bp) were removed, and coding complete (16) genome sequences for each virus were acquired using the *de novo* assembly algorithm in CLC Genomics Workbench (version 6.5) software. The average coverage over each genome was 225 times (range, 24 to 837 times) for all viruses. Viral genomes were annotated in CLC Genomics Workbench (version 6.5), and putative open reading frames (ORFs) were confirmed by querying the NCBI GenBank database (18).

Phylogenetic analysis. Nineteen ORF1b sequences representing the known diversity of simian arteriviruses, including all sequences reported here, were aligned using a codon-based version of the open-source software multiple-sequence alignment using fast Fourier transform (MAFFT) (19), implemented in the TranslatorX web server, without GBLOCKS gene fragment cleaning (20). The phylogenetic history was inferred from the aligned nucleotide sequences using the maximum likelihood method (1,000 bootstrap replicates) via molecular evolutionary genetics analysis, version 6 (MEGA6), open-source software (21). The best nucleotide substitution model, a general time reversible model coupled with a Γ distribution for rate variation (GTR+Γ; with five rate categories, +Γ parameter = 1.1092), was estimated using MEGA6. All positions containing gaps and missing data were eliminated, resulting in a final data set of 4,380 positions. The initial tree for the heuristic search was obtained by applying the neighbor-joining method to a matrix of pairwise distances, estimated using the maximum composite likelihood approach.

SimPlot analysis. Viral genomes were annotated with CLC Genomics Workbench (version 7.1) software (CLC Bio, Aarhus, Denmark), and putative ORFs were confirmed by querying the NCBI GenBank database (18). ORFs were individually aligned with the sequences of the prototype SHFV variant LVR 42-0/M6941 (GenBank accession number NC003092), red colo-

bus variants KRCV-1 (previously referred to as SHFV-krc1; GenBank accession number HQ845737) and KRCV-2 (previously referred to as SHFV-krc2; GenBank accession number KC787631), and red-tailed guenon variants KRTGV-1 (previously referred to as SHFV-krtg1; GenBank accession number JX473847) and KRTGV-2 (previously referred to as SHFV-krtg2; GenBank accession number JX473849) using a codon-based version of the MAFFT algorithm (19) implemented in TranslatorX (20). Individual ORF alignments were then concatenated, the nucleotide-level similarities of the resulting full-length coding genomes were calculated using MEGA5 (22), and sliding-window plots of inferred amino acid similarity were created with open-source software (SimPlot, version 3.5.1) (23).

qRT-PCR. A TaqMan quantitative reverse transcriptase-PCR (qRT-PCR) assay (Life Technologies, Grand Island, NY) was developed to quantify plasma RNA of both MYBV-1 and the SWBV-1 in each sample (forward primer, 5′-GCTTGCTGGTAAGATTGCCA-3′; reverse primer, 5′-GCAGCGGATCTTTGTGGAA-3′; probe, 5′-FAM-TGATTAACCTGAGGAAGTATGGCTGGC-BHQ1-3′, where FAM is 6-carboxyfluorescein and BHQ1 is black hole quencher 1). A standard curve was established by cloning a 738-bp fragment of the SWBV-1_16986_11.4.2013 genome (forward primer, 5′-GCGCCACACTAATTTCATCA-3′; reverse primer, 5′-GCAGCGGATCTTTGTGGAA-3′) into the Zero Blunt PCR vector (Invitrogen, Carlsbad, CA), followed by linearization (HindIII; New England BioLabs, Ipswich, MA). The fragment was transcribed *in vitro* for 6 h (MEGAscript T7 transcription kit; Invitrogen, Carlsbad, CA), purified (MEGAclear transcription cleanup kit; Invitrogen, Carlsbad, CA), quantified (Qubit RNA high-sensitivity assay kit; Invitrogen), and diluted to a concentration of 1×10^{10} transcript copies/μl. Tenfold dilutions of this transcript were used as a standard curve.

Viral RNA was extracted from plasma samples using a QIAamp MinElute virus spin kit (Qiagen, Hilden, Germany) with carrier RNA. RNA was reverse transcribed and amplified using a SuperScript III one-step qRT-PCR system (Invitrogen, Carlsbad, CA) on a LightCycler 480 apparatus (Roche, Indianapolis, IN). Reverse transcription was carried out at 37°C for 15 min and then 50°C for 30 min, followed by 2 min at 95°C. Amplification was accomplished over 50 cycles, as follows: 95°C for 15 s and 60°C for 1 min. The reaction mixture contained $MgSO_4$ at a final concentration of 3.0 mM, the two amplification primers at a concentration of 500 nM, and a probe at a concentration of 100 nM. The standard curve was linear over (at least) 8 orders of magnitude and was sensitive down to 10 copies of RNA transcript per reaction.

Pan-simian arterivirus RT-PCR. A set of primers was designed on the basis of a multiple-sequence comparison by log expectation alignment (with MUSCLE open-source software) of all currently known simian arterivirus genomic sequences. Primer 3 (24) was used to identify suitable primer binding sites within regions of high conservation, and degenerate bases and inosine bases were added to accommodate polymorphic sites [forward primer, ACCT(G/C/T)A(G/A)GAAGTATGGCTGGCA; reverse primer, GGGTTCIGC(A/G)AAIACA(A/T)AATG]. RT-PCR was optimized for this primer set using RNA extracted from plasma from SNPRC baboon 16986 under the following conditions using the SuperScript III One-Step qRT-PCR system (Invitrogen, Carlsbad, CA): reverse transcription at 45°C for 30 min, followed by 2 min at 94°C and then 40 cycles of amplification consisting of 94°C for 15 s, 50°C for 30 s, and 68°C for 25 s. The reaction mixture contained $MgSO_4$ at a final concentration of 3.0 mM and the two amplification primers at a concentration of 200 nM.

Nucleotide sequence accession numbers. The coding complete genome sequences of the 11 MYBV-1 strains detected in this study have been placed in GenBank under accession numbers KM110935 to KM110945. The coding complete genome sequences of the SWBV-1 strains have been placed in GenBank under accession numbers KM110946 to KM110948.

TABLE 1 Demographic information for Mikumi yellow baboons (*Papio cynocephalus*)[a]

Animal identifier	MYBV-1 load[b]	Wt (lb)	Social group	Age group
M01	1.03E+08	53	Punk	Adult
M03	0.00E+00	41	Kizorobi	Sub-Ad/Juv
M05	0.00E+00	49	Nevsi	Sub-Ad/Juv
M06	6.53E+07	51	Punk	Adult
M09	0.00E+00	60	Barabara	Adult
M100	2.20E+07	41	Punk	Sub-Ad/Juv
M11	0.00E+00	47	Punk	Adult
M13	7.23E+05	49	Kizorobi	Adult
M14	8.82E+07	60	Barabara	Adult
M16	9.75E+02	38	Punk	Sub-Ad/Juv
M17	0.00E+00	50	VI	Sub-Ad/Juv
M18	0.00E+00	35	Kizorobi	Sub-Ad/Juv
M22	8.91E+04	52	VI	Adult
M23	0.00E+00	27	Kizorobi	Sub-Ad/Juv
M26	2.94E+08	58	Punk	Adult
M27	0.00E+00	55	VI	Adult
M28	1.76E+07	50	Kizorobi	Adult
M31	0.00E+00	32	Punk	Sub-Ad/Juv
M33	1.97E+07	48	Kizorobi	Adult
M36	0.00E+00	35	Punk	Sub-Ad/Juv
M38	0.00E+00	42	Kizorobi	Adult
M43	0.00E+00	41	Kizorobi	Sub-Ad/Juv
M45	0.00E+00	39		Sub-Ad/Juv
M46	0.00E+00	27	Punk	Sub-Ad/Juv
M49	0.00E+00	37	Punk	Sub-Ad/Juv
M51	7.77E+06	50	Kizorobi	Adult
M52	0.00E+00	48.5	Punk	Adult
M56	0.00E+00	20	VI	Sub-Ad/Juv
M58	5.40E+07	47	Punk	Adult
M59	9.29E+05	49	VI	Adult
M62	1.22E+05	25	VI	Sub-Ad/Juv
M63	2.17E+04	52.5	Kizorobi	Adult
M65	0.00E+00	26	VI	Sub-Ad/Juv
M71	1.77E+07	61	Born in VII	Adult
M73	0.00E+00	61	Punk	Adult
M74	0.00E+00	36	VI	Sub-Ad/Juv
M75	1.15E+07	47	Kizorobi	Adult
M76	0.00E+00	49	Punk	Adult
M77	0.00E+00	38	VI	Sub-Ad/Juv
M79	0.00E+00	57	Barabara	Adult
M82	0.00E+00	49	Punk	Adult
M83	1.23E+07	56	VI	Adult
M91	0.00E+00	37	Punk	Sub-Ad/Juv

[a] All baboons were male. Sub-Ad/Juv, subadult or juvenile.
[b] Number of genome copies per ml of plasma.

RESULTS

Discovery of two novel simian arteriviruses in wild and captive baboons. We performed unbiased deep sequencing of RNA extracted from blood samples obtained from 23 wild Kibale olive baboons, 43 wild Mikumi yellow baboons, and 31 captive olive and hybrid olive/yellow baboons at SNPRC. From this sequencing we discovered a novel, highly divergent simian arterivirus in samples from 14 Mikumi yellow baboons (Mikumi yellow baboon virus 1 [MYBV-1]) and assembled coding complete genome sequences for viruses from 11 of these samples. We assembled coding complete genome sequences for a second divergent simian arterivirus (Southwest baboon virus 1 [SWBV-1])

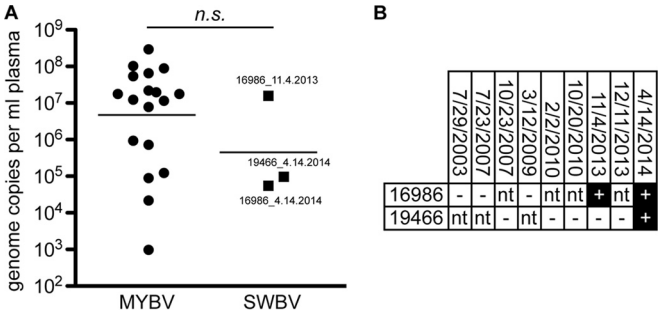

FIG 2 Plasma viral loads of simian arterivirus and longitudinal sampling of SWBV-1. RNA was isolated from plasma, and qRT-PCR was performed using primers and probes designed from deep sequencing data. (A) The difference in viral loads between MYBV-1 (circles) and SWBV-1 (squares) was not significant (n.s.; two-tailed *t* test performed on log-transformed 95% confidence interval values). Baboon identifiers and sample collection dates are indicated for SWBV-1-infected baboons. (B) Collection dates and results of testing of banked samples from SNPRC olive baboons 16986 and 19466: not tested (nt), SWBV-1 positive (+), and SWBV-1 negative (−).

from two SNPRC olive baboons but could not detect simian arteriviruses by unbiased deep sequencing in captive SNPRC hybrid olive/yellow baboons or wild Kibale olive baboons.

Prevalence and plasma viral loads of MYBV-1 and SWBV-1. We designed a highly sensitive qRT-PCR assay capable of detecting both MYBV-1 and SWBV-1 to verify the prevalence of the viruses in the baboons studied and determine the plasma concentrations of these viruses. Results from this assay were largely congruent with our deep sequencing findings, identifying MYBV-1 infection in 18 of 43 (41.9%) Mikumi yellow baboons, including all 14 animals that were determined to be MYBV-1 infected by deep sequencing. qRT-PCR also confirmed the presence of SWBV-1 in the 2 SNPRC olive baboons identified by deep sequencing (2/31, 6.5%) and the absence of SWBV-1 in the 29 other SNPRC baboons tested (Fig. 1). Similarly, RT-PCR of RNA extracted from the plasma of each Kibale olive, Mikumi yellow, and SNPRC olive and hybrid olive/yellow baboon using our pan-simian arterivirus PCR assay did not identify any additional simian arterivirus infections but produced an amplicon of the expected size for both SWBV-1-positive SNPRC olive baboons and all MYBV-1-positive Mikumi yellow baboons identified to by qRT-PCR, except the single Mikumi yellow baboon with the lowest plasma MYBV-1 load (M16; Table 1). MYBV-1 titers were high, averaging 4.0×10^7 genome copies/ml of plasma, whereas SWBV-1 titers in infected SNPRC olive baboons were lower, averaging 5.3×10^6 genome copies/ml of plasma (Fig. 2A). Multiple attempts to propagate MYBV-1 and SWBV-1 on cell lines permissive for other arteriviruses (MA-104, MARC, and BSC-1) and on primary rhesus macaque monkey bronchoalveolar lavage (BAL) cells were not successful (data not shown) (25).

The distribution of MYBV-1 infection among the Mikumi yellow baboons showed an interesting and potentially important pattern. All of the 43 yellow baboons tested were male and belonged to one of five social groups located within a limited area of Mikumi National Park (Table 1). Each social group with the exception of the fifth group contained animals infected with MYBV-1; only one baboon from the fifth group was tested. There were 24 adult Mikumi baboons tested, with 15 found to be MYBV-1 positive (62.5%); in comparison, of 19 juvenile and subadult Mikumi baboons tested, only 3 were positive (15.8%). The difference in MYBV-1 prevalence between adults and younger animals was statistically significant (Fisher's exact test, $P < 0.01$).

TABLE 2 Demographic information for SNPRC baboons[a]

Animal identifier	SWBV-1 infection status	Date of birth (mo/day/yr)	Approx age (yr)	Sex	Pedigree group	Species
12491	−	01/25/95	19	F	Baboon1	PCX
12552	−	03/10/95	19	F	Baboon1	PCA
13387	−	08/02/96	18	M	Baboon1	PCX
14421	−	02/15/98	16	F	Baboon1	PCX
14930	−	10/01/98	16	M	Baboon1	PCA
15633	−	09/20/99	15	M	Baboon1	PCA
28002	−	05/02/06	8	F	Baboon1	PCA
28143	−	06/28/06	8	F	Baboon1	PCA
28779	−	03/30/07	7	F	Baboon1	PCA
28866	−	05/12/07	7	F	Baboon1	PCX
31879	−	06/05/12	2	F	Baboon1	PCX
31958	−	07/06/12	2	M	Baboon1	PCX
31966	−	07/14/12	2	M	Baboon1	PCX
31979	−	07/21/12	2	M	Baboon1	PCA
32007	−	08/21/12	2	M	Baboon1	PCA
14641	−	04/10/98	16	F	Baboon3	PCA
14943	−	10/10/98	16	F	Baboon3	PCX
18150	−	04/25/02	12	F	Baboon3	PCA
18503[b,c]	−	08/19/02	12	M	Baboon3	HAN
19910[b]	−	02/01/04	10	M	Baboon3	PCA
19914	−	03/15/04	10	M	Baboon3	PCA
19950[b]	−	02/07/04	10	M	Baboon3	PCA
28941	−	07/05/07	7	F	Baboon3	PCA
29833	−	11/10/08	6	F	Baboon3	PCA
29945	−	01/24/09	5	F	Baboon3	PCA
30091	−	04/20/09	5	F	Baboon3	PCA
30104	−	05/04/09	5	F	Baboon3	PCX
30222	−	07/26/09	5	F	Baboon3	PCA
30264	−	09/16/09	5	F	Baboon3	PCA
31984	−	07/25/12	2	F	Baboon3	PCX
16986	+	04/19/01	13	M	Baboon2	PCA
19466	+	05/15/03	11	M	Baboon3	PCA

[a] F, female; M, male; PCA, olive baboon (*Papio anubis*); PCX, olive/yellow hybrid baboon (*Papio anubis* × *Papio cynocephalus*); HAN, Ethiopian sacred baboon (*Papio hamadryas*).
[b] Cage mate (or former cage mate) of SWBV-1-positive baboons.
[c] Not included in analysis.

Transmission of SWBV-1 in captivity. To assess transmission in captivity, we used qRT-PCR to examine banked samples that had been collected from the two baboons at SNPRC found to be infected with SWBV-1. For one olive baboon (baboon 16986), only the two most recent samples, collected in 2013 and 2014, tested positive for SWBV-1, indicating that baboon 16986 became infected at some point between 2009 and 2013 (Fig. 2B). All samples from the second olive baboon (baboon 19466) except the sample collected most recently were negative for SWBV-1, indicating that this animal became infected in late 2013 or early 2014. Notably, these two animals have been housed together at SNPRC since December 2012. Animals in this housing group have had frequent close contact. For example, veterinary medical records indicate that baboon 19466 was admitted to the clinic for lacerations due to trauma in May and July 2013. However, no other baboons from this housing group tested positive for SWBV-1 (Table 2). According to SNPRC

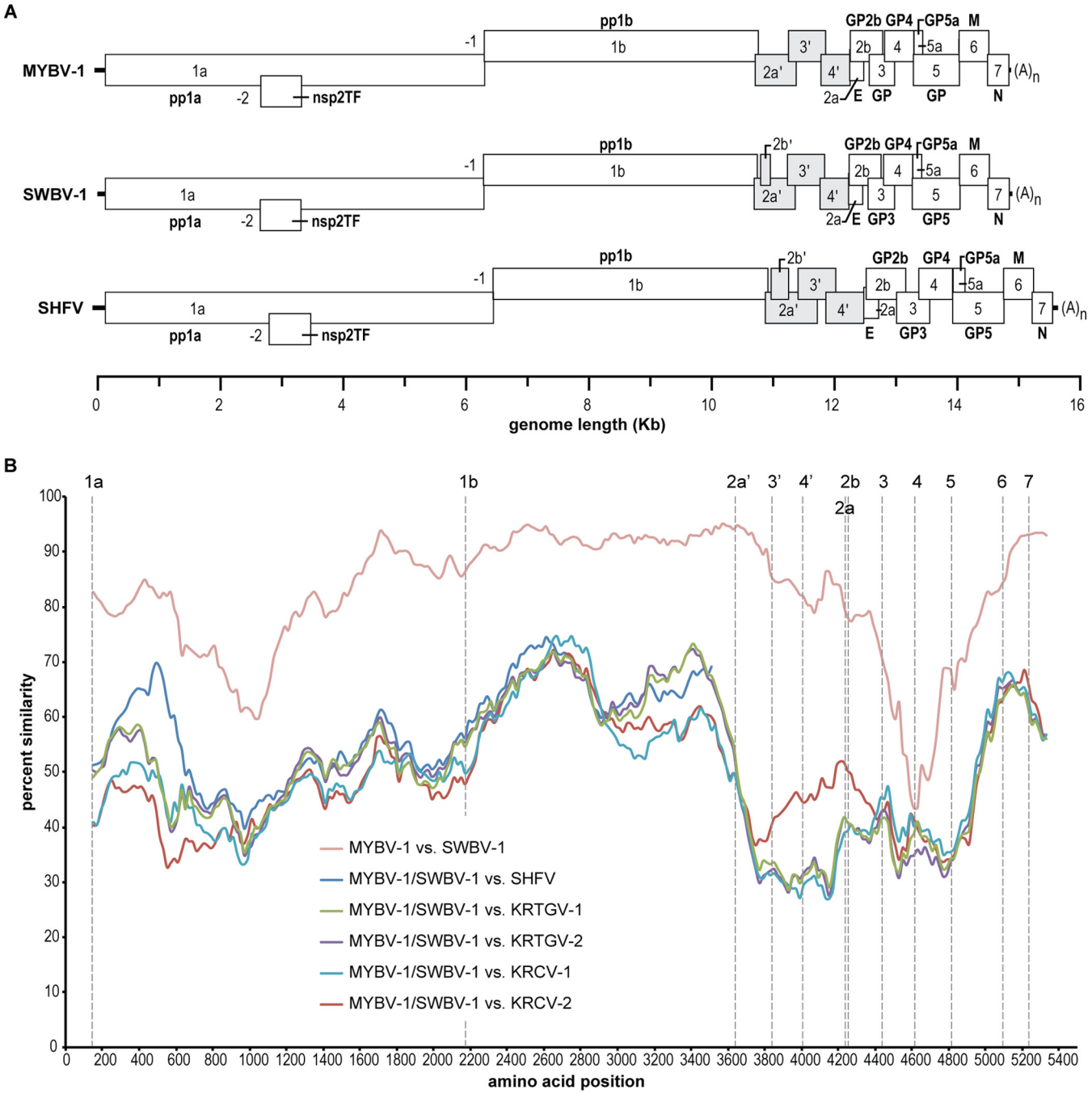

FIG 3 Genome organization and plot of the similarity of SWBV-1 and MYBV-1 sequences to those of other simian arteriviruses. (A) The genome organization of SWBV-1 and MYBV-1 is shown in comparison to that of SHFV, the prototype simian arterivirus. Boxes represent open reading frames and are drawn to scale. ORFs unique to the simian arteriviruses are shown in gray. For ORFs that produce a defined protein homologue in other arteriviruses, the name of the putative protein product is given in bold. (B) Sliding-window similarity plots of percent amino acid identity among select SHFV variants across aligned coding genomes. The analysis was performed with a window size of 300 and a step size of 25. Dashed vertical lines, the start positions of each ORF.

veterinary medical records, both baboon 16986 and baboon 19466 were afebrile and neither baboon showed signs of disease during the period of initial infection. Both animals remain at SNPRC and are in good health.

Viral sequence analysis. The genomes of MYBV-1 and SWBV-1 are organized similarly to one another and to those of the other described simian arteriviruses (KRCV-1, KRCV-2, KRTGV-1, KRTGV-2, SHFV; Fig. 3A). In particular, all genomes contain four open reading frames (ORFs) coding for putative structural proteins that are not present in the genomes of other, nonsimian arteriviruses. MYBV-1 differs from the other simian arteriviruses in that one of these additional ORFs (ORF2b′) is truncated by a premature stop codon. Sliding-window similarity analysis showed that these viruses are the most divergent from one another in the 3′ region of the ge-

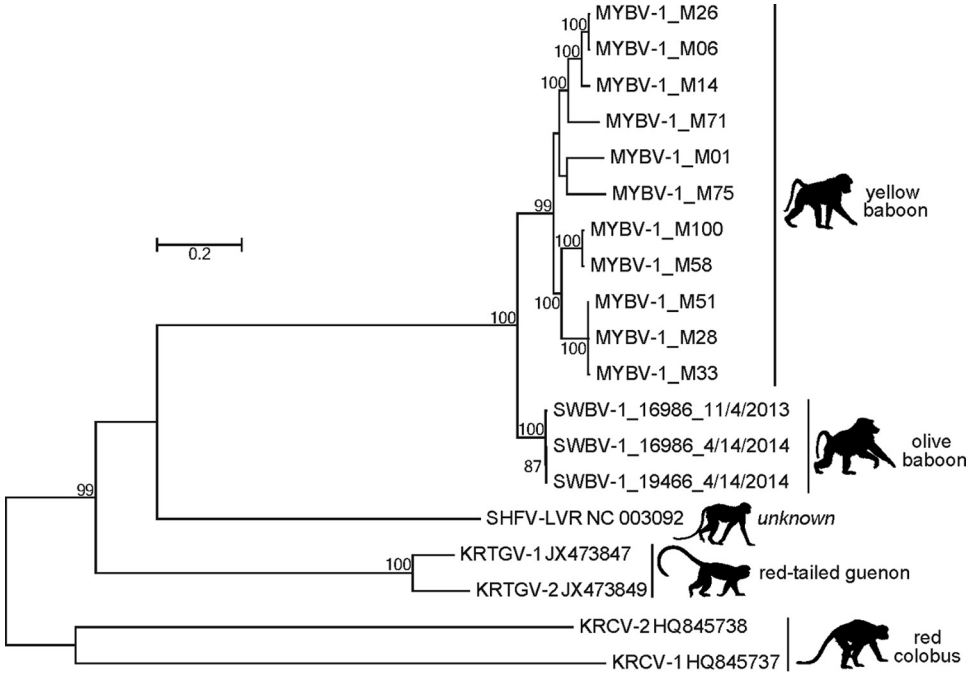

FIG 4 Simian arterivirus phylogeny based on ORF1b nucleotide sequence alignment. The simian arterivirus phylogenetic history was inferred using the maximum likelihood method (1,000 bootstrap replicates) with a best-fit substitution model of the form GTR+Γ (with five rate categories, +Γ parameter = 1.1092). All positions containing gaps and missing data were eliminated, resulting in a final data set of 4,380 positions. Bootstrap values greater than 70% are shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Analyses were conducted using MEGA6 (22).

nome, where ORFs 3, 4, and 5, which code for putative envelope glycoproteins, are present (Fig. 3B). Construction of an interactive heat map of genetic diversity using our custom-built Layercake software (26) revealed a similar pattern (http://graphics.cs.wisc.edu/WP/layercake/shfv-dataset/). Phylogenetic analysis revealed that the MYBV-1 and SWBV-1 genomic sequences are both highly divergent from all previously described simian arterivirus sequences (49.4% nucleotide similarity, on average) but are relatively closely related to one another (80.8% nucleotide similarity, on average) (Fig. 4). Sequences from variants within MYBV-1 were diverse (85.7% nucleotide similarity, on average).

Analysis of the SWBV-1 sequences showed that both of the SWBV-1 sequences from SNPRC olive baboons 16986 and 19466 obtained in 2014 were more distantly related to the SWBV-1 sequence obtained from baboon 16986 in 2013 than they were to each other (Fig. 5A to C). Interestingly, 35% (12/40) of all amino acid differences between the SWBV-1 sequence obtained from baboon 16986 in 2013 and the SWBV-1 sequence obtained from the same animal in 2014 were clustered in a small region in the 5′ portion of ORF5 (Fig. 5D). SWBV-1 sequences from baboons 16986 and 19466 collected in 2014 were very closely related, with only 33 nucleotide differences differentiating the two viruses over the entire coding complete genome at the consensus level (Fig. 5A to C). We detected low-frequency variants within the viral population of baboon 16986 with variations at 20 of these 33 sites, whereas we detected low-frequency variants within the viral population of baboon 19466 with variations at only 8 of these 33 sites. When the low-frequency variants were compared, the variations in a number of low-frequency variants within the viral population of baboon 16986 were fixed (16/20) or were the predominant base (4/20) in the viral population of baboon 19466 (Fig. 5E).

DISCUSSION

Our finding of simian arterivirus (SWBV-1) infection in captive olive baboons at SNPRC definitively demonstrates that captive baboons can harbor simian arteriviruses, although the frequency of SWBV-1 infection across baboon colonies is not clear at this time. Moreover, our discovery of simian arterivirus (MYBV) infections in wild yellow baboons from Mikumi National Park, Tanzania, extends the geographic and natural host range of this rapidly expanding taxon of primate viruses. Interestingly, not all wild baboon populations seem to harbor simian arteriviruses: our data show that while MYBV-1 is highly prevalent in yellow baboons from Tanzania, no simian arteriviruses were found in olive baboons from Kibale National Park in nearby Uganda (a distance of approximately 1,200 km), despite our detection of other viruses in these samples (27; unpublished data). In contrast, the opposite was true of the captive baboons that we surveyed: SWBV-1 was found in olive baboons at SNPRC but not hybrid olive/yellow baboons. This discrepancy underscores the likelihood that simian arteriviruses are widespread and prevalent among some, but not all, populations of African monkeys. Additionally, our data show that the prevalence of MYBV-1 is greater in adult male yellow baboons than juvenile or subadult males of the same social groups. Although only samples from Mikumi male baboons were available for testing, this observation may be relevant to the mode of simian arterivirus transmission, as aggressive encounters between male primates is a common mode of transmission for other bloodborne viruses (28). A prolonged duration of infection could also potentially explain the higher prevalence of infection in adult baboons. Consistent with these suggestions, the two SNPRC olive baboons that tested positive for SWBV-1 were older males, aged

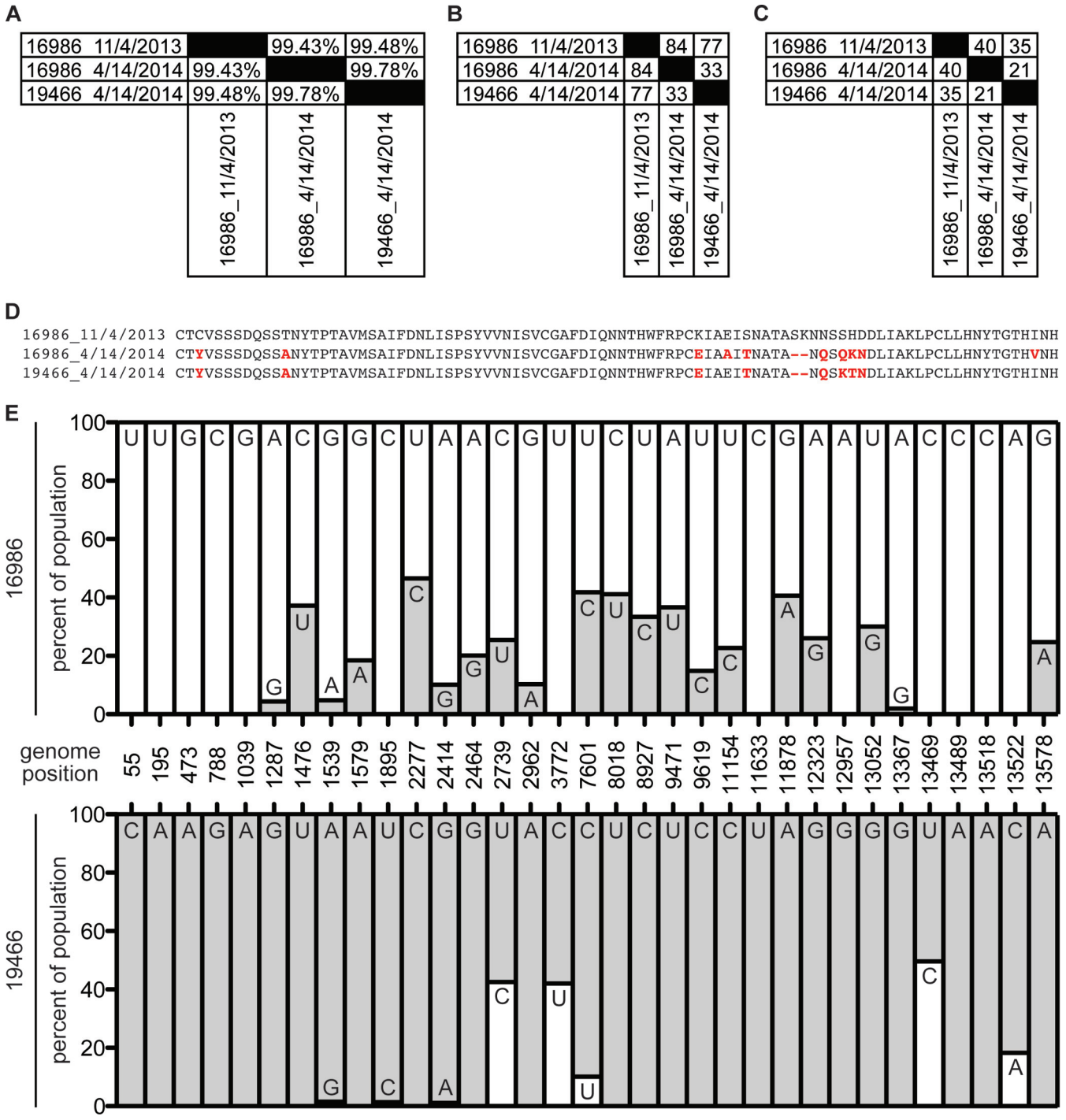

FIG 5 Comparison of SWBV-1 sequences. Consensus nucleotide sequences were extracted from deep sequencing read mappings and aligned using the MUSCLE algorithm in CLC Genomics Workbench (version 6.5). Pairwise comparisons were used to calculate percent nucleotide similarity (A), nucleotide differences (B), and amino acid differences (C). For amino acid comparisons, consensus sequences were annotated and each ORF was translated, aligned, and concatenated. Baboon identifiers and sample collection dates are indicated. (D) Alignment showing the region of SWBV-1 ORF5 with a high density of amino acid changes (red) relative to the sequence collected from baboon 16986 on 4 November 2013. (E) Frequencies of minor nucleotide variants (gray, baboon 16986; white, baboon 19466) at the 33 sites that differentiate SWBV-1 consensus sequences from baboon 16986 (white) and baboon 19466 (gray) collected in 2014.

11 and 13 years (Table 2). However, an assessment of both males and females in a population with a high frequency of simian arterivirus infection will be required to more fully understand the transmission of these viruses.

The sequence diversity of MYBV-1 variants possibly reflects the evolution of MYBV-1 within persistently infected individuals or the complex migratory dynamics of the yellow baboon population in Mikumi (29). In contrast, the SWBV-1 sequences from

SNPRC olive baboons 16986 and 19466 were nearly identical. Interestingly, however, the sequences from baboons 16986 and 19466 obtained in 2014 were more similar to each other than either was to the sequence from baboon 16986 obtained in 2013. This observation is consistent with a very recent transmission of SWBV-1 from baboon 16986 to baboon 19466, which is supported by the timing of infection in these two animals, their cohoused status, and the lack of SWBV-1 viremia in the other baboons in this housing group. How baboon 16986 became infected remains to be determined, but our data suggest that SWBV-1 may be circulating at a low frequency in the population of >1,000 baboons kept at SNPRC that were not tested. The history of SWBV-1 infection at SNPRC is also difficult to determine. SNPRC (at Texas Biomed, formerly the Southwest Foundation for Biomedical Research) began importing (mostly olive) baboons from Kenya in the 1960s, and over 600 baboons were imported during the 1970s. However, given that some of the founding baboons at SNPRC were trapped near the olive baboon-yellow baboon hybrid zone in southern Kenya and that olive and hybrid olive/yellow baboons have frequent contact at SNPRC, it is possible that SWBV-1 was introduced to the SNPRC baboon colony by a SWBV-1-infected wild yellow (or hybrid olive/yellow) baboon that was brought into captivity (30).

The high frequency of MYBV-1 infection in the Mikumi baboons, the diversity of MYBV-1 variants, the high prevalence of MYBV-1 in older animals, the presence of SWBV-1 for several months in animal 16986, and the evolution of SWBV-1 sequences in this animal together strongly suggest that these infections are long lived and rapidly evolving in these hosts. Longitudinal sampling of SNPRC olive baboon 16986 showed that SWBV-1 has a high capacity for adaptation, acquiring 84 consensus-level nucleotide changes and 40 amino acid changes in 161 days. Twelve of the amino acid changes were in a region of ORF5, the putative major envelope glycoprotein that corresponds to the primary neutralizing antibody epitope of other arteriviruses (2). Amino acid changes in this region may enable simian arteriviruses to maintain persistent infection in an individual via escape of the adaptive immune response. Such mutations have unknown consequences for pathogenesis, tissue tropism, and cross-species transmission.

Although MYBV-1 and SWBV-1 have never been associated with an outbreak of SHF, viruses from many past SHF outbreaks were not culturable (12, 25) and viral sequences were never obtained. In accordance with the guidance provided in the *Guide for the Care and Use of Laboratory Animals* (31), SNPRC practices strict separation of African and Asian monkeys, and an outbreak of SHF has never occurred in its macaque colony. However, of all the African monkeys known to harbor simian arteriviruses, baboons are the most closely related to macaques, with both macaques and baboons belonging to the Papionini tribe (Cercopithecinae subfamily, Cercopithecidae family) of Old World monkeys (32). Given this phylogenetic relationship and the fact that many strains of simian arteriviruses from more distantly related species of African Old World monkeys are capable of causing febrile disease in macaques (4, 33, 34; our unpublished data), SWBV-1 and MYBV-1 should be considered pathogenic to macaques until proven otherwise. Experimental inoculation of macaques with SWBV-1 or MYBV-1 would be informative with regard to the epizootic potential of these viruses, although the infectivity of individual virus isolates could vary, given the range of genetic diversity within MYBV-1. Future work should focus on identifying additional arteriviruses responsible for past outbreaks of SHF, defining the primate species that support natural simian arterivirus infections, and characterizing the viral and host factors that facilitate cross-species transmission.

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