Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company’s public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Research paper

Identification of group A rotaviruses from Zambian fruit bats provides evidence for long-distance dispersal events in Africa

Michihito Sasakia,b,⁎, Masahiro Kajiharaa, Katendi Changula, Akina Mori-Kajiharaa, Hirohito Ogawa d,1, Bernard M. Hang'ombe cf, Aaron S. Mweene c,fg, Martin Simunzae, Reiko Yoshidab, Michael Carr i, Yasuko Orbaa, Ayato Takad lh, Hirofumi Sawa a,g,h, Hirohito Ogawad,1, Bernard M. Hang’ombec,f, Aaron S. Mweenee,f,g, Martin Simunzae,

a Division of Molecular Pathobiology, Research Center for Zoonosis Control, Hokkaido University, Sapporo 001-0020, Japan
b Division of Global Epidemiology, Research Center for Zoonosis Control, Hokkaido University, Sapporo 001-0020, Japan
c Department of Paracutinal Studies, School of Veterinary and Medicine, University of Zambia, PO Box 32379, Lusaka, Zambia
d Hokudai Center for Zoonosis Control in Zambia, School of Veterinary Medicine, University of Zambia, PO Box 32379, Lusaka, Zambia
e Department of Disease Control, School of Veterinary and Medicine, University of Zambia, PO Box 32379, Lusaka, Zambia
f Africa Center of Excellence for Infectious Diseases of Humans and Animals, University of Zambia, PO Box 32379, Lusaka, Zambia
g Global Virus Network, Baltimore, MD 21201, USA
h Global Institution for Collaborative Research and Education (GI-CoRE), Hokkaido University, Sapporo 001-0020, Japan
i National Virus Reference Laboratory, School of Medicine, University College Dublin, Dublin 4, Ireland

⁎ Corresponding authors at: Division of Molecular Pathobiology, Research Center for Zoonosis Control, Hokkaido University, Sapporo 001-0020, Japan.
E-mail addresses: m-sasaki@czc.hokudai.ac.jp (M. Sasaki), b-sawa@czc.hokudai.ac.jp (H. Sawa).
1 Present address: Department of Virology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8558, Japan

ARTICLE INFO

Keywords:
Rotavirus
African fruit bats
Long-distance dispersal
Interspecies transmission
Phylogenetic analysis
Novel RVA genotypes

ABSTRACT

Group A rotavirus (RVA) is a major cause of diarrhea in children worldwide. Although RVA infects many animals, little is known about RVA in bats. The present study investigated the genetic diversity of RVA in Zambian bats. We identified RVA from two straw-colored fruit bats (Eidolon helvum) and an Egyptian fruit bat (Rousettus aegyptiacus), and analyzed the genome sequences of these strains. Genome segments of the RVA strains from Zambian E. helvum showed 97%–99% nucleotide sequence identity with those of other RVA strains from E. helvum in Cameroon, which is 2800 km from the sampling locations. These findings suggest that migratory straw-colored fruit bat species, distributed across sub-Saharan Africa, have the potential to disseminate RVA across long distances. By contrast, the RVA strain from Zambian R. aegyptiacus carried highly divergent NSP2 and NSP4 genes, leading us to propose novel genotypes N21 and E27, respectively. Notably, this RVA strain also shared the same genotype for VP6 and NSP3 with the RVA strains from Zambian E. helvum, suggesting interspecies transmission and genetic reassortment may have occurred between these two bat species in the past. Our study has important implications for RVA dispersal in bat populations, and expands our knowledge of the ecology, diversity and evolutionary relationships of RVA.

1. Introduction

Rotavirus is a major causative agent of gastroenteritis in children under five, with >120,000 cases of diarrheal death annually worldwide (Clark et al., 2017). Among nine species of rotavirus (groups A to I), group A rotavirus (RVA) is the major species and the most well studied to date. RVA has a genome of 11 segments of double-stranded RNA, which encode the viral structural proteins (VP1-4, VP6 and VP7) and the non-structural proteins (NSP1-6). The current nomenclature system of RVA defines the genotype as: Gx-P[y]-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx for the VP7-, VP4-, VP6-, VP1-, VP2-, VP3-, NSP1-, NSP2-, NSP3-, NSP4- and NSP5/6-encoding genes, respectively (Matthijnssens et al., 2008, 2011a). Based on the genome sequence, all RVA isolates are classified into genotypes in accordance with the recommendations of the Rotavirus Classification Working Group (RCWG) to ensure uniformity (Matthijnssens et al., 2008). This classification system has been widely adopted and has greatly facilitated the analysis of RVA sequence data, which has uncovered high genetic diversity and proposed new genotypes (Esona et al., 2018; He et al., 2017; Ianiro et al., 2017; Li et al., 2016; Rojas et al., 2016, 2017; Yinda et al., 2016).

Bats harbor numerous pathogens and act as reservoir hosts of high-consequence zoonotic viruses (Hayman, 2016; Olival et al., 2017). A limited number of studies have reported on RVA from frugivorous bats: Eidolon helvum in Kenya and Cameroon (Esona et al., 2010; Yinda et al., 2011). Based on the genome sequence, all RVA isolates are classified into genotypes in accordance with the recommendations of the Rotavirus Classification Working Group (RCWG) to ensure uniformity (Matthijnssens et al., 2008). This classification system has been widely adopted and has greatly facilitated the analysis of RVA sequence data, which has uncovered high genetic diversity and proposed new genotypes (Esona et al., 2018; He et al., 2017; Ianiro et al., 2017; Li et al., 2016; Rojas et al., 2016, 2017; Yinda et al., 2016).

Bats harbor numerous pathogens and act as reservoir hosts of high-consequence zoonotic viruses (Hayman, 2016; Olival et al., 2017). A limited number of studies have reported on RVA from frugivorous bats: Eidolon helvum in Kenya and Cameroon (Esona et al., 2010; Yinda et al., 2011). Based on the genome sequence, all RVA isolates are classified into genotypes in accordance with the recommendations of the Rotavirus Classification Working Group (RCWG) to ensure uniformity (Matthijnssens et al., 2008). This classification system has been widely adopted and has greatly facilitated the analysis of RVA sequence data, which has uncovered high genetic diversity and proposed new genotypes (Esona et al., 2018; He et al., 2017; Ianiro et al., 2017; Li et al., 2016; Rojas et al., 2016, 2017; Yinda et al., 2016).

Bats harbor numerous pathogens and act as reservoir hosts of high-consequence zoonotic viruses (Hayman, 2016; Olival et al., 2017). A limited number of studies have reported on RVA from frugivorous bats: Eidolon helvum in Kenya and Cameroon (Esona et al., 2010; Yinda et al., 2011). Based on the genome sequence, all RVA isolates are classified into genotypes in accordance with the recommendations of the Rotavirus Classification Working Group (RCWG) to ensure uniformity (Matthijnssens et al., 2008). This classification system has been widely adopted and has greatly facilitated the analysis of RVA sequence data, which has uncovered high genetic diversity and proposed new genotypes (Esona et al., 2018; He et al., 2017; Ianiro et al., 2017; Li et al., 2016; Rojas et al., 2016, 2017; Yinda et al., 2016).

https://doi.org/10.1016/j.meegid.2018.05.016
Received 20 April 2018; Received in revised form 17 May 2018; Accepted 18 May 2018
Available online 21 May 2018
1567-1348/ © 2018 Elsevier B.V. All rights reserved.
Infection, Genetics and Evolution 63 (2018) 104–109

2. Materials and methods

2.1. Sample collection and ethics statement

From 2014 to 2015, 60 frugivorous and 40 insectivorous bat species were captured at five different locations in Zambia, with permission from the Department of National Parks and Wildlife (formerly the Zambia Wildlife Authority), Ministry of Tourism and Arts (Act No. 12 of 1998). Spleen, liver, kidney and colon tissues were collected through dissection. Bats were speciated based on morphology and sequencing of ribosomal RNA and cytochrome b loci, as previously described (Sasaki et al., 2012). Sample information is summarized in Table 1.

2.2. Nested RT-PCR screening for RVA

Total RNA was extracted from bat colon tissue using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. For nested RT-PCR screening, cDNA was synthesized using random hexamers and SuperScript IV Reverse Transcriptase (Invitrogen; Thermo Fisher Scientific, Waltham, MA, USA), and subjected to nested PCR amplification employing the Tks Gflex DNA polymerase (Takara Bio, Kusatsu, Japan) and oligonucleotide primers targeting RVA VP7 as follows: RotexF (5′- MDCGGWTA-GMYBBTWTAAAATG-3′) and RotexR (5′- CCCATNGMDATCCAYTTRT-3′) for the 1st round PCR, and RotinF (5′- TAGGYYBTTTTRATGAT-GGKAT-3′) and RotinR (5′- TCCTANGGRTTRCAHARCC-3′) for the 2nd round PCR (Li et al., 2016). The thermostability conditions were: cycle of 94 °C for 2 min followed by 35 cycles of 98 °C for 10 s, 46 °C (1st PCR) or 50 °C (2nd PCR) for 15 s and 68 °C for 30 s. Amplicons were purified with the MonoFas DNA Purification Kit 1 (GL Sciences, Tokyo, Japan) and sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems; Thermo Fisher Scientific).

2.3. Amplification and sequencing of RVA genome segments

Each genome segment was separately amplified by a nested RT-PCR strategy. After denaturation at 95 °C for 5 min, RNA samples were reverse transcribed with SuperScript IV Reverse Transcriptase and specific primer sets targeting the 5′ and 3′ ends of each of the 11 RVA genome segments, referred to as exoF or exor, as described previously (Li et al., 2016). The 1st round PCR was performed with Tks Gflex DNA polymerase and the gene-specific primer pairs that were used in the reverse transcription step. The 2nd round PCR was performed with Tks Gflex DNA polymerase and the inner primer set, referred to as inF or inR as described previously (Li et al., 2016). The PCR amplicons were sequenced as described above.

2.4. Assignment of RVA genotypes

Genotypes of the identified segments were determined using the online tool Rotac (http://rotac.regatools.be) or following the judgment of RCWG (https://rega.kuleuven.be/cev/viralmetagenomics/virus-classification/rcwg) (Maes et al., 2009).

2.5. Phylogenetic analysis

Maximum likelihood phylogenetic trees with 500 bootstrap replicates were inferred from multiple nucleotide sequence alignments of full-length genes of RVA reference strains and bat RVAs using MEGA7 software (Kumar et al., 2016). For the maximum likelihood analyses, the GTR + G + 1 model for VP1 and VP6, the GTR + G model for VP7, NSP2 and NSP3, and the TN93 + G model for NSP4 were employed based on the “Find best DNA/protein model” in the MEGA7 software.

2.6. Nucleotide sequence accession numbers

The determined RVA genome sequences were deposited in the DDBJ/EMBL/GenBank database under accession no. LC277159-LC277170.

3. Results

3.1. Detection of RVA VP7 genome segments in Zambian fruit bats

During 2014–2015, three frugivorous bat species (E. helvum, Epomophorus crypturus, R. aegyptiacus) and three insectivorous bat species (Hipposideros gigas, Nycteris sp., Miniopterus schreibersii) were captured in Zambia (Table 1). No bats showed signs of serious infection, including diarrhea. RNA was extracted from 100 bat colon samples and subjected to nested RT-PCR screening targeting the conserved VP7 gene of RVA. The screening identified three VP7 positive samples from Zambian fruit bats: strain ZFB14-52 from an adult male E. helvum, ZFB14-135 from an adult female E. helvum and ZFB14-126 from an adult female R. aegyptiacus. To determine the genotype, we attempted to amplify the near-complete sequence of the VP7 gene and recovered it from ZFB14-52 and ZFB14-135, but not ZFB14-126.

3.2. Detection of RVA genome segments from VP7-positive bats

To further characterize the RVA strains detected in Zambian fruit

Table 1

| Bat species                  | Location           | RT-PCR positive/total |
|-----------------------------|--------------------|-----------------------|
| Fruit bats                  |                    |                       |
| Eidolon helvum              | Ndola              | 1/10                  |
| Eidolon helvum              | Kasanka national park | 1/10                |
| Epomophorus crypturus       | Monge              | 0/20                  |
| Rousettus aegyptiacus       | Lusaka             | 1/20                  |
| Insectivorous bats          |                    |                       |
| Hipposideros gigas          | Lusaka             | 0/10                  |
| Miniopterus schreibersii    | Lusaka             | 0/10                  |
| Nycteris sp.                | Livingstone        | 0/20                  |
bats, we sought to identify the remaining 10 genome segments of RVA in the VP7-positive specimens. The genome segments were amplified by nested RT-PCR. All RT-PCR products were sequenced directly and in the bats, we sought to identify the remaining 10 genome segments of RVA strain ZFB14-126, and NSP2-4 were not observed in the sequencing electropherogram, suggesting each amplicon originated from a single RVA strain. We determined the sequences of VP6 and NSP3 from strain ZFB14-52, VP6 and NSP2-4 from strain ZFB14-126, and VP1, VP6, and NSP3 from strain ZFB14-135 (Table 2). Despite multiple attempts by RT-PCR, the sequences of the other RVA genome segments remain to be elucidated.

3.3. Sequence comparison and phylogenetic analysis of VP7, VP1, VP6 and NSP3

Genotype identification was performed employing the RotaC online tool, which indicated that VP7 of ZFB14-52 and ZFB14-135 could be assigned to the G31 genotype (Table 2). The sequence of these VP7 genome segments showed 98% nucleotide identity to RVA strain BatLi08, belonging to the G31 genotype, which was previously discovered from E. helvum in the South West region of Cameroon (Yinda et al., 2016). Phylogenetic analysis of VP7 showed that ZFB14-52 and ZFB14-135 clustered with BatLi08 and were distantly related to BatLi09, BatLi10 and BatLy17 belonging to the G30 genotype (Fig. 1), which were also identified from E. helvum in Cameroon (Yinda et al., 2016).

The VP1 of ZFB14-135 showed 94% nucleotide identity with that of BatLi08 belonging to the R15 genotype. The VP6 of ZFB14-52, ZFB14-126 and ZFB14-135 showed 97%, 90% and 99% nucleotide identities with that of BatLi08 belonging to the I22 genotype, respectively. The NSP3 of ZFB14-52, ZFB14-126 and ZFB14-135 showed 98%, 90% and 99% nucleotide identities with that of BatLi08 belonging to the T17 genotype, respectively. Phylogenetic analyses of these genome segments revealed that ZFB14-52, ZFB14-126 and ZFB14-135 formed a discrete cluster with Cameroonian bat RVAs (BatLi08, BatLi09, BatLi10 and BatLy17) and were clearly separable from other previously described bat RVAs (Fig. 1). Collectively, these results indicated that Zambian fruit bat RVAs harbor the same genotypes of VP1, VP6, VP7 and NSP3 as Cameroonian fruit bat RVAs and exhibit high nucleotide sequence identities with these genome segments.

3.4. Identification of novel NSP2 and NSP4 genotypes

The NSP2 and NSP4 genotypes of ZFB14-126 could not be determined by RotaC due to their nucleotide sequence divergence. BLAST search analyses indicated that both NSP2 and NSP4 of ZFB14-126 showed < 80% nucleotide sequence identity with all available RVA sequence data deposited in the DDBJ/EMBL/GenBank public databases. Therefore, these sequences were submitted to RCGW and were approved as new genotypes: N21 for NSP2 and E27 for NSP4 (RCGW, 2018). Phylogenetic analyses revealed that NSP2 of ZFB14-126 was distantly related to other RVAs and segregated in a different clade from the Cameroonian bat RVA N15 genotype (Fig. 2). Furthermore, NSP4 of ZFB14-126 was highly divergent from all other RVAs and represented a distinct lineage of NSP4 (Fig. 2). These findings indicate that RVA strain ZFB14-126 possessed discordant NSP2 and NSP4 gene segments when compared with other genome segments.

4. Discussion

E. helvum is distributed across sub-Saharan Africa and previous studies revealed that the mean migratory distance of E. helvum was 860 km with a range from 270 to 3000 km (Ossa et al., 2012; Richter and Cumming, 2008). Prior genetic studies revealed a pandemic population of E. helvum across continental Africa, suggesting that this bat species travels and interbreeds over long distances (Peel et al., 2013). In Zambia, over one million E. helvum roost from October to December (Peel et al., 2017). Previous reports have suggested that migration of E. helvum facilitates the introduction of viruses into the bat population, such as filoviruses, henipaviruses, lyssaviruses and coronaviruses (Drexler et al., 2012; Leopardi et al., 2016; Ogawa et al., 2015; Peel et al., 2013).

In this study, we identified bat RVA strains ZFB14-52 and ZFB14-135 from E. helvum in Zambia, which belong to genotypes G31 for VP7, R15 for VP1, I22 for VP6, and T17 for NSP3. These genotypes were initially identified from Cameroonian E. helvum by another research group who proposed that novel RVA genotype constellations exist in E. helvum, such as has been determined in humans and domesticated animals (Mathijssens et al., 2011b; Mathijssens and Van Ranst, 2012; Yinda et al., 2016). Our results support this view that certain RVA genotype constellations exist in this bat species. Interestingly, these Zambian bat RVA strains (ZFB14-52 and ZFB14-135) carried VP7, VP6 and NSP3 genome segments that shared 97%–99% nucleotide sequence identity with those of BatLi08 from E. helvum in Limbe, Cameroon, at least 2800 km apart from our sampling locations. Notably, it has been reported that RVA strain BatLy03 from Cameroonian E. helvum shared the same genotypes for VP2, VP6, VP7, NSP2, NSP3 and NSP5 as strain KE4852 from Kenyan E. helvum (Yinda et al., 2016). These findings suggest that the migration of E. helvum may have the potential to spread RVA across long distances and impact on the viral ecology.

Recent genetic analyses of bat RVAs have discovered new genotypes of this virus (Asano et al., 2016; Esona et al., 2016; He et al., 2017; Yinda et al., 2016). In this study, we identified the previously unrecognized genotypes N21 for NSP2 and E27 for NSP4 in RVA strain ZFB14-126 from R. aegyptiacus in Zambia. Both N21 and E27 were distinguished from other mammalian RVAs by long branch lengths in their phylogenies (Fig. 2). These results indicate that previously unrecognized genotypes are harbored by bats with unique evolutionary histories. In addition, ZFB14-126 shared the same I22 and T17 genotypes with ZFB14-52 and ZFB14-135 from E. helvum (Table 2), suggesting interspecies transmission and genetic reassortment may have
occurred between these two bat species in the past. However, we could not formally exclude the possibility of mixed infection with different RVA strains in this individual bat. Unfortunately, we failed to recover all RVA gene segments of these RVA strains and their complete genotype constellations remain to be elucidated. Although several universal primer sets targeting the 5' and 3' regions of each genome segment were employed to amplify RVA genomes and determine the genotypes (Fujii et al., 2012; Gentsch et al., 1992; Gouvea et al., 1990; Li et al., 2016), there are significant nucleotide mismatches between these primers and recently described bat RVA genomes (Yinda et al., 2016). A high-throughput sequencing approach may help to identify divergent bat RVA genomes and determine the genotype constellations (He et al., 2017; Yinda et al., 2016).

Previous studies reported that bat RVAs carry genome segments closely related to other mammalian RVAs, including human RVAs (Asano et al., 2016; He et al., 2017; Sasaki et al., 2016). In this study, all detected genome segments were divergent to those of other mammalian RVAs and are tentatively considered to be bat-specific. However, Esona et al. recently identified RVA strain KE4852 from E. helvum, which carried VP4 and NSP4 genes with shorter genetic distances to other mammalian RVAs providing evidence of interspecies transmission between E. helvum and other mammal host species (Esona et al., 2010). Therefore, further studies with increasing numbers of specimens are required to evaluate the public health risk of RVA harbored by Zambian bats and to delineate further the genetic diversity and evolutionary history of these viruses.
Fig. 2. Phylogenetic analysis based on the nucleotide sequences of the NSP2 and NSP4 genes. The group A rotavirus (RVA) strain ZFB14-126 identified in this study is highlighted in red. Other reference bat RVAs included in the analysis are colored in blue. Genotypes are shown to the left of each taxon. The bootstrap values above 70 after 500 replicates are shown at tree nodes. The scale bars represent the numbers of nucleotide substitutions per site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Acknowledgments

We thank Sakae Kashihara, Emiko Nakagawa, Edgar Simulundu, Ryo Nakao, Wakako Furuyama, Chiho Kaneko, Joseph Ndebe, Penjaniinge Kapila, Ladslav Moonga, John Yabe and the Department of National Parks and Wildlife of the Ministry of Tourism and Arts (formerly ZAWA) for technical assistance in Zambia. We also thank Dr. Jelle Matthijnssens and RCWG for help with RVA genotyping, and Kate Fox from Edanz Group for editing a draft of this manuscript.

Funding

This work was supported by the Japan Initiative for Global Research Network of Infectious Diseases (J-GRID) from Japan Agency for Medical Research and Development (AMED) (JP18fm0108008); AMED/Japan International Cooperation Agency (JICA) within the framework of the Science and Technology Research Partnership for Sustainable Development (SATREPS); Grants-in-Aid for Scientific Research on Innovative Areas from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan (16H06429, 16H06431, 16K21723); and Japan Society for the Promotion of Science (JSPS) KAKENHI (16H05805).

Declaration of interest

The authors declare that they have no conflicts of interest.

References

Asano, K.M., Gregori, F., Hora, A.S., Scheiffer, K.C., Fahl, W.O., Iamamoto, K., Mori, E., Silva, F.D., Tanimaki, S.A., Brandão, P.E. 2016. Group A rotavirus in Brazilian bats: description of novel T15 and H15 genotypes. Arch. Virol. 161 (11), 3225–3230.
Clark, A., Black, R., Tate, J., Roos, A., Kotloff, K., Lamm, D., Blackwelder, W., Parashar, U., Lanata, C., Kang, G., Troeger, C., Platt–Mills, J., Mokdad, A., Sanderson, C., Lambertie, L., Levine, M., Santoshi, M., Steele, D., Network, G.R.S., 2017. Estimating global, regional and national rotavirus deaths in children aged <5 years: current approaches, new analyses and proposed improvements. PLoS One 12 (9), e0183392.
Drexler, J.F., Corman, V.M., Müller, M.A., Maganga, G.D., Vallo, P., Binger, T., Gloza-Rausch, F., Cottontail, V.M., Rasche, A., Yordanov, S., Seebens, A., Knosenchild, M., Oppong, S., And Sarkodie, Y., Pongombo, C., Lukanen, A., Schmidt-Chanasit, J., Stocker, A., Carneiro, A.J., Erbar, S., Mainzer, A., Fronenhoffs, F., Buettner, R., Kalko, E.K., Kruppa, T., Franke, C.R., Kallies, R., Vandoko, E.R., Herfier, G., Reusken, C., Hassanin, A., Krüger, D.H., Matthée, S., Ulbrich, R.G., Leroy, E.M., Drosten, C., 2012. Bats host major mammalian paramyxoviruses. Nat. Commun. 3, 796.
Eeno, M.D., Mjøstadvik-Rustempas, S., Conrady, C., Tong, S., Kuzmin, I.V., Agwanda, B., Breiman, R.P., Banyai, K., Niezgoda, M., Rupprecht, C.E., Gentch, J.R., Bowen, M.D., 2010. Reassortant group A rotavirus from straw-colored fruit bat (Eidolon helvum). Emerg. Infect. Dis. 16 (12), 1844–1852.
Eeno, M.D., Roy, S., Rungsirisiruyachai, K., Gautam, R., Hermelin, S., Rey-Benito, G., Bowen, M.D., 2018. Molecular characterization of a human G20P[28] rotavirus strain with multiple genes related to bat rotavirus. Infect. Genet. Evol. 57, 166–170.
Fujii, Y., Shimokoe, T., Takagi, H., Murakami, K., Tochado-Takai, R., Park, Y., Katayama, K., 2012. Amplification of all 11 RNA segments of group A rotaviruses based on reverse transcription polymerase chain reaction. Microbiol. Immunol. 56 (9), 630–638.
Gentch, J.R., Glass, R.L., Woods, P., Gouveia, V., Gorzgizia, M., Flores, J., Das, R.K., Bhan, M.K., 1992. Identification of group A rotavirus 4 genes by polymerase chain reaction. J. Clin. Microbiol. 30 (6), 1365–1373.
Gouveia, V., Glass, R.L., Woods, P., Taniguchi, K., Clark, H.F., Forrester, B., Fang, Z.Y., 1990. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J. Clin. Microbiol. 28 (2), 276–282.
Hayman, D.T., 2016. Bats as viral reservoirs. Annu Rev Virol 3 (1), 77–99.
He, B., Yang, F., Yang, W., Zhang, Y., Feng, Y., Zhou, J., Xie, J., Bao, X., Guo, H., Li, Y., Xu, L., Li, N., Matthijnssens, J., Zhang, H., Tu, C., 2013. Characterization of a novel G3P[3] rotavirus isolated from a lesser horseshoe bat: a distant relative of feline/canine rotaviruses. J. Virol. 87 (22), 12357–12366.
He, B., Huang, X., Zhang, F., Tan, W., Matthijnssens, J., Qin, S., Xu, L., Zhao, Z., Yang, L., Wang, Q., Hu, T., Bao, X., Wu, J., Tu, C., 2017. Group A rotaviruses in Chinese bats: basic genetic composition, serology, and evidence for bat-to-human transmission and reassortment. J. Virol. 91 (12).
Inamori, G., Di Bartolo, I., De Sabato, L., Pampiglione, G., Ruggieri, F.M., Ostanello, F., 2017. Detection of uncommon G3P[3] rotavirus a (RVA) strain in rat possessing a genetic composition, serology, and evidence for bat-to-human transmission and reassortment. J. Virol. 91 (7).
J.G., Li, M.H., Hong, W.S., Holmes, E.C., Zhang, Y.Z., 2016. Identification of novel T15 and H15 genotypes. Arch. Virol. 161 (11), 3225–3230.
Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33 (7), 1870–1874.
Leopardi, S., Oluwayelu, D., Meseko, C., Marciano, S., Tassoni, L., Bakarey, S., Monne, I., Cattolì, G., De Benedictis, P., 2016. The close genetic relationship of lineage D Betacoronavirus from Nigerian and Kenyan straw-colored fruit bats (Eidolon helvum). Emerg. Infect. Dis. 16 (12), 1844–1852.
Maes, P., Matthijnssens, J., Van Ranst, M., 2009. Rotavirus genotyping and evolution of group A rotaviruses infecting humans. Curr. Opin. Virol. 2 (4), 426–433.
Matthijnssens, J., Clariet, M., Rahman, M., Attoui, H., Bányai, K., Estes, M.K., Gentch, J.R., Iurza-Gómez, M., Kirkwood, C.D., Martella, V., Mertens, P.P., Nakagomi, O., Patton, J.T., Ruggeri, F.M., Saif, L.J., Santos, N., Steyer, A., Taniguchi, K., Desselberger, U., Van Ranst, M., 2008. Recommendations for the classification of group A rotavirus. Infection, Genetics and Evolution 63 (2018) 104–109.
group A rotaviruses using all 11 genomic RNA segments. Arch. Virol. 153 (8), 1621–1629.

Matthijnssens, J., Ciarlet, M., McDonald, S.M., Attoui, H., Bánayi, K., Brister, J.R., Buesa, J., Esson, M.D., Estes, M.K., Gentech, J.R., Iturriza-Gómez, M., Johne, R., Kirkwood, C.D., Martella, V., Mertens, P.P., Nakagomi, O., Parreño, V., Rahman, M., Raggetter, F.M., Saif, L.J., Santos, N., Steyer, A., Taniguchi, K., Patton, J.T., Desselberger, U., Van Ranst, M., 2011a. Uniformity of rotavirus strain nomenclature proposed by the rotavirus classification working group (RCWG). Arch. Virol. 156 (8), 1397–1413.

Matthijnssens, J., De Grazia, S., Piensens, J., Heylen, E., Zeller, M., 2011b. Multiple reassortment and interspecies transmission events contribute to the diversity of feline, canine and feline/canine-like human group A rotavirus strains. Infect. Genet. Evol. 11 (6), 1396–1406.

Olm, K.J., Hosseini, P.R., Zambrana-Torrelio, C., Ross, N., Bogich, T.L., Daszak, P., 2017. Host and viral traits predict zoonotic spillover from mammals. Nature 546 (7660), 646–650.

Ossa, G., Kramer-Schadt, S., Peel, A.J., Scharf, A.K., Voigt, C.C., 2012. The movement ecology of the straw-colored fruit bat, Eidolon helvum, in sub-Saharan Africa assessed by stable isotope ratios. PLoS One 7 (9), e45729.

Peel, A.J., Sargan, D.R., Baker, K.S., Hayman, D.T.S., Bart, J.A., Cranmer, G., Suu-Ire, R., Broder, C.C., Lembo, T., Wang, L.F., Fooks, A.R., Rossiter, S.J., Wood, J.L.N., Cunningham, A.A., 2013. Continent-wide panmixia of an African fruit bat facilitates transmission of potentially zoonotic viruses. Nat. Commun. 4, 2770.

Peel, A.J., Wood, J.L., Baker, K.S., Breed, A.C., Carvalho, A.D., Fernández-Loras, A., Gabrieli, H.S., Gembu, G.C., Kalibata, P.M., 2017. How does Africa’s most hunted bat vary across the continent? Population traits of the straw-Coloured fruit bat (eidoson helvu m) and its interactions with humans. Acta Chiropterol. 19 (1), 77–92.

Richter, H., Cumming, G., 2008. First application of satellite telemetry to track African straw-coloured fruit bat migration. J. Zool. 275 (2), 172–176.

Rojas, M., Gonçalves, J.L., Dias, H.G., Manchecho, A., Pezo, D., Santos, N., 2016. Whole-genome characterization of a Peruvian alpaca rotavirus isolate expressing a novel VP4 genotype. Vet. Microbiol. 196, 27–35.

Rojas, M.A., Gonçalves, J.L.S., Dias, H.G., Manchecho, A., Santos, N., 2017. Identification of two novel rotavirus a genotypes, G3S and P[50], from Peruvian alpaca faeces. Infect. Genet. Evol. 55, 71–74.

RotaVirus Classification Working Group (RCWG), 2018. List of Accepted Genotypes. http://rega.kuleuven.be/cev/viralmetagenomics/virus-classification/rcwg.

Sasaki, M., Setiyono, A., Handharyani, E., Rahmadani, I., Taha, S., Adianhi, S., Subanghit, M., Sawa, H., Nakamura, I., Kimura, T., 2012. Molecular detection of a novel paramyxovirus in fruit bats from Indonesia. Virol. J. 9, 240.

Sasaki, M., Orba, Y., Sasaki, S., González, G., Ishii, A., Hang’ombe, B.M., Mweene, A.S., Ito, K., Sawa, H., 2016. Multi-reassortant G3P[3] group A rotavirus in a horseshoe bat in Zamb. J. Gen. Virol. 97 (10), 2488–2493.

Waruhu, C., Ommehe, S., Obanda, V., Agwanda, V., Gakuya, F., Ge, X.Y., Yang, X.L., Wu, L.J., Zohaib, A., Hu, B., Shi, Z.L., 2017. Molecular detection of viruses in Kenyan bats and discovery of novel astroviruses, caliciviruses and rotaviruses. Virol. Sin. 32 (2), 101–114.

Xia, L., Fan, Q., He, B., Xu, L., Zhang, F., Hu, T., Wang, Y., Li, N., Qiu, W., Zheng, Y., Matthijnssens, J., Tu, C., 2014. The complete genome sequence of a G3P[10] Chinese bat rotavirus suggests multiple bat rotavirus inter-host species transmission events. Infect. Genet. Evol. 28, 1–4.

Yinda, C.K., Zeller, M., Conceição-Neto, N., Mba, P., Deboerre, W., Beller, L., Heylen, E., Ghogomu, S.M., Van Ranst, M., Matthijnssens, J., 2016. Novel highly divergent reassortant bat rotaviruses in Cameroon, without evidence of zoonosis. Sci. Rep. 6, 34209.