<https://helda.helsinki.fi>

Sera of Peruvians with fever of unknown origins include viral nucleic acids from non-vertebrate hosts

Phan, Tung Gia

2018-02

Phan , T G , del Valle Mendoza , J , Sadeghi , M , Altan , E , Deng , X & Delwart , E 2018 , '
Sera of Peruvians with fever of unknown origins include viral nucleic acids from
non-vertebrate hosts ' , Virus Genes , vol. 54 , no. 1 , pp. 33-40 . <https://doi.org/10.1007/s11262-017-1514-3>

<http://hdl.handle.net/10138/301081>

<https://doi.org/10.1007/s11262-017-1514-3>

publishedVersion

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.

Sera of Peruvians with fever of unknown origins include viral nucleic acids from non-vertebrate hosts

Tung Gia Phan^{1,2} · Juana del Valle Mendoza^{3,4} · Mohammadreza Sadeghi^{1,2,5} · Eda Altan^{1,2} · Xutao Deng¹ · Eric Delwart^{1,2}

Received: 2 June 2017 / Accepted: 6 October 2017 / Published online: 17 October 2017
© Springer Science+Business Media, LLC 2017

Abstract Serum samples collected from 88 Peruvians with unexplained fever were analyzed for viral sequences using metagenomics. Nucleic acids of anelloviruses, pegivirus A (GBV-C), HIV, Dengue virus, and Oropouche virus were detected. We also characterized from two sera the RNA genomes of new species of partitivirus and dicistrovirus belonging to viral families known to infect fungi or arthropod, respectively. Genomic DNA of a putative fungal cellular host could be PCR amplified from the partitivirus-containing serum sample. The detection in human serum of nucleic acids from viral families not known to infect vertebrates may indicate contamination during sample collection and aliquoting or human infection by their presumed cellular host, here a fungus. The role, if any, of the non-vertebrate infecting viruses detected in serum in inducing fever is unknown.

Keywords Dicistrovirus · Partitivirus · Fever · Metagenomics · Sera

Edited by Detlev H. Kruger.

✉ Eric Delwart
delwarte@medicine.ucsf.edu

¹ Blood Systems Research Institute, San Francisco, CA 94118, USA

² Department of Laboratory Medicine, University of California at San Francisco, San Francisco, CA 94118, USA

³ School of Medicine, Research and Innovation Centre of the Faculty of Health Sciences, Universidad Peruana de Ciencias Aplicadas, Lima, Peru

⁴ Instituto de Investigación Nutricional, Lima, Peru

⁵ Department of Virology, University of Helsinki, Helsinki, Finland

Introduction

Fever can often remain unexplained despite testing for specific human pathogens. Fever of unknown origin may reflect infections with known but untested pathogens, still uncharacterized and possibly emerging infectious agents, or be due to non-infectious causes. Testing of blood samples from such unexplained febrile cases has revealed the presence of human viruses including anelloviruses and pegivirus A (GBV-C), hepatitis A, HHV-6, Dengue 2, and parvovirus B19 in febrile patients from Nicaragua [1] and the same viruses plus HIV, HBV, rhinovirus C, Merkel cell polyomavirus, and the enteric norovirus and rotavirus in febrile Kenyans [2]. Plasma from febrile Nigerians yielded Dengue 2 virus, Lassa virus, pegivirus A, herpesvirus 4/5/8, HIV, HBV, HCV, and two novel rhabdoviruses detected in febrile as well as healthy individuals [3]. This study indicated that sequencing blood from febrile patients can result in the identification of previously unknown and possibly emerging viruses.

Here, we analyzed human sera from individuals from Peru with unexplained fever and identified both known commensal and pathogenic human viruses as well as viruses whose tropism are not known to include vertebrates.

Materials and methods

Clinical samples

Serum samples were collected from patients with unexplained fever in Peru. These samples had pre-tested negative for dengue virus [4], Zika virus [5], chikungunya virus [6], Oropouche virus [7], Mayaro virus [8], Bartonella [9], and Leptospira [10] bacteria. The self-defined inclusion

criteria were patients who presented in the outpatient clinics with acute, undifferentiated, febrile illness (greater than or equal to 38 °C axillar temperature for 7 days of duration or less) along with one or more of the following symptoms: headache, muscle pain, ocular and/or joint pain, nausea, vomiting, sore throat, cough, rhinorrhea, difficulty breathing, diarrhea, jaundice, generalized fatigue, and cough. Exclusion criteria were if an identifiable focus of infection was diagnosed, such as sinusitis, pneumonia, acute otitis media, or other acute infections. This study was approved by the Research Ethics Board of the Hospital Regional de Cajamarca, Peru. A written informed consent was signed before enrollment; for participants under 18-year old, the informed consent was signed by parents or children caregivers before enrollment.

Viral metagenomics

A total of 88 serum samples from different patients were first analyzed in pools of 4–5 samples using a viral metagenomic approach. Each pool was clarified by 15,000×g centrifugation for 10 min, and then filtered through a 0.45-μm filter (Millipore). The filtrate was treated with a mixture of nuclease enzymes to digest unprotected nucleic acids and also to deplete ribosomal RNA [11]. Viral nucleic acids were extracted using a Maxwell 16 automated extractor (Promega). cDNA and DNA were then generated by using random RT-PCR followed by the use of the Nextera XT Sample Preparation Kit (Illumina) to construct a DNA library [11]. An Illumina MiSeq run of 250 bases paired-end reads was generated whose data were deposited in GenBank's short read archive (SRP102377). After quality control removal of duplicate reads and reads shorter than 50 bases, the VecScreen program was used to remove Illumina primers potentially missed by Illumina's default primer removal. Viral sequences were then identified through translated protein sequence similarity search (BLASTx) to all annotated viral proteins available in GenBank's viral RefSeq database.

Genome acquisition of new RNA viruses

Complete genomes of dicistrovirus (GenBank KY973643) and partitivirus (GenBank KY973644 and KY973645) were recovered by de novo assembly using the Ensemble program integrating the sequential use of de Bruijn graph (DBG) and overlap-layout-consensus assemblers (OLC) with a partitioned sub-assembly approach [12]. Putative ORFs in the new genomes were identified using the NCBI ORF finder. All alignments and phylogenetic analyses were based on in silico translated amino acid sequences. Sequence alignment was performed using CLUSTAL X [13]. Sequence identity was determined using BioEdit. Phylogenetic trees with bootstrap resampling of the alignment datasets were generated

using the maximum likelihood method with the WAG model that was tested and recommended by the program MEGA version 6 [14]. Bootstrap values (based on 100 replicates) for each node are shown if > 70%.

PCR assays for dicistrovirus and partitivirus

In order to identify the individual sample within the pools that contained dicistrovirus or partitivirus viral RNA initially detected by deep sequencing, two sets of PCR primers were used for nested RT-PCR assays with primers designed based on the short read sequence data. For the RT step, viral nucleic acids were added to a reagent mixture consisting of first-strand buffer (Invitrogen), dNTP (Thermo Scientific), superscript reverse transcriptase III (Invitrogen), random primer, and RNase inhibitor (Thermo Scientific). The RT step was carried out at 25 °C for 5 min, 50 °C for 1 h, 70 °C for 15 min, and then the mixture was held at 4 °C.

For dicistrovirus, primers PEDiV-F1 (5'-⁶⁸¹CAC CCC GTT GCA TTT GTT CGC TCT⁷⁰⁴-3') and PEDiV-R1 (5'-⁷¹⁵TCA CAG TCT CCT TCA ACA CAG AAA TCC AT⁷⁴⁴-3') were used for the first round of PCR, and primers PEDiV-F2 (5'-¹¹⁷³AAG TGA ACA TAG TTG ATG AAA GAA CCC CGA¹¹⁹⁷-3') and PEDiV-R2 (5'-¹²¹²GCA TAG CAA CGT CCA TCC TCC TAC A¹²⁴⁰-3') for the second round of PCR. The PCR conditions in these two assays were 95 °C for 5 min, 35 cycles 95 °C for 30 s, 55 °C (for the first or second round) for 30 s, and 72 °C for 1 min, a final extension at 72 °C for 10 min, resulting in an expected amplicon of 483 bp for dicistrovirus.

For partitivirus, segment-2 primers PEPartiV-F1 (5'-¹⁶⁷CAA CTG GAA ATA CAG CAT CGT CTG A¹⁹¹-3') and PEPartiV-R1 (5'-²⁰⁵GAG TCG GGA GCG GGT ATC AAG T²²⁵-3') were used for the first round of PCR, and primers PEPartiV-F2 (5'-⁵⁹⁶ACC CTT GAC CTT CCC AAC CGA⁶¹⁷-3') and PEPartiV-R2 (5'-⁶²¹TGA GGA AGC ATG GAT TGA AGG A⁶⁴²-3') for the second round of PCR. The PCR conditions in these two assays were 95 °C for 5 min, 35 cycles 95 °C for 30 s, 52 °C (for the first or second round) for 30 s, and 72 °C for 1 min, a final extension at 72 °C for 10 min, resulting in an expected amplicon of 413 bp for partitivirus.

PCR assay for fungi ITS

PCRs with two sets of universal primers for fungal ITS-1 and ITS-2 amplification were performed [15, 16]. Primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS2 (5'-GCT GCG TTC TTC ATC GAT GC-3') should yield a 145–695 bp amplicon. Primers 5.8S-Fun (5'-AAC TTT YRR CAA YGG ATC WCT-3') and ITS4-Fun (5'-AGC CTC CGC TTA TTG ATA TGC TTA ART-3') should yield a 267–511 bp amplicon. The PCR condition in these two

assays were 96 °C for 2 min, 40 cycles 94 °C for 30 s, 55 °C for 40 s, and 72 °C for 2 min, a final extension at 72 °C for 10 min.

PCR assay for insects COI

PCRs with two sets of universal primers with some modifications for insect COI-5P amplification were performed [17, 18]. Primers C_LepFolF (5'-RKT CAA CMA ATC ATA AAG ATA TTG G-3') and MLepR2 (5'-GTT CAW CCW GTW CCW GCY CCA TTT TC-3') should yield a 307 bp amplicon. Primers MLepF1 (5'-GCT TTC CCA CGA ATA AAT AAT A-3') and C_LepFolR (5'-TAA ACT TCW GGR TGW CCA AAA AAT CA-3') should yield a 407 bp amplicon. The PCR condition was followed as previously published [18].

Results

Eighty-eight serum samples from Peruvian patients with unexplained fever were analyzed in 18 pools of 4–5 samples using one Illumina MiSeq run of 250 base paired-ends sequencing. Using a stringent BLASTx cutoff of E scores $< 10^{-20}$, the most numerous viral sequence reads were

from diverse anelloviruses (18 positive pools), followed by pegivirus A (aka GBV-C) (5 pools), dengue virus 2 (DENV2: 4 pools), Oropouche virus (OROV: 2 pools), and HIV (1 pool) (Table 1).

In order to search for novel viral sequences (without very close relatives in GenBank), we also lowered the stringency of the protein matches to BLASTx E score of 10^{-5} . We detected a large number of dicistrovirus and partitivirus protein coding reads in two different serum pools. One pool which generated 1.1×10^5 sequence reads included 2232 reads related to dicistroviruses, and another pool which generated 5.2×10^4 sequence reads included 372 reads related to partitiviruses. The nucleic acids of the four sera in the partitivirus-positive pool and the five sera in the dicistrovirus-positive pool were individually extracted using a different extraction method (QIAamp Viral RNA Mini Kit) and tested for the dicistrovirus and partitivirus RNA by RT-PCR (Materials and methods section), to identify the virus positive serum samples within each pool. These two sera were then individually analyzed by viral metagenomics and the data were deposited in GenBank's short read archive (SRP103886). Sequencing again revealed partitivirus and anelloviruses in one serum sample and dicistrovirus in the other sample as the only viral sequences using an E score cutoff of $< 10^{-5}$.

Table 1 Distribution of sequence reads to different viruses in 18 sera pools from Peru

Pool ID	No. of reads	Anellovirus	Pegivirus	Dengue virus	Oropouche virus	HIV	Dicistrovirus	Partitivirus
21	125715	26	6					
22	241021	53						
23	50623	195						
24	85094	563		26				
25	589125	18						
26	100744	43	165					
27	172978	429		66842	6			
28	105338	4143						
29	154675	47815						
30	60665	685						
31	34905	21		374				
32*	110015	396				28802	2232	
33	95898	66	24	17				
34*	52584	416	21					372
35	73536	285						
36	38957	3054						
37	24199	71						
38	60026	3839	1780		10062			
Individual SF573	47589						78	
Individual C-11	42727	2761						11574

*Pools highlighted contain the initial new dicistrovirus and partitivirus sequence reads further analyzed in individually infected samples SF573 and C-11

The nearly complete genomes of the dicistrovirus and partitivirus were generated using de novo assembly (Materials and methods section).

The dicistrovirus was named human blood-associated dicistrovirus—HuBDV, and was 9566 bases in length, including two major ORF-1 and -2 (Fig. 1a). The first ORF (5' ORF) coded for the non-structural polyprotein (1959 aa), and showed the highest protein identity (18 or 26% similarity) to that of wenzhou picorna-like virus 26 reported in snails (GenBank KX884379) [19]. The second ORF (3' ORF) coded for the structural polyprotein (944 aa), and shared the best identity of 19% (25% similarity) to that of aphid lethal paralysis virus (GenBank AF536531) [20] belonging to the genus *Cripavirus*. The conserved motifs of RNA helicase [**GKTGVGK**] [21] and protease [**GDCG**] [22] were found in the non-structural polyprotein [conserved amino acid in boldface and underlined]. Additionally, three repeated VPg-like sequences [**SxQxRTxGxxRRNVE**] were found [23]. The C-terminal region of the HuBDV's non-structural polyprotein contained a RNA-dependent RNA polymerase (RdRp). A putative non-ATG start codon was identified for the HuBDV's structural polyprotein, a phenomenon also observed in other dicistroviruses such as cricket paralysis virus (Genbank KP974706), drosophila C

virus (GenBank AF014388), and aphid lethal paralysis virus (Genbank AF536531). The pair-wise amino acid sequence analysis demonstrated that the structural polyprotein contained capsid proteins VP1-VP4 (Fig. 1a). A phylogenetic analysis of both polyproteins is shown (Fig. 1b). Based on ICTV, the members of a dicistrovirus species should share above 90% aa identity in their capsid region [24]. Since very low identities ($\leq 19\%$) were measured between HuBDV's capsid and its closest relatives, HuBDV can be considered a member of a new species or even a new genus in the family *Dicistroviridae* [24].

The genome of the novel partitivirus, referred to as human blood-associated partitivirus—HuBPV, consisted of two segments (Fig. 2a). The first segment was 2168 bp in length with GC content of 44%, and contained a single ORF encoding a RNA-dependent RNA polymerase (RdRp). The second segment was 2161 bp in length with GC content of 47%, and contained a single ORF encoding a capsid protein. The RdRp (675 aa in length) and capsid (653 aa in length) proteins showed the closest identities of 63% (65% similarity) and 55% (55% similarity) to those of a fungus (*atkinsonella hypoxylon*) partitivirus (GenBank L39125 and L39126), with the next closest identities of 59% (61% similarity) and 43% (46% similarity) to fungus (*ceratocystis polonica*)

Fig. 1 New dicistrovirus. **a** Genome organization. **b** Phylogenetic trees generated with non-structural and structural polyproteins of dicistrovirus detected in a Peruvian patient with unexplained fever

and genetically related dicistroviruses in the family *Dicistroviridae*. The scale indicates amino acid substitutions per position

Fig. 2 New partitivirus. **a** Genome organization. **b** Phylogenetic trees generated with non-structural and structural proteins of partitivirus detected in a Peruvian patient with unexplained fever and geneti-

cally related partitiviruses in the family *Partitiviridae*. The scale indicates amino acid substitutions per position

partitivirus (GenBank AY260756 and AY247205). Analysis of the deduced amino acid RdRp sequences revealed six conserved motifs among mycoviruses, **SHPDKL****KVR**PVY-NAPMIYLR, **IDWS****RED**HLAP, **GVP****SGIL**MTQFFDS-FVNLTIL, **LIM****GDD**NVALT, **GMKINID****KSS**STSIRRKI-EVLGY, and **RSISK**LVGQLAYPER [conserved amino acid in boldface and underlined] [25]. Phylogenetic analysis of the RdRp and capsid proteins showed that HuBPV grouped together with other partitiviruses in the genus *Betapartitivirus* (Fig. 2b). According to the ICTV, partitiviruses in the same genus should share at least 40% aa identity in the RdRp region (Delmas, 2012). Genome analysis therefore indicated that HuBPV should be considered a new member of the genus *Betapartitivirus*.

In order to further investigate the origins in human serum samples of the partitivirus and dicistrovirus (known to infect fungi and arthropod, respectively), we used PCR assays to test for DNA from their putative cellular hosts in the human sera. Two sets of PCR primers targeting the highly conserved internal transcribed spacer region between the small and large rRNA genes (ITS1/2) of fungi were used to test for the presence of fungal DNA [15, 16]. Arthropod DNA was tested for using a PCR targeting the conserved cytochrome

c oxidase subunit I gene (COI-5P) [18] of arthropods (**Materials and methods** section). The DNA of twenty individual Peruvian serum samples including the two positive for the dicistrovirus and the partitivirus were extracted (QIAamp Viral RNA Mini Kit) and tested by PCR. No sample was positive for the arthropod DNA CROI region. Only one serum was positive for the fungi ITS1/2 DNA, the same sample positive (C-11) for the partitivirus RNA (Fig. 3). Both fungal ITS1/2 PCRs were positive. Analysis of the longer 566-base-long ITS fragment (Genbank KY973646) by Sanger sequencing indicated that its closest relative (563/566 bases identity) was from *bipolaris sorokiniana* (GenBank JQ936306.1) a fungus reported to infect cereals. The presence of three mismatches between these ITS1/2 sequences prevent a definitive assignment of the origin of the fungal DNA in the human sera.

Discussion

In the study, we describe the viruses identified in sera from people with fever from Peru, the nearly complete genomes of dicistrovirus and partitivirus in two such sera,

Fig. 3 Fungal DNA amplification. PCR assay using universal fungal primers targeting ITS1 and ITS2 was performed. The HuBDV partitivirus RNA positive serum sample was found positive for fungal ITS DNA while eighteen other serum samples were negative

and the detection of fungal DNA in the partitivirus containing serum. The known human viruses detected are either considered commensal (anelloviruses and pegivirus A) or are known pathogens (Dengue 2, Orouche, HIV). GBV-C, recently renamed pegivirus A, is a common flavivirus infection associated with blood contact but has not been associated with fever or other diseases [26]. Infections with anelloviruses are also considered non-pathogenic [27]. The pathogenicity of Dengue 2 virus, a flavivirus arbovirus, and its detection in South America has been extensively documented [28]. The samples analyzed were pre-screened using a real-time RT-PCR assay to exclude Dengue virus RNA-positive samples. Our metagenomics detection of Dengue viral sequences indicates that some positive samples were missed by that assay either due to low viral loads or in the case of pool 27 with > 60,000 viral reads possibly due to sample or data mix up. Oropouche, an orthobunyavirus arbovirus, has been reported in the Western hemisphere initially in the Caribbean in 1955 [29] and most recently in Peru in 2016 [30]. Here two pools of serum collected in Peru in January–February 2016 contained Oropouche sequences. Oropouche virus is a frequent cause of arboviral disease in Latin America believed to be transmitted in its urban/epidemic cycle by the mosquito *Culex quinquefasciatus* and/or the biting midge *Culicoides paraensis* [31]. Detection of Oropouche together with Dengue 2 virus further highlight that sampling of febrile patients can detect the short term viremia typical of such arboviral infections [1–3].

The detection of viral nucleic acids from viral families not known to infect vertebrates was further evaluated to exclude possible contamination during nucleic acid extraction or library preparation. The presence of contaminating viral nucleic acids have been reported from samples extracted [2, 32–34] using Qiagen silica-based extraction columns. Sera were therefore re-extracted using an alternative method and the presence of dicistrovirus and partitivirus viral RNA in the two sera samples was confirmed by RT-PCR.

Dicistroviruses belong to the family *Dicistroviridae* with small linear ssRNA genome of approximately 8–10 kb and an internal ribosome entry site separating two main ORFs encoding non-structural and structural proteins whose functional domains distantly resemble those

of picornaviruses. Dicistrovirus is known to infect invertebrates and is responsible for several arthropod diseases, often causing paralysis in their hosts [35]. Dicistroviruses have also been reported in the feces of insectivorous bats [36] and goose [37]. The dicistrovirus genome amplified from serum is deeply rooted within the *Dicistroviridae* phylogeny and highly divergent from its closest known relatives, the Taura syndrome virus [38] infecting Pacific white shrimp *Litopenaeus vannamei* [39] in the genus *Aparavirus* and an unclassified dicistrovirus from a mud crab (GenBank RefSeq accession number NC_014793). As no arthropod DNA was identified using PCR targeting a highly conserved arthropod locus, the presence of the dicistrovirus RNA genome in the serum of a febrile patient remains unexplained. Patient infection with an arthropod refractory to the PCR primers used or a non-arthropod invertebrate infected with the dicistrovirus remain a possibility as does an insect bite transferring dicistrovirus particles but little insect DNA. Replication in human cells seems unlikely given the currently known tropism of other dicistroviruses.

The family *Partitiviridae* is a group of viruses with bisegmented genomes comprising two dsRNA segments, of 1.4–2.3 kb currently classified into 5 genera, 4 of which infect plant or fungi (*Alphapartitivirus*, *Betapartitivirus*, *Gammapartitivirus*, *Deltapartitivirus*), while the fifth genus (*Cryspovirus*) infects a protozoa (*Cryptosporidium* spp.). Partitiviruses are often associated with latent infections with no apparent symptoms on their fungal hosts [40] but which may lead to either hyper or hypovirulence of the infected fungi upon their hosts [41–44]. A new partitivirus was recently found infecting an emerging North American, but not European, fungal pathogen causing fatal white-nose syndrome of hibernating bats in North America [45].

The serum-associated partitivirus was found in the presence of fungal ITS ribosomal DNA most closely related to that of cereals-infecting *Bipolaris sorokiniana* (Common Root Rot and Spot Blotch of Barley and Wheat) in the fungal family *Pleosporaceae* but sequence mismatches prevented a definitive assignment to a specific fungal species. The genus *Bipolaris* includes several significant plant pathogens of the family *Poaceae* (grasses), including rice, maize, wheat, and sorghum [46], and is closely related to

the genus *Curvularia* which includes several human and plant fungal pathogens [47].

Possible explanations for the detection of partitivirus RNA in human serum include transfer of viral particles from fungi in the digestive track to the blood, contamination from fungi present on the skin during phlebotomy or airborne fungal spores during serum sample aliquoting. We recently described the presence of another putative fungal virus in large plasma pools used for biological products production [48]. A more intriguing possibility from our current finding is that the fungal virus found in a human serum reflects infection of this febrile patient with a partitivirus-infected fungus. While fungal infections are rare relative to bacterial infections, they can also result in fever and be particularly difficult to diagnose and treat [49].

Acknowledgements We acknowledge the support from Blood Systems Research Institute. We thanks Dr. Paul D. N. Hebert and Sean Prosser from the Center for Biodiversity Genomics at the University of Guelph, Ontario, Canada, with crucial assistance selecting the arthropod specific PCR primers.

Funding Funding was provided by National Heart, Lung, and Blood Institute (Grant No. R01 HL105770).

Author contributions TP, JM, and ED: designed the study; TP, MS, EA, and XD: performed experiments; TP, JM, and ED: wrote the manuscript; JM: provided clinical information and samples.

Compliance with ethical standards

Conflicts of interest None.

Research involving Human Participants and/or Animals All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

- N.L. Yozwiak, P. Skewes-Cox, M.D. Stenglein, A. Balmaseda, E. Harris, J.L. DeRisi, PLoS Negl. Trop. Dis. **6**, e1485 (2012)
- C.N. Ngoi, J. Siqueira, L. Li, X. Deng, P. Mugo, S.M. Graham, M.A. Price, E.J. Sanders, E. Delwart, J. Gen. Virol. **97**, 3359–3367 (2016)
- M.H. Stremlau, K.G. Andersen, O.A. Folarin, J.N. Grove, I. Odia, P.E. Ehiane, O. Omoniwa, O. Omoregie, P.P. Jiang, N.L. Yozwiak, C.B. Matrangola, X. Yang, S.K. Gire, S. Winnicki, R. Tariyal, S.F. Schaffner, P.O. Okokhere, S. Okogbenin, G.O. Akpede, D.A. Asogun, D.E. Agbonlahor, P.J. Walker, R.B. Tesh, J.Z. Levin, R.F. Garry, P.C. Sabeti, C.T. Happi, PLoS Negl. Trop. Dis. **9**, e0003631 (2015)
- I. Leparç-Goffart, M. Baragatti, S. Temmam, A. Tuiskunen, G. Moureau, R. Charrel, X. de Lamballerie, J. Clin. Virol. **45**, 61–66 (2009)
- O. Faye, D. Diallo, M. Diallo, M. Weidmann, A.A. Sall, Virol. J. **10**, 311 (2013)
- C.N. Peyrefitte, B.A. Pastorino, M. Bessaud, P. Gravier, F. Tock, P. Couissinier-Paris, J. Martial, P. Huc-Anais, R. Cesaïre, M. Grandadam, H.J. Tolou, Emerg. Infect. Dis. **11**, 757–761 (2005)
- M.L. Moreli, V.H. Aquino, A.C. Cruz, L.T. Figueiredo, J. Med. Virol. **66**, 139–142 (2002)
- M. Llagonne-Barets, V. Icard, I. Leparç-Goffart, C. Prat, T. Perpoint, P. Andre, C. Ramiere, J. Clin. Virol. **77**, 66–68 (2016)
- L. Dong-Mei, L. Yun-Yan, D. Peng-Cheng, S. Xiu-Ping, Qi-Yong, Microbiol. China **42**, 427–435 (2015)
- P. Bourhy, S. Bremont, F. Zinini, C. Giry, M. Picardeau, J. Clin. Microbiol. **49**, 2154–2160 (2011)
- L. Li, X. Deng, E.T. Mee, S. Collot-Teixeira, R. Anderson, S. Schepelmann, P.D. Minor, E. Delwart, J. Virol. Methods **213**, 139–146 (2015)
- X. Deng, S.N. Naccache, T. Ng, S. Federman, L. Li, C.Y. Chiu, E.L. Delwart, Nucleic Acids Res. **43**, e46 (2015)
- N. Saitou, M. Nei, Mol. Biol. Evol. **4**, 406–425 (1987)
- K. Tamura, G. Stecher, D. Peterson, A. Filipski, S. Kumar, Mol. Biol. Evol. **30**, 2725–2729 (2013)
- D.L. Taylor, W.A. Walters, N.J. Lennon, J. Bochicchio, A. Krohn, J.G. Caporaso, T. Pennanen, Appl. Environ. Microbiol. **82**, 7217–7226 (2016)
- N.A. Bokulich, D.A. Mills, Appl. Environ. Microbiol. **79**, 2519–2526 (2013)
- S.W. Prosser, M.G. Velarde-Aguilar, V. Leon-Regagnon, P.D. Hebert, Mol. Ecol. Res. **13**, 1108–1115 (2013)
- P.D. Hebert, J.R. Dewaard, E.V. Zakharov, S.W. Prosser, J.E. Sones, J.T. McKeown, B. Mantle, J. La Salle, PLoS ONE **8**, e68535 (2013)
- M. Shi, X.D. Lin, J.H. Tian, L.J. Chen, X. Chen, C.X. Li, X.C. Qin, J. Li, J.P. Cao, J.S. Eden, J. Buchmann, W. Wang, J. Xu, E.C. Holmes, Y.Z. Zhang, Nature **540**, 539–543 (2016)
- M. Van Munster, A.M. Dulleman, M. Verbeek, J.F. Van Den Heuvel, A. Clerivet, F. Van Der Wilk, J. Gen. Virol. **83**, 3131–3138 (2002)
- A.E. Gorbalenya, E.V. Koonin, A.P. Donchenko, V.M. Blinov, Nucleic Acids Res. **17**, 4713–4730 (1989)
- A.E. Gorbalenya, A.P. Donchenko, V.M. Blinov, E.V. Koonin, FEBS Lett. **243**, 103–114 (1989)
- N. Nakashima, N. Shibuya, J. Invertebr. Pathol. **92**, 100–104 (2006)
- S.M. Valles, Y. Chen, A.E. Firth, D.M. Guerin, Y. Hashimoto, S. Herrero, J.R. de Miranda, E. Ryabov, Report C. Ictv, J. Gen. Virol. **98**, 355–356 (2017)
- H. Liu, Y. Fu, J. Xie, J. Cheng, S.A. Ghabrial, G. Li, Y. Peng, X. Yi, D. Jiang, BMC Evol. Biol. **12**, 91 (2012)
- N. Bhattarai, J.T. Stapleton, Trends Microbiol. **20**, 124–130 (2012)
- D. Focosi, G. Antonelli, M. Pistello, F. Maggi, Clin. Microbiol. Infect. **22**, 589–593 (2016)
- J.R. Torres, T.A. Orduna, M. Pina-Pozas, D. Vazquez-Vega, E. Sarti, J. Trop. Med. **2017**, 8045435 (2017)
- C.R. Anderson, L. Spence, W.G. Downs, T.H. Aitken, Am. J. Trop. Med. Hyg. **10**, 574–578 (1961)
- D. Romero-Alvarez, L.E. Escobar, Mem. Inst. Oswaldo Cruz **112**, 292–298 (2017)
- da Rosa J.F., de Souza W.M., de Paula Pinheiro F., Figueiredo M.L., Cardoso J.F., Acrani G.O., Nunes M.R., Am. J. Trop. Med. Hyg. 2017

32. F. Lysholm, A. Wetterbom, C. Lindau, H. Darban, A. Bjerkner, K. Fahlander, A.M. Lindberg, B. Persson, T. Allander, B. Andersson, *PLoS ONE* **7**, e30875 (2012)
33. S.N. Naccache, A.L. Greninger, D. Lee, L.L. Coffey, T. Phan, A. Rein-Weston, A. Aronsohn, J. Hackett Jr., E.L. Delwart, C.Y. Chiu, *J. Virol.* **87**, 11966–11977 (2013)
34. C.N. Ngoi, J. Siqueira, L. Li, X. Deng, P. Mugo, S.M. Graham, M.A. Price, E.J. Sanders, E. Delwart, *J. Gen. Virol.* **98**, 517 (2017)
35. B.C. Bonning, W.A. Miller, *Annu. Rev. Entomol.* **55**, 129–150 (2010)
36. L. Li, J.G. Victoria, C. Wang, M. Jones, G.M. Fellers, T.H. Kunz, E. Delwart, *J. Virol.* **84**, 6955–6965 (2010)
37. Greninger A.L., Jerome K.R., *Genome Announc.* **4**, 2016
38. J. Mari, B.T. Poulos, D.V. Lightner, J.R. Bonami, *J. Gen. Virol.* **83**, 915–926 (2002)
39. K.W. Hasson, D.V. Lightner, B.T. Poulos, R.M. Redman, B.L. White, J.A. Brock, J.R. Bonami, *Dis. Aquatic Org.* **23**, 115–126 (1995)
40. S.A. Ghabrial, N. Suzuki, *Annu. Rev. Phytopathol.* **47**, 353–384 (2009)
41. S.A. Ghabrial, J.R. Caston, D. Jiang, M.L. Nibert, N. Suzuki, *Virology* **479–480**, 356–368 (2015)
42. A.I. Soldevila, W.M. Havens, S.A. Ghabrial, *Virology* **272**, 183–190 (2000)
43. I.P. Ahn, Y.H. Lee, *Mol Plant Microbe Interact.* **14**, 496–507 (2001)
44. S. Ozkan, R.H. Coutts, *Fungal Genet. Biol.* **76**, 20–26 (2015)
45. V. Thapa, G.G. Turner, S. Hafenstein, B.E. Overton, K.J. Vanderwolf, M.J. Roossinck, *PLoS Pathog.* **12**, e1006076 (2016)
46. D.S. Manamgoda, A.Y. Rossman, L.A. Castlebury, P.W. Crous, H. Madrid, E. Chukeniroti, K.D. Hyde, *Stud. Mycol.* **79**, 221–288 (2014)
47. H. Madrid, K.C. da Cunha, J. Gene, J. Dijksterhuis, J. Cano, D.A. Sutton, J. Guarro, P.W. Crous, *Persoonia* **33**, 48–60 (2014)
48. W. Zhang, L. Li, X. Deng, J. Blumel, C.M. Nubling, A. Hunfeld, S.A. Baylis, E. Delwart, *Transfusion* **56**, 2248–2255 (2016)
49. D.D. Garbee, S.S. Pierce, J. Manning, *Crit. Care Nurs. Clin. N. Am.* **29**, 67–79 (2017)