Discovery of a novel simian pegivirus in common marmosets (*Callithrix jacchus*) with lymphocytic enteritis

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Abstract

From 2010 to 2015, 73 common marmosets (*Callithrix jacchus*) housed at the Wisconsin National Primate Research Center (WNPRC) were diagnosed postmortem with lymphocytic enteritis. We used unbiased deep-sequencing to screen the blood of deceased enteritis-positive marmosets for the presence of RNA viruses. In five out of eight marmosets found to have lymphocytic enteritis, we discovered a novel pegivirus not present in ten subsequently deep-sequenced healthy marmosets. The novel virus, which we have named Southwest bike trail virus (SOBV), is most closely related to a strain of simian pegivirus A (68% nucleotide identity) that was previously isolated from a three-striped night monkey (*Aotus trivirgatus*). To determine the prevalence of this novel virus within the WNPRC marmoset colony, we screened 146 living animals and found an overall prevalence of 34% (50/146). Over the next four years, 85 of the 146 screened marmosets were examined histologically for lymphocytic enteritis. Out of these 85 animals, 27 SOBV-infected common marmosets had developed lymphocytic enteritis, compared to 42 uninfected common marmosets, indicating no association between this virus and development of enteritis (p=0.820). The novel pegivirus was also found in 2 of 32 (6.25%) healthy marmosets screened while in quarantine during the transfer from the New England Primate Research Center to the WNPRC.

Importance

Common marmosets (*Callithrix jacchus*) are a valuable model species. We discovered two variants of a novel pegivirus, which we named the Southwest bike trail virus (SOBV), in common marmosets which had postmortem histologic diagnosis of lymphocytic enteritis. We screened 146 live healthy marmosets in the Wisconsin National Primate Research Center colony and found 34% (50/146) of the animals were infected. SOBV was also present in 2 of 32 (6.25%) healthy marmosets from the New England Primate Research Center. These findings have implications for animal studies in which infection-free animals are desired, and they demonstrate the need for further investigations to increase understanding of this genus of viruses.

Introduction

Common marmosets (*Callithrix jacchus*) are a valuable model species due to their small body size, communal monogamous familial behavior, birth of hematopoietic chimeric litters, short parturition intervals, and status as members of a non-endangered primate species.[1-4] The utility of common marmosets in research has resulted in a recent increase in demand for these animals.[5] The Wisconsin National Primate Research Center (WNPRC) in Wisconsin, Madison, USA, houses a common marmoset colony typically consisting of about 240 common marmosets, which are used by researchers at the University of Wisconsin-Madison for groundbreaking research in neurological, neurobehavioral, and pharmacologic studies, and many others.[6-16]

From 2010 to 2015, 73 common marmosets housed at the WNPRC were euthanized due experimental end point, chronic, intractable diarrhea, or chronic severe weight loss, underwent necropsy with histology and were diagnosed with lymphocytic enteritis. Beyond the regrettable loss of animal life, common marmoset demise due to enteritis is harmful to both colony success and to the scientific studies to which these animals are assigned. Though lymphocytic enteritis is one of the most common causes of death in captive common marmosets,[17-22] the epizootic at WNPRC was associated with an unusually high disease incidence, prompting investigations into a possible infectious contributor. Unbiased deep-sequencing led to the discovery of two closely related variants of a novel pegivirus, most closely related to a variant of simian pegivirus A (SPgV-A) previously isolated from a three-striped night monkey (*Aotus trivirgatus*). This novel pegivirus was present in a subset of deceased common marmosets diagnosed postmortem with lymphocytic enteritis and not present in matched healthy controls. Pegiviruses, members of genus *Pegivirus (Amarillovirales*: *Flaviviridae)*, are ubiquitous in animal populations,[23-34] but their biological consequences are poorly understood. Given the importance of common marmosets as a model species and the disease burden caused by lymphocytic enteritis, we set out to characterize the possible link between these new viruses and the disease state.

Here, we report the discovery of two variants of a novel pegivirus in a captive common marmoset colony. We establish phylogenetic relationships with other known pegiviruses. Since this virus was discovered in common marmosets with lymphocytic enteritis and was absent in healthy controls, we measured the prevalence of the virus in the colony. We investigated the potential association between the virus and the occurrence of lymphocytic enteritis. We ultimately found no statistically significant association between infection status and disease. Still, we did find the virus to be highly prevalent (34%) in our colony, less prevalent (6.25%) in a comparison colony, and significantly associated with increasing age (p=0.03237). These findings have implications for animal studies in which infection-free animals are desired, and

83 they demonstrate the need for further investigations to increase understanding of this genus of
84 viruses.

85 Results

86 Captive common marmosets harbor a novel pegivirus

87 To examine the etiology of the unusually high rate of lymphocytic enteritis in deceased WNPRC
88 common marmosets, banked plasma samples from eight common marmosets diagnosed with
89 lymphocytic enteritis and from 10 healthy, live common marmosets to be used as controls were
90 screened by deep sequencing for the presence of DNA and RNA viruses. Five of eight
91 deceased marmosets with lymphocytic enteritis were infected with the previously undocumented
92 pegivirus. We propose this novel virus (BioProject accession number PRJNA613737), be
93 formally named the Southwest bike trail virus (SOBV). The ten healthy common marmoset
94 controls were found to be negative for this virus.
95
96 SOBV consists of a 9.8-kb-long contig that is highly similar to the genome of simian pegivirus A
97 (SPgV A) trivirgatus, a simian pegivirus previously discovered in a three-striped night monkey
98 (*Aotus trivirgatus*)[26] (Figure 1), with 68% nucleotide identity across the coding sequence when
99 aligned using ClustalW with an IUB cost matrix (gap extension cost, 6.66; gap open cost,
100 15.00). Four of the five marmosets infected with SOBV had variants of the virus having 98-99%
101 sequence identity, while one marmoset was infected with a variant with 88% sequence identity
102 to the others. We have named these variants SOBV-1 and -2.
103
104 Pairwise comparisons of nucleotide identity across the entire coding region further illustrate the
105 similarity of SOBV-1 and SOBV-2 and the divergence between these novel virus strains and the
106 next most closely-related viruses (Figure 2, Figure 3), most of which were simian pegiviruses.
107 Interestingly, a pegivirus isolate found in a bat, BPgV 737,[35] also shared a high degree of
108 similarity with the novel pegivirus and with similar simian pegiviruses.
109

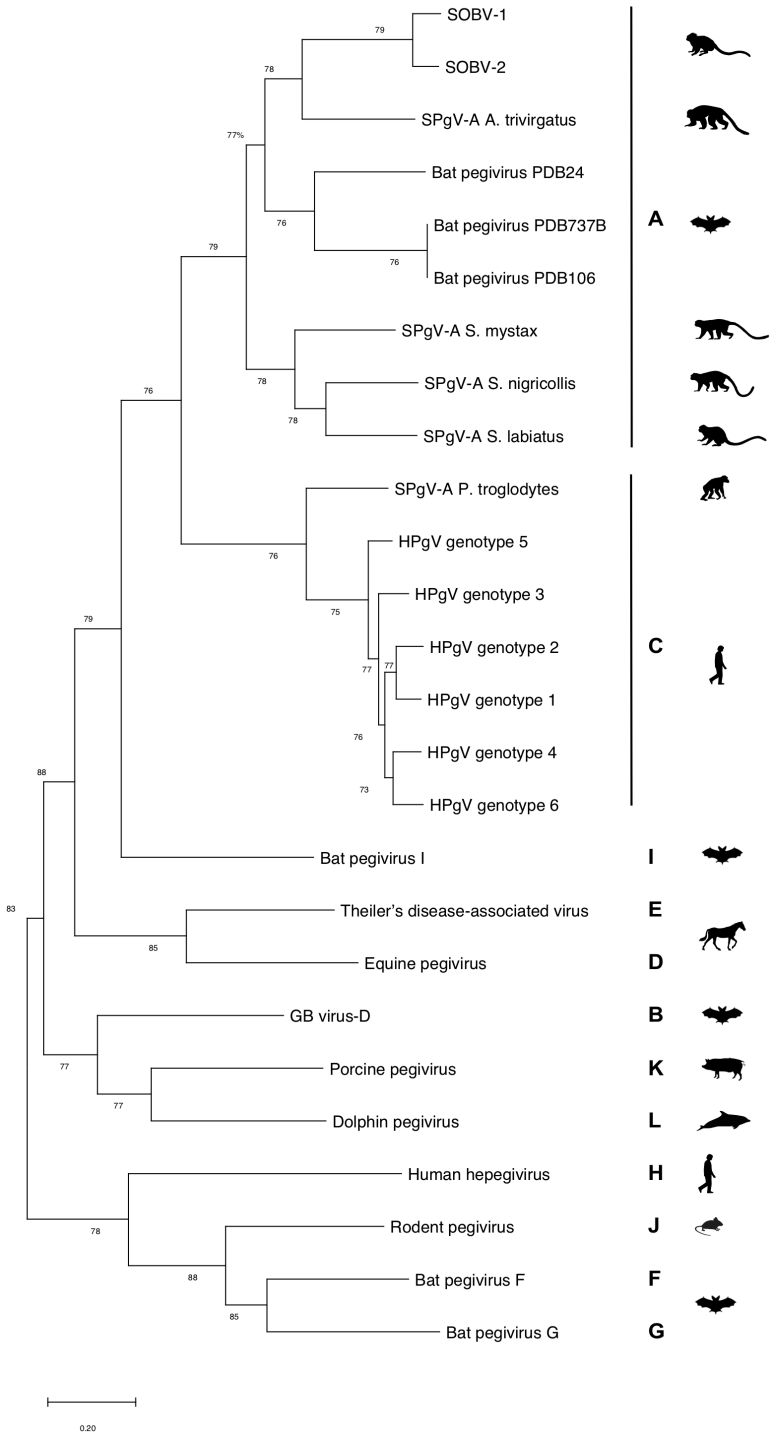


Figure 1. A phylogenetic tree of newly discovered pegivirus Southwest bike trail virus (SOBV strains 1 and 2) shows it is most closely related to pegiviruses found in other New World monkeys. We generated maximum likelihood trees using MEGA6.06 (1,000 bootstrap replicates, GTR+I+γ model) from codon-based alignments (via MAFFT); Bootstrap values of less than 70 are omitted.
Abbreviations: HPgV = human pegivirus; SPgV = simian pegivirus; GBV-C = G.B. virus C; BPgV = bat pegivirus; EqPgV = equine pegivirus; HePegi = hepegivirus; RPgV = rodent pegivirus

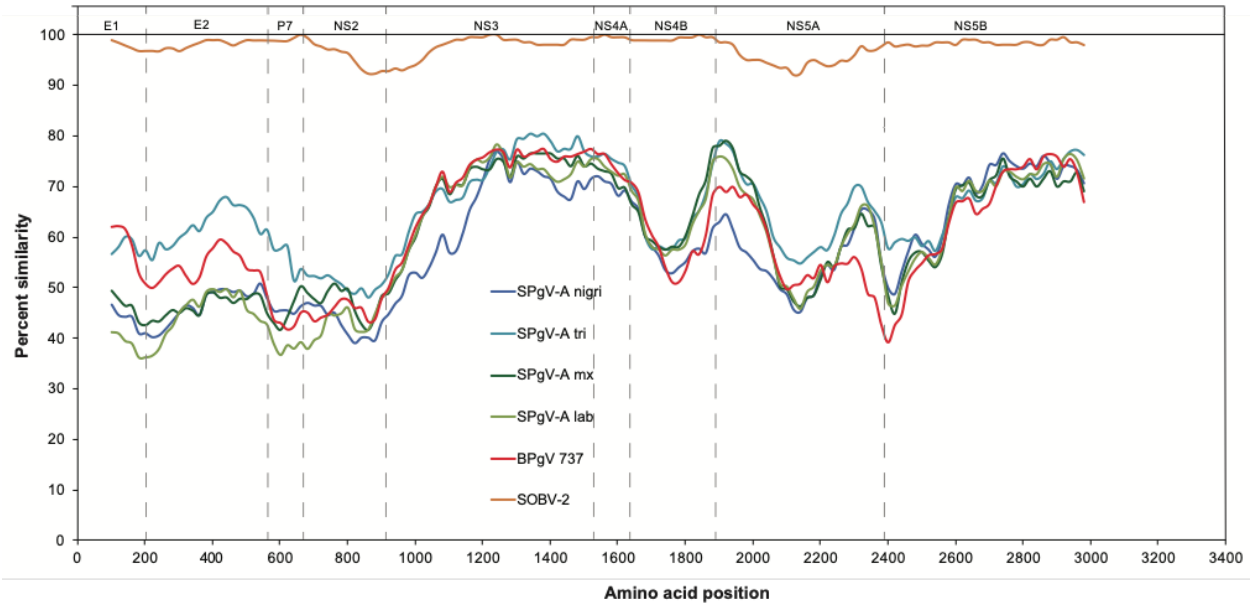


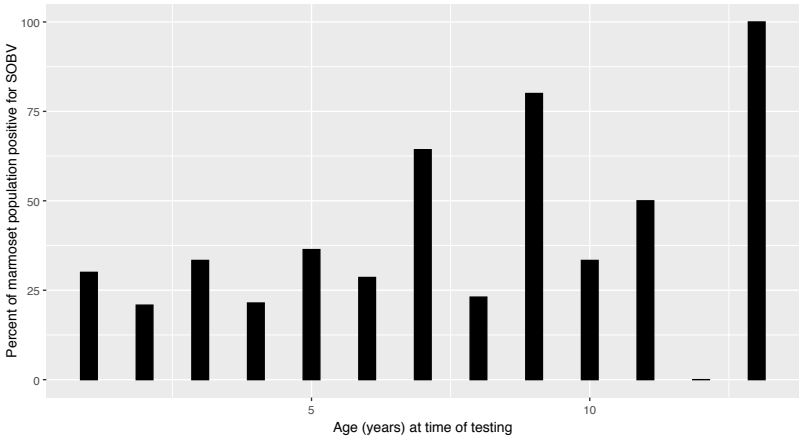
Figure 2. Sliding window similarity plots[36] show the relatedness of the amino acid sequences of SOBV-2 and other closely related pegiviruses to SOBV-1. Dashed vertical lines indicate the putative approximate start positions of inferred viral proteins, from left to right: E1, E2, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.[37]
Abbreviations: SPgV-A nigri = GBV-A-like virus recovered from *Saguinus nigricollis*; SPgV-A tri = GBV-A-like virus recovered from *Aotus trivirgatus*; SPgV-A mx = GBV-A-like virus recovered from *Saguinus mystax*; SPgV-A lab = GBV-A-like virus recovered from *Saguinus labiatus*; BPgV = bat pegivirus recovered from *Eidolon helvum*

	Pegivirus L - Dolphin pegivirus (MK059751)	Pegivirus C - Human pegivirus genotype 2 (U44402)	Pegivirus A - GB virus-A; simian pegivirus (U22303)	SOBV-1	SOBV-2	Pegivirus K - Porcine pegivirus (KU351669)	Pegivirus B - GB virus D (GU566734)	Pegivirus I - Bag pegivirus I (KC796088)	Pegivirus D - Theiler's disease associated virus (KC145265)	Pegivirus E - Equine pegivirus (KC410872)	Pegivirus H - Human hepegivirus; human pegivirus 2 (KT439329)	Pegivirus G - Bat pegivirus G (KC796076)	Pegivirus F - Bat pegivirus F (KC796080)	Pegivirus J - Rodent pegivirus (KC815311)
Pegivirus L - Dolphin pegivirus (MK059751)	100.0													
Pegivirus C - Human pegivirus genotype 2 (U44402)	35.1	100.0												
Pegivirus A - GB virus-A; simian pegivirus (U22303)	34.8	43.8	100.0											
SOBV-1	35.3	43.9	57.9	100.0										
SOBV-2	35.4	43.7	57.8	97.6	100.0									
Pegivirus K - Porcine pegivirus (KU351669)	35.7	35.9	35.7	36.3	36.2	100.0								
Pegivirus B - GB virus D (GU566734)	36.6	36.0	35.9	36.4	36.2	36.6	100.0							
Pegivirus I - Bag pegivirus I (KC796088)	37.5	37.8	37.2	37.8	37.5	36.8	40.9	100.0						
Pegivirus D - Theiler's disease associated virus (KC145265)	36.0	33.2	32.2	32.9	32.8	33.8	35.8	36.0	100.0					
Pegivirus E - Equine pegivirus (KC410872)	32.9	30.2	30.4	30.5	30.6	31.4	32.6	33.1	39.1	100.0				
Pegivirus H - Human hepegivirus; human pegivirus 2 (KT439329)	26.2	25.4	26.2	25.6	25.4	26.2	25.2	25.5	24.7	23.4	100.0			
Pegivirus G - Bat pegivirus G (KC796076)	28.9	26.2	27.5	27.0	27.0	27.2	28.6	27.2	28.4	27.5	30.1	100.0		
Pegivirus F - Bat pegivirus F (KC796080)	30.1	28.4	27.8	28.1	28.0	29.2	29.3	28.4	28.8	28.6	31.8	42.8	100.0	
Pegivirus J - Rodent pegivirus (KC815311)	29.4	27.0	28.0	27.5	27.4	28.2	29.0	28.0	29.6	28.6	30.8	40.4	44.6	100.0

Figure 3. Sequence identity matrix based on amino acid alignment of the newly discovered SOBV-1 and SOBV-2 (red box) compared to members of the 11 recognized pegivirus species and of one* proposed species.[37]
*The classification of dolphin into species "Pegivirus L" has been suggested.[38]

Novel pegivirus infects up to 35% of a captive common marmoset colony

Having identified the novel pegivirus, we sought to determine its prevalence within the WNPRC common marmoset colony. We developed an RT-PCR assay to detect a conserved region of the putative helicase protein of SOBV and used this to screen plasma collected from 146 healthy live common marmosets in the WNPRC colony, confirming results through deep-sequencing of the amplicons. At the time of the initial screening in March–April 2014, 50 of the 146 (34.25%) healthy screened animals tested positive for SOBV. Nineteen of 60 females (31.67%) and 31 of 86 males (36.05%) tested positive at the time of screening. Sex was not associated with the likelihood of infection using univariate logistic regression (p=0.5834). Age at the time of screening was mildly associated with the likelihood of infection (p=0.03237), with the likelihood of positivity increasing with age (Figure 4).



WNPRC common marmosets screened for SOBV			
Age (years) at time of screening	**Total number common marmosets**	**Number common marmosets infected**	**Number common marmosets non-infected**
1	20	6	14
2	24	5	19
3	18	6	12
4	14	3	11
5	22	8	14
6	7	2	5
7	14	9	5
8	13	3	10
9	5	4	1
10	6	2	4
11	2	1	1
12	0	0	0
13	1	1	0

Figure 4. Prevalence of infection with Southwest bike trail virus (SOBV) in common marmosets at the WNPRC increases with age. One hundred forty-six live, healthy common marmosets in the WNPRC captive common marmoset colony were screened for SOBV using RT-PCR and deep sequencing methods. The likelihood of infection with these viruses was significantly statistically associated with increasing age (p=0.03237) using univariate logistic regression.

In November 2014, 82 common marmosets were transferred from the New England Primate Research Center (NEPRC) to the WNPRC. Samples from 32 NEPRC animals were collected while the animals were in quarantine. Two (6.25%) were found to be infected with SOBV when screened by RT-PCR in quarantine.

Presence of novel pegivirus is not statistically significantly associated with common marmoset lymphocytic enteritis

We next sought to determine whether infected animals were more likely to develop lymphocytic enteritis. Typical enteric architecture consists of slender, often branching villi, with short intestinal glands, small numbers of lymphocytes in the lamina propria, and prominent B cell aggregates dispersed throughout the length of the intestines (Figure 5, control). Lymphocytic enteritis was diagnosed as a disruption of this architecture, with lymphocytic infiltration that expands the lamina propria, resulting in widening and shortening of villi and hyperplasia of crypt epithelium (Figure 5, E1-E3). Cases varied in severity, with mild cases showing only slight

expansion of the lamina propria and advanced cases showing complete loss of villus architecture due to infiltration of the lamina propria with large numbers of CD3-positive lymphocytes. Eighty-five of the live WNPRC animals initially screened for SOBV in 2014 were euthanized for experimental end points or clinical illness between their screening and May 3, 2019. Sixty-nine (81.18%) of these animals were diagnosed by postmortem histological analysis with lymphocytic enteritis of the small intestine, or large intestine, or both. Two animals were removed from this analysis due to confounding factors (one animal had severe tissue autolysis, and the other animal had B cell lymphoma of the small and large intestines).

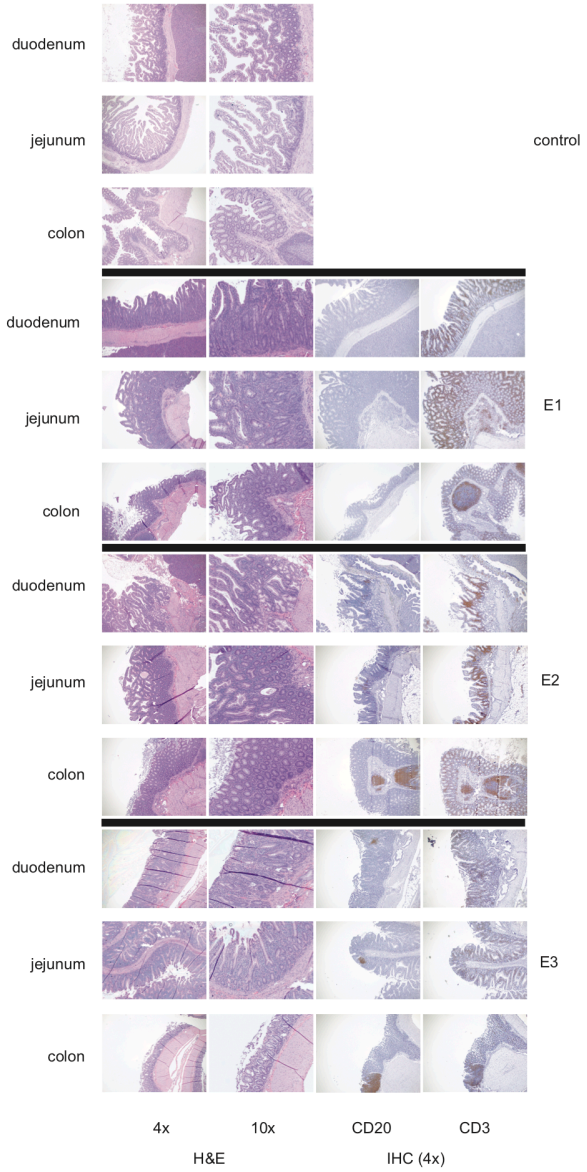


Figure 5. Representative photomicrographs show lymphocytic enteritis distorting normal intestinal structures in the duodenum, jejunum, and colon of common marmosets. Histology performed upon intestinal samples from 85 common marmosets. All intestinal sections were stained with hematoxylin and eosin (H&E). Intestinal sections from animals with enteritis were stained using B cell-specific and T cell-specific staining procedures (immunohistochemistry) with monoclonal antibodies to CD20 or CD79 (B cell markers) and CD3 (T cell marker), respectively.

Pegivirus infection was not found to be associated with an increased likelihood of developing lymphocytic enteritis in the large intestine (p=0.196), small intestines (p=0.779), both small and large intestines (p=0.0798), or either the large or small intestines (p=0.820) (Figure 6).

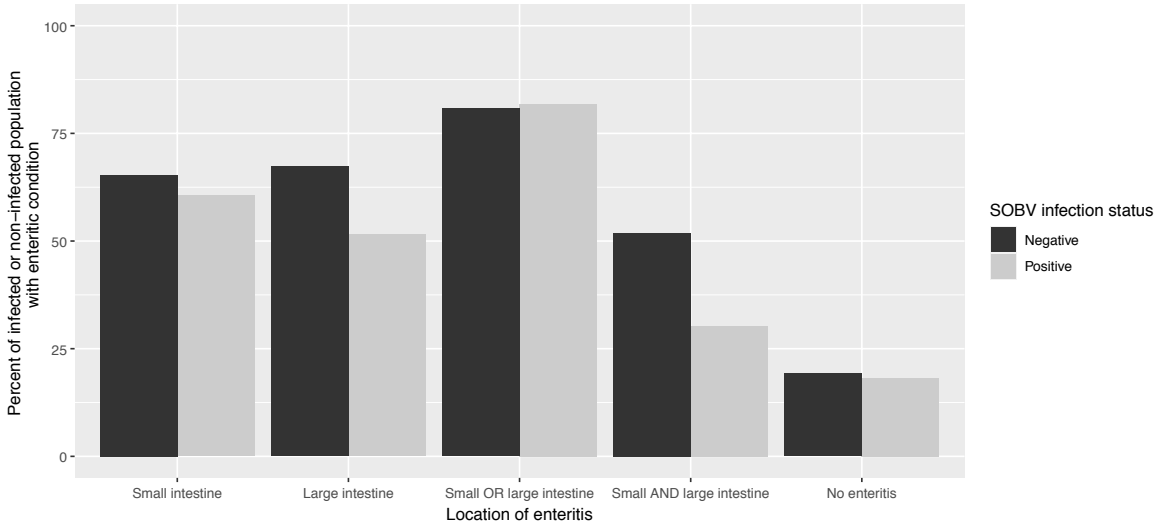


Figure 6. Infection with Southwest bike trail virus (SOBV) is not associated with the likelihood of developing lymphocytic enteritis. Eighty-five common marmosets at the WNPRC, which had been previously screened for SOBV by RT-PCR or deep-sequencing of plasma samples, were examined postmortem for histological evidence of lymphocytic enteritis. Pegivirus infection was not found to be associated with an increased likelihood of developing lymphocytic enteritis in the large intestine (p=0.196), small intestines (p=0.779), both small and large intestines (p=0.0798), or either the large or small intestines (p=0.820) using univariate logistic regression.

Discussion

We describe the discovery of a novel simian pegivirus first identified in common marmosets diagnosed with lymphocytic enteritis. We show this pegivirus was prevalent in our colony during a period of increased incidence of lymphocytic enteritis and that it was less prevalent in a similar, healthy colony. The novel virus was not significantly associated with the likelihood of developing lymphocytic enteritis, though prevalence of the virus increased with increasing age in the common marmoset. With an average prevalence of 34%, SOBV appears common throughout the WNPRC common marmoset colony.

Pegiviruses, the members of genus *Pegivirus (Amarillovirales*: *Flaviviridae)*, have single-stranded, positive-sense RNA genomes and produce enveloped virions.[39] The first members of the genus were identified about twenty years ago,[40] and since that time pegiviruses have been found in many animal populations.[23-34,38] Pegiviruses have never been shown to be causative agents of any disease or alteration in physiology,[41-58] though human pegivirus (HPgV; species *Pegivirus A*) has been linked to incidence of various types of lymphoma,[59-66] though this remains controversial.[67-71] HPgV has, intriguingly, been linked to improved outcomes in coinfection with HIV-1[72-88] and Ebola virus,[87] leading some to propose the use of HPgV as a biotherapy.[89]

It is not known whether these viruses are unique to the captive common marmoset populations or whether they are also present in wild populations.[90] Other pegiviruses have been discovered

in wild common marmosets in the 1990s,[91] but their prevalence has never been examined. The prevalence of these viruses in our captive common marmoset population was quite high compared to the best-studied pegivirus, HPgV, which is found in about 1–4% of human populations.[92-99] The novel pegiviruses were most similar to a pegivirus discovered in a three-striped night monkey (*Aotus trivirgatus*),[26] a monkey used in malaria research at other primate research facilities.[100-102] Interestingly, SOBV was highly similar to several variants of a bat pegivirus isolated from African straw-coloured fruit bats (*Eidolon helvum*). This was somewhat surprising given that the bat pegivirus isolates fall into Pegivirus species B. However, bat pegiviruses have been found to be prevalent (up to 5%) among wild bats around the globe and to span the full diversity of pegivirus species.[35] Our findings suggest common ancestry or an otherwise close relationship between these variants.

The routes of transmission of SOBV and of other simian pegiviruses have not been examined. Human pegivirus transmission has been extensively studied and is known to be occur efficiently through blood products or dialysis,[43,103-107] intravenous drug use and needle sticks,[104,108-110] sexual intercourse,[104,108,111,112] and from mother to infant.[104,113-117] In humans, HPgV can replicate at high titers in a host for more than a decade[103,118,119] with an unusually low mutation rate compared to other RNA viruses,[120,121] with the host's production of anti-pegivirus antibodies resulting in protective sterilizing immunity once the immune system recognizes and clears the virus.[73,119] The duration of pegivirus infections in humans indicates SOBV may be similarly long-lasting commensals of common marmosets, potentially impacting common marmoset lives in unknown ways. Defining mechanisms of transmission will be important in preventing infection and thereby allowing the study of these viruses' effects.

The high prevalence of this virus at the WNPRC raises essential considerations about potential effects on common marmoset experiments. Facilities working with common marmosets should prescreen the animals to establish the pegivirus infection status of animals in research. Future investigations, perhaps involving the isolation of common marmosets for years at a time to follow the natural history of long-term pegivirus infection in these animals, could examine the long-term effects of infecting common marmosets with SOBV.

This study has several limitations. First, this study was observational in nature and could not examine a causal link between viral positivity and the development of enteritis. Definitive establishment of causation would require demonstrating that animals infected experimentally develop lymphocytic enteritis. Second, not all of the animals initially screened were deceased at the time of this analysis and future necropsies of these animals may contribute additional data concerning the likelihood of enteritis development. Third, we were unable to culture this virus; difficulties in culturing pegiviruses have been documented.[37,122] Finally, some animals in this study may have cleared the virus before the samples we tested were collected. Consequently, these animals could have been mistakenly classified as virus-naïve; others may have acquired the virus after our initial screening. Serial testing of animals would have counteracted this problem; however, this was a retrospective study, so serial testing was impossible. Development of a SOBV-specific ELISA or other serodiagnosis tools would enable deeper appropriate analyses of SOBV infection rates both prospectively and retrospectively.

In summary, this work describes the discovery of a novel simian pegivirus and investigates its relationship with a widespread and devastating cause of common marmoset mortality. Our study lays the groundwork for the future development of a nonhuman primate model system using this natural infection as a potential model for studying the mechanisms of these enigmatic viruses and providing a greater understanding of their genus as a whole.

Materials and methods

Animals

All animals in this study were common marmosets (*Callithrix jacchus* Linnaeus, 1758) housed at the Wisconsin National Primate Research Center (WNPRC) in Madison, WI, USA. The common marmoset colony at the WNPRC was established in 1960. The original animals were imported from northeastern Brazil, with the final importation occurring in the early 1970s. The average yearly population of the colony each year from 2010 to 2019 was approximately 240 animals, all of which were born in captivity. WNPRC animals screened were 41% (60 animals) female and 59% (86 animals) male. Age at the time of screening ranged from 0.82–12.82 years (mean 4.65+/-2.83 years, median 4.26 years).

The New England Primate Research Center (NEPRC), Southborough, MA, USA, was closed in 2015, resulting in a transfer of 82 common marmosets to WNPRC before closure in November 2014. Plasma samples were collected from these animals upon their arrival at WNPRC (November–December 2014) while quarantined in a separate building and location from the WNPRC marmoset colony. In the population initially from the NEPRC, 45 (55%) of the screened animals were female, and 37 (45%) were male. Age at the time of screening ranged from 0.65–10.66 years (mean 3.74+/-2.60, median 2.51 years) in this population.

Ethics

All common marmosets were cared for by WNPRC staff according to the regulations and guidelines outlined in the National Research Council's Guide for the Care and Use of Laboratory Animals, the Animal Welfare Act, the Public Health Service Policy on the Humane Care and Use of Laboratory Animals, and the recommendations of the Weatherall report (https://royalsociety.org/topics-policy/publications/2006/weatherall-report/). Per WNPRC standard operating procedures for animals assigned to protocols involving the experimental inoculation of infectious pathogens, environmental enhancement included constant visual, auditory, and olfactory contact with conspecifics, the provision of feeding devices that inspire foraging behavior, the provision and rotation of novel manipulanda, and enclosure furniture (i.e., perches, shelves). The common marmosets were housed socially in enclosures measuring 0.6m D × 0.9m W × 1.8 m H or 0.6m D × 1.2m W × 1.8 m H. The WNPRC maintains an exemption from the USDA for these enclosures as they do not meet the Animal Welfare Act regulations for floor space but greatly exceed height requirements as the species are arboreal. This study was approved by the University of Wisconsin-Madison College of Letters and Sciences and Vice Chancellor for Research and Graduate Education Centers Institutional Animal Care and Use Committee (animal protocol numbers G005401 and G005443).

Unbiased deep-sequencing

Samples from 18 common marmosets (8 deceased common marmosets diagnosed with lymphocytic enteritis through necropsy and 10 live, healthy common marmosets) from the WNPRC and 12 common marmosets (all live and healthy) from the NEPRC were screened for the presence of viruses using unbiased deep-sequencing. The live WNPRC common marmosets and the live NEPRC common marmosets were selected randomly for deep-sequencing.

313 DNA and RNA were isolated from plasma. Common marmoset plasma (1 ml/animal) was
314 centrifuged at 5,000 x *g* for 5 min at 4°C. Supernatants were removed and filtered through a
315 0.45-µm filter, then centrifuged at maximum speed (20,817 g) for 5 min at 4°C. Supernatants
316 were removed and incubated for 90 min at 37°C with a DNA/RNA digest cocktail consisting of 4
317 µl DNAfree DNAse (0.04 U/µl; Ambion, Austin, TX, USA), 6 µl Baseline Zero DNAse (0.1 U/µl,
318 Epicentre Technologies, Madison, WI, USA), 1 µl Benzonase (1 U/µl, Sigma-Adrich, St. Louis,
319 MO, USA), and 12 µl DNAse 10x buffer. Viral nucleic acids were then isolated using the Qiagen
320 QIAamp MinElute Virus Spin Kit without the use of AW1 buffer or carrier RNA (Qiagen,
321 Valencia, CA, USA). Random hexamers were used to prime cDNA synthesis (Life
322 Technologies, Grand Island, NY, USA), followed by DNA purification using Ampure XP beads,
323 as previously described[123,124]. Deep-sequencing libraries were prepared using the Nextera XT
324 DNA Library Prep Kit (Illumina, San Diego, CA, USA) and sequenced on MiSeq (Illumina).

325 Viral sequence and phylogenetic analysis

326 Sequence data were analyzed using CLC Genomics Workbench 5.5 (CLC bio, Aarhus,
327 Denmark). Low-quality reads (Phred <Q30) and short reads (<100 bp) were removed with CLC
328 Genomics Workbench 7.1 (CLC bio, Aarhus, Denmark), and the remaining reads were
329 assembled *de novo* using the MEGAHIT assembler. Assembled contiguous sequences
330 (contigs) and singleton reads were queried against GenBank database nt using the basic local
331 alignment search tools blastn. Nucleotide sequences were codon aligned individually for all
332 known pegiviruses with complete genomes using ClustalW in the alignment editor program in
333 MEGA v5.10 and edited manually. The best-fitting distance model of nucleotide substitution for
334 each alignment was inferred using the maximum likelihood (ML) method with goodness of fit
335 measured by the Bayesian information criterion in MEGA6.06. The best-fitting nucleotide
336 substitution model for the phylogenetic alignments was inferred to be the GTR model with
337 discrete gamma and invariant among-site rate variation.
338
339 Protein family analysis and putative protein predictions were performed using Pfam
340 (http://pfam.xfam.org/). The nucleotide similarity of the novel pegivirus with related pegivirus
341 lineages was determined across the polyprotein using SimPlot v3.5.1[36] following TranslatorX
342 alignment (MAAFT) without Gblocks cleaning.

343 Screening for SOBV by RT-PCR

344 Plasma samples from 136 healthy WNPRC common marmosets were screened specifically for
345 SOBV by RT-PCR. Twenty plasma samples collected from NEPRC animals were likewise
346 screened by RT-PCR as they contained less than 100 µl.
347
348 Screening of the 136 animals was performed with samples from animals positive for SOBV by
349 deep-sequencing as positive controls. RNA was isolated from 200–500 µl of plasma using the
350 QIAamp Viral RNA Mini Kit (Qiagen). A primer set (forward primer:
351 GGTGGTCCACGAGTGATGA; reverse primer: AGGTACCGCCTGGGGTTAG) targeting a
352 region of the viral helicase which was conserved among the animals initially positive by deep
353 sequencing was designed, resulting in a 615-bp amplicon. Viral RNA was reverse-transcribed
354 and amplified using the SuperScript III High Fidelity One-Step RT-PCR kit (Invitrogen, Life
355 Technologies, Carlsbad, CA, USA). The reverse transcription-PCR conditions were as follows:
356 50°C for 30 min; 94°C for 2 min; 40 cycles of 94°C for 15 s, 55°C for 30 s, and 68°C for 1 min;
357 and 68°C for 5 min. Following PCR, amplicons were purified from excised gel slices (1%
358 agarose) using the Qiagen MinElute Gel Extraction kit (Qiagen). Each amplicon was quantified

using Quant-IT HS reagents (Invitrogen), and approximately 1 ng of each was used in a tagmentation reaction with the Nextera XT DNA Library Prep Kit. Final libraries representing each amplicon were characterized for average length using a DNA high sensitivity chip on a 2100 bioanalyzer (Agilent Technologies, Loveland, CO, USA) and quantitated with Quant-IT HS reagents. Libraries were sequenced on a MiSeq.

Postmortem diagnosis of lymphocytic enteritis

All animals humanely euthanized or found dead at the WNPRC undergo complete post mortem examination (necropsy) with histology. Standard hematoxylin and eosin (H&E) stains are used for histological examinations to determine whether normal tissue architecture and cellular populations are present. In this study, immunohistochemical (IHC) CD3 and CD20 or CD79 staining was performed on any samples with lymphocytic enteritis to differentiate lymphocyte populations (primarily T cells, B cells, or mixed T and B cells). Diagnosis of T-cell rich lymphocytic enteritis was based on abnormal villus architecture of the intestines and IHC staining.[21,125] If confounding factors hampered diagnosis (e.g., severe B cell lymphoma or autolysis), the animal was removed from the analysis.

Statistical analysis

We used univariate logistic regression to evaluate the associations of SOBV viremia with enteritis risk. Analyses were repeated to determine association with enteritis in small bowel only, colon only, both small bowel and colon, and either small bowel or colon. All reported P-values are two-sided and $P<0.05$ was used to define statistical significance. Statistical analyses were conducted using R version 3.6.3 in RStudio version 1.1.383.

Data accessibility and management

Metagenomic sequencing data have been deposited in the Sequence Read Archive (SRA) under Bioproject PRJNA613737. Derived data, analysis pipelines, and figures have been made available for easy replication of these results at a publicly-accessible GitHub (https://github.com/aheffron/SPgVwnprc_in_marmosets).

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