Running title: novel rhabdovirus in Kenya bat

Short Communication

Isolation and molecular characterization of Fikirini rhabdovirus, a novel virus from a Kenya bat

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The GenBank accession number for the genome of Fikirini rhabdovirus is KC676792.

Abstract

Zoonotic and vector-borne pathogens have comprised a significant component of emerging human infections in recent decades, and bats are increasingly recognized as reservoirs for many of these disease agents. To identify novel pathogens associated with bats, we screened tissues of bats collected in Kenya. Virus isolates were identified by next generation sequencing of viral nucleic acid preparations from the infected cell culture supernatant and characterized molecularly. Here we report the identification of Fikirini rhabdovirus, a novel rhabdovirus isolated from a bat, Hipposideros vittatus, captured along the Kenyan coast.

Since 1980, novel human pathogens have been discovered at a rate of over three per year, and approximately 75% of these have been viruses, with 80% associated with non-human vertebrate reservoirs (Woolhouse and Gaunt, 2007). Bats have been recognized as reservoir hosts for highly virulent viruses including lyssaviruses (Rupprecht et al. 2011), henipaviruses (Young et al 1996, Chua et al. 2000), SARS-related coronaviruses (Li et al. 2005), and filoviruses (Leroy et al., 2005, 2009; Towner et al. 2007, 2009; Negredo et al. 2011). Evolutionarily, bats have also been identified as major natural reservoirs of hepaciviruses and paramyxoviruses, two taxa which contain viruses responsible for prominent human diseases (Drexler et al. 2012, Quan et al. 2013). Together, these discoveries have made bats a focal target for virus discovery efforts.

Little is known about the public health burden, prevalence, and distribution of African bat lyssaviruses (Markotter et al. 2008). For example, in Kenya, van Thiel et al. (2009) reported a fatal human case of Duvenhage virus following exposure to a bat. In response to this knowledge gap, enhanced surveillance for lyssaviruses and other zoonotic pathogens among bats in Kenya has uncovered multiple viruses including the novel Shimoni bat virus (*Rhabdoviridae: Lyssavirus*) from *Hipposideros vittatus* (formerly

known as *H. commersoni*) (Kuzmin et al. 2010a), Lagos bat virus (*Rhabdoviridae: Lyssavirus*) from *Eidolon helvum* and *Rousettus aegyptiacus* (Kuzmin et al. 2008a), and serologic evidence for West Caucasian bat virus (*Rhabdoviridae: Lyssavirus*) in *Miniopterus* spp. (Kuzmin et al. 2008b). In addition, viruses from numerous families have been isolated and/or detected from a diversity of bat species such as Marburg virus (*Filoviridae: Marburgvirus*) from *R. aegyptiacus* (Kuzmin et al. 2010b), a rotavirus (*Reoviridae: Rotavirus*) from *E. helvum* (Esona et al. 2010), 41 coronaviruses (*Coronaviridae: Alphacoronavirus, Betacoronavirus*) from 14 genera of bats (Tong et al. 2009, Tao et al. 2012), 11 distinct lineages of polyomaviruses (*Polyomaviridae: Polyomavirus)* (Tao et al 2013), and a variety of hepaciviruses (Quan et al., 2013). Historically, Mount Elgon bat virus (*Rhabdoviridae:* Oita group) was isolated from *Rhinolophus hildebrandtii eloquens* (Metselaar et al. 1969). Given these prior data, the specific aim of this study was to screen tissues of bats captured in Kenya for the presence of additional novel pathogens.

Bats were captured throughout Kenya during August – September 2011 (Fig. 1) Bat capture and tissue sampling techniques were performed as described by Kuzmin et al. (2008a) under animal protocol 2096FRAMULX-A3 approved by the Institutional Animal Care and Use Committee of the U.S. Centers for Disease Control and Prevention (Atlanta, GA) and local authorities (permit KWS/5001). All bats were euthanized to harvest tissues for virus isolations. In total, tissues were analyzed from 447 bats, including 31 *Coleura afra*, 8 *Eidolon helvum*, 78 *Hipposideros vittatus*, 153 *Miniopterus minor*, 80 *Miniopterus* spp., 2 *Nycteris* spp., 12 *Otomops martiensseni*, 9 *Rhinolophus landeri*, 7 R*hinolophus* spp., 56 *Rousettus aegyptiacus*, 1 *Taphozous* spp., and 12 *Triaenops afer* (formerly known as *T. persicus*). Liver and/or spleen specimens were homogenized for virus isolation.

Approximately 0.5 – 1 cm^3 sections of tissue were mechanically homogenized in a 2.0 mL snap cap tube containing 1.5 mL BA1 medium (Hanks M-199 salts, 0.05M Tris pH 7.6, 1% bovine serum albumin, 0.35 g/L sodium bicarbonate, 100 U/mL streptomycin, 1μg/mL Fungizone) and one or two stainless steel
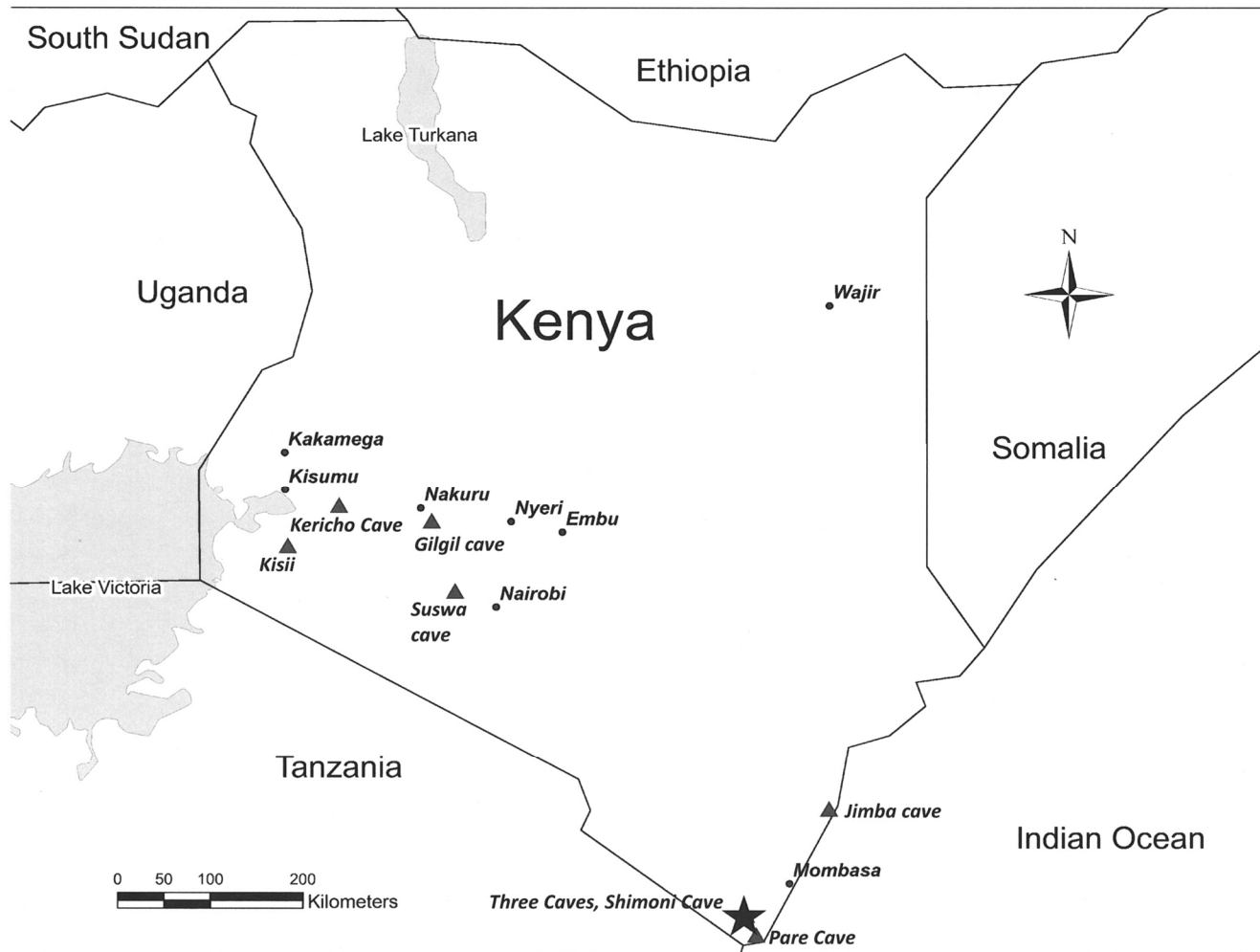
Figure 1. Map of bat collection sites in Kenya, August – September 2011. The collection sites are shown with triangles and the site where Fikirini rhabdovirus was isolated is denoted by a star.

5 mm beads in a Qiagen mixer mill (Qiagen, Valencia, CA) at 25 cycles/sec for four minutes.

Homogenates were clarified by centrifugation at approximately 12,800 x g for 8 min at 4°C and stored

at -80 °C. One hundred microliters of homogenized tissue supernatant was inoculated directly onto

Vero cell monolayers, with one sample per well on a 6-well plate for virus isolation by double-overlay

plaque assay (Miller et al. 1989). A second overlay containing neutral red was added four days post

infection, and plates were observed for plaques up to 10 days post infection. Plaque-positive wells were

harvested into 1 mL DMEM+10% fetal bovine serum and clarified by centrifugation and the infected

supernatant was stored at -80°C.   The viral RNA from plaque-positive samples was extracted from 200µl

of the supernatant and eluted into a final volume of 140 µl AE buffer using the Qiagen BioRobot EZ1

Workstation using the EZ1 Virus Mini Kit v2.0.

Virus isolates were subjected to next generation sequencing. High-quality viral nucleic acid was

prepared by digesting extracted RNA with DNase I (Invitrogen/Life Technologies, Grand Island, NY)

according to the manufacturer's instructions followed by purification using the Qiagen RNeasy mini kit.

The cDNA was generated from 100 ng digested, purified viral RNA using the Ovation® RNA-Seq System

V2 kit (NuGEN Technologies, Inc., San Carlos, CA). A cDNA library was then prepared from 400 ng

purified cDNA using the Ion Xpress™ Plus gDNA and Amplicon Library Preparation kit (Ion Torrent, Life

Technologies, South San Francisco, CA) according to the manufacturer's instructions and using an

enzymatic shear-time of eight minutes. The amplified cDNA library was quantified on the 2100

Bioanalyzer (Agilent Technologies, Santa Clara, CA) and diluted to a concentration of 280 million

molecules per microliter in deionized water. Template-positive Ion Sphere Particles were generated and

enriched using the Ion OneTouch™ 200 Template Kit v2 or the v2 DL kit (Ion Torrent, Life Technologies,

South San Francisco, CA). Enriched Ion Sphere Particles were then sequenced on the Ion Personal

Genome Machine using the Ion PGM™ Sequencing 200 Kit or Ion PGM™ Sequencing 300 Kit and an Ion

316™ Chip. *De novo* sequence assemblies were performed using both the Lasergene Genomics Suite

(DNA STAR, Inc., Madison, WI) and CLC Genomics Workbench 5.1.1 (CLC Bio, Cambridge, MA) software packages. Contigs were searched against the GenBank database to identify the closest sequence relatives, genomic location, and orientation.

Based on these efforts, a novel rhabdovirus was identified from the liver of *H. vittatus*. This virus was named Fikirini rhabdovirus after the forests immediately surrounding the caves where the infected bat was captured (Fig. 1). The complete 11,139-nt genome sequence of the rhabdovirus (GenBank accession number KC676792) was confirmed via conventional RT-PCR and direct sequencing using novel primers (Supplementary Tables S1 – S2). Sequences of the rhabdovirus genome termini were determined using the 5'/3'RACE kit, 2nd generation (Roche, Indianapolis, IN) per manufacturer's instructions. Virus-specific primers used for RACE are available upon request. The genome of Fikirini rhabdovirus contained the expected rhabdovirus open reading frames (ORFs): nucleoprotein (N), phosphoprotein (P), matrix (M), glycoprotein (G), and polymerase (L) (Fig. 2). There were no additional accessory proteins or ORFs in alternate reading frames.
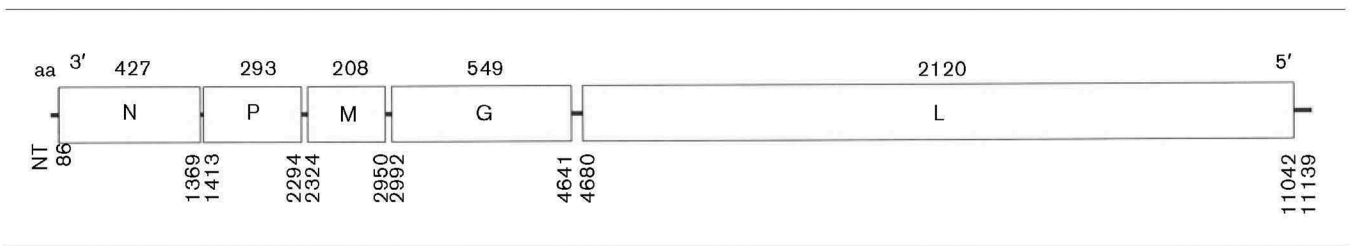

Figure 2. Schematic diagram of the genome and open reading frames of Fikirini rhabdovirus.

Maximum Likelihood phylogenetic trees were generated in MEGA 5.05 (Tamura et al. 2011) for different fragments of rhabdovirus genomes available in GenBank. Among these, only N sequences were available for the viruses most closely related to Fikirini rhabdovirus. The analysis was performed using a complete deletion substitution model and 1000 bootstrap replicates (Fig. 3).
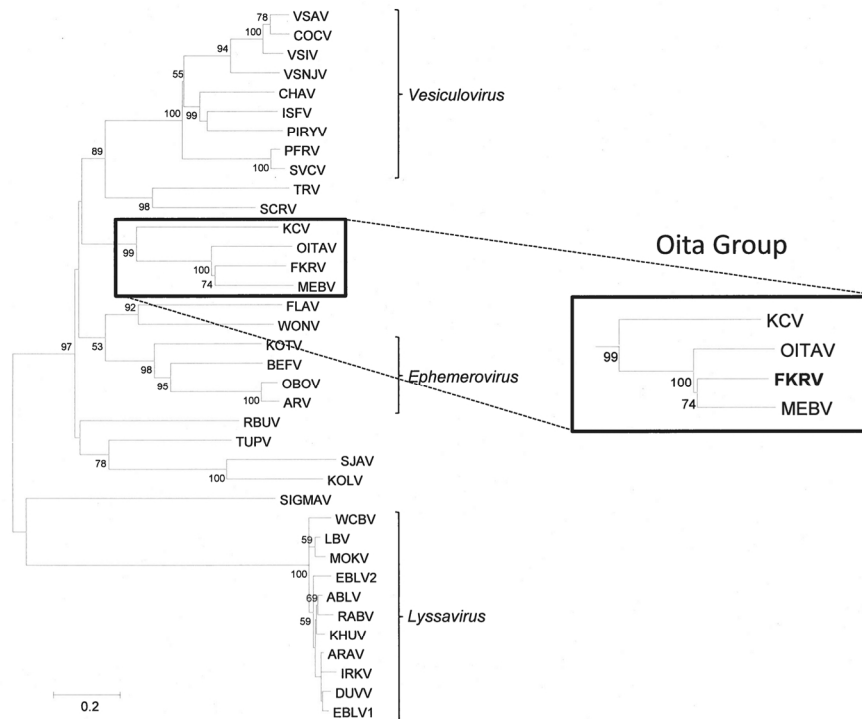
Figure 3. Maximum likelihood tree based on partial amino acid nucleoprotein gene sequences (complete gap deletion). The shortest nucleoprotein sequence used to construct the phylogeny was Mount Elgon bat virus which was 302 amino acids in length. Scale bar indicates substitutions per site. Bootstrap values >50% are shown (1000 replicates). Fikirini rhabdovirus (FKRV), Adelaide River virus (ARV) Q65111, Aravan virus (ARAV) Q6X1D8, Australian bat lyssavirus (ABLV) AAD01267, Bovine ephemeral fever virus (BEFV) NP_065398, Chandipura virus (CHAV) P11211, Cocal virus (COCV) ACB47434, Drosophila melanogaster sigma virus (SIGMAV) ACV67011, Duvenhage virus (DUVV) Q66453, European bat lyssavirus 1 (EBLV1) AAX62875, European bat lyssavirus 2 (EBLV2) YP_001285393, Flanders virus (FLAV) AAN73283, Isfahan virus (ISFV) Q5K2K7, Kern Canyon virus (KCV) ABE69215, Khujand virus (KHUV) Q6X1D4, Kolongo virus (KOLV) ABE69214, Kotonkon virus (KOTV) ABE69213, Lagos bat virus (LBV) ABF56214, Mokola virus (MOKV) YP_142350, Mount Elgon bat virus (MEBV) ABE69217, Obodhiang virus (OBOV) ABE69212, Oita rhabdovirus (OITAV) BAD13431, Pike fry rhabdovirus (PFRV) ACP27998, Piry virus (PIRYV) P26037, Rabies virus (RABV) ACN51666 , Rochambeau virus (RBUV) ABE69218, Sandjimba virus (SJAV) ABE69216, Siniperca chuatsi rhabdovirus (SCRV) YP_802937, Spring viraemia of carp virus (SVCV) ABW24033, Trout rhabdovirus 903/87 (TRV) AAL35756, Tupaia virus (TUPV) YP_238528, Vesicular stomatitis Alagoas virus (VSAV) ACB47439, Vesicular stomatitis Indiana virus (VSIV) P11212, Vesicular stomatitis New Jersey virus (VSNJV) P04881, West Caucasian bat virus (WCBV) Q5VKP2, Wongabel virus (WONV) YP_002333271.

From the infected bat, additional sections of lung, kidney, brain, and intestine as well as oral and fecal swabs were also processed for virus isolation as above, and screened for viral nucleic acids. The samples were screened by RT-PCR using primers designed from the nucleoprotein gene: KEN352-00046F ACGGTCTTTCGACTGGCTTC / KEN352-00729R AAGAGCCGAGCAATCCTTGA and polymerase gene: KEN352-10555F GTTGGGAGCTGGCTATTGGA / KEN352-11036R CCCACGGAGTTTGAGATCCT. All tested tissues (e.g. liver, spleen, lung, kidney, intestine, brain), and feces contained infectious rhabdovirus as well as viral RNA. Only the oral swab from this bat was negative for both infectious virus and viral RNA.

Worldwide, bats have been found associated with a diversity of rhabdoviruses (Calisher et al. 2006, Kuzmin et al. 2009, Aznar-Lopez et al. 2013). Many of these bat rhabdoviruses have been classified in the genus *Lyssavirus*, including Shimoni bat virus which was also isolated from coastal Kenya from *H. vittatus* (Kuzmin et al. 2010a). Based on the available N gene sequences, *Mount Elgon bat virus*, *Kern Canyon virus*, and *Oita virus* constitute a distinct monophyletic clade (Kuzmin et al. 2006) (Fig. 3). Viruses in this group appear to be associated with insectivorous bats throughout the world, with Oita virus found in *R. cornutus* in Japan (Iwasaki et al. 2004), Mount Elgon bat virus in *R. hildebrandtii eloquens* in Kenya (Metselaar et al. 1969), and Kern Canyon virus in *Myotis yumanensis* in the United States (Murphy and Fields 1967). *Fikirini rhabdovirus* also falls in this taxanomic group and is associated with an insectivorous bat host (Fig. 3). Overall, Fikirini rhabdovirus and Oita virus share 57% amino acid identity and 71% similarity across the nucleoprotein with long regions of extremely high identity between these two viruses (i.e. amino acids 272-348 of N show 91% identity and 95% similarity). Serologically, *Nkolbisson*, *Barur*, and *Fukuoka viruses* also fall within this group of rhabdoviruses (Calisher et al. 1989), although sequence data supporting this classification are limited. Aznar-Lopez et al. (2013) recently published a rhabdovirus phylogeny based on a short fragment of the polymerase gene. In this analysis, *Nkolbisson*, *Barur*, and *Fukuoka viruses* also clustered in the same group with *Kern Canyon, Oita,* and *Mount Elgon bat viruses*. However, more complete sequence data are needed to further resolve their

phylogenetic relationships, and ascertain the exact placement of *Fikirini rhabdovirus* within this complex.

The finding of infectious virus and viral nucleic acid in multiple tissues of the bat provides further insight into host tropisms and potential mechanisms of transmission, although we did not did not confirm virus presence in specific tissues through histopathology. Infectious rhabdovirus particles and viral RNA were present in every tissue tested and in the feces, but not the oral swab. This finding suggests that the main exit portal for this virus may be through feces as opposed to saliva via a bite. The finding of lyssavirus nucleic acid in the feces has been reported previously (Allendorf et al. 2012). However, even for lyssaviruses the presence in saliva is intermittent and depends on the stage of the disease. Arthropod transmission of Fikirini rhabdovirus remains a possibility worthy of exploration. Nkolbisson virus was isolated from mosquitoes in Cameroon, Barur virus from a rat and ticks in India, mosquitoes in Kenya, and ticks in Somalia, and Fukuoka virus from midges and cattle in Japan (Calisher et al. 1989, Noda 1992). A Barur-like virus was also isolated from ticks in Kenya (Sang et al. 2006). While Nkolbisson virus has also been isolated from a human in the Central African Republic (Ndiaye et al. 1987), the broader public health significance of these rhabdoviruses, and the potential of Fikirini rhabdovirus to be transmitted by arthropods are unknown.

The extent of human exposure to Fikirini rhabdovirus is also unclear. *Hipposideros vittatus* is a cave-roosting bat. At present, the caves are visited by guano collectors, tourists, and researchers, which could each serve as potential opportunities for exposure especially with regard to viral shedding in the feces. Fikirini rhabdovirus grew to high titers and formed plaques in Vero cells. *In vivo*, Oita virus and Mount Elgon bat virus caused lethal encephalitis in mice (Murphy et al. 1970, Catalog of Arboviruses 1970, Iwasaki et al. 2004), suggesting that Fikirini rhabdovirus is likely to be similarly pathogenic, although these studies have yet to be completed. Because this virus causes pathology in non-human primate cells

and is closely related to viruses that cause morbidity and/or mortality in other vertebrates, further study is warranted to assess its zoonotic potential.

References

Allendorf, S.D., Cortez, A., Heinemann, M.B., Harary, C.M., Antunes, J.M., Peres, M.G., Vicente, A.F., Sodré, M.M., da Rosa, A.R., & other authors. (2012). Rabies virus distribution in tissues and molecular characterization of strains from naturally infected non-hematophagous bats**. *Virus Res* **165**, 119—125.

Aznar-Lopez C., Vazquez-Moron, S., Marston, D.A., Juste, J., Ibáñez, C., Berciano, J.M., Salsamendi, E., Aihartza, J., Banyard, A.C., & other authors. (2013). Detection of rhabdovirus viral RNA in oropharyngeal swabs and ectoparasites of Spanish bats. *J Gen Virol* **94,** 69—75.

Catalog of Arboviruses. (1970). No. 224. Mount Elgon bat (MEB), Strain BP846. *Am J Trop Med Hyg* **19**, 1119 – 1120.

Calisher, C.H., Karabatsos, N., Zeller, H., Digoutte, J-P., Tesh, R.B., Shope, R.E., Travassos da Rosa, A.P.A., St. George, T.D. (1989). Antigenic relationships among rhabdoviruses from vertebrates and hematophagous arthropods. *Intervirology* **30**, 241–257.

Calisher, C.H., Childs, J.E., Field, H.E., Holmes, K.V., Schountz, T. (2006) Bats: Important Reservoir Hosts of Emerging Viruses. *Clin Microbiol Rev* **19,** 531—545.

Chua, K.B., Bellini, W.J., Rota, P.A., Harcourt, B.H., Tamin, A., Lam, S.K., Ksiazek, T.G., Rollin, P.E., Zaki, S.R., & other authors. (2000). Nipah virus: a recently emergent deadly paramyxovirus. *Science* **288**, 1432 – 1435.

Esona, M.D., Mijatovic-Rustempasic, S., Conrardy, C., Tong, S., Kuzmin, I., Agwanda, B., Breiman, R.F., Banyai, K., Niezgoda, M., & other authors. (2010). Reassortant group A rotavirus from straw-colored fruit bat (*Eidolon helvum*). *Emerg Inf Dis* **16**, 1844–1852.

Drexler, J.F., Corman, V.M., Müller, M.A., Maganga, G.D., Vallo, P., Binger, T., Gloza-Rausch, F., Rasche, A., Yordanov, S., & other authors. (2012). Bats host major mammalian paramyxoviruses.

Nat Commun 3,796.

Iwasaki, T., Inoue, S., Tanaka, K., Sato, Y., Morikawa, S., Hayasaka, D., Moriyama, M., Ono, T., Kanai, S., & other authors. (2004). Characterization of Oita virus 296/1972 of Rhabdoviridae isolated from a horseshoe bat bearing characteristics of both lyssavirus and vesiculovirus. *Arch Virol* **149**,1139– 1154.

Kuzmin, I.V., Hughes, G.J., & Rupprecht, C.E. (2006). Phylogenetic relationships of seven previously unclassified viruses within the family *Rhabdoviridae* using partial nucleoprotein gene sequences. *J Gen Virol* **87**, 2323 – 2331.

Kuzmin, I.V., Niezgoda, M., Franka, R., Agwanda, B., Markotter, W., Beagley, J.C., Urazova, O.Y., Breiman, R.F. & Rupprecht, C.E. (2008a). Lagos Bat Virus in Kenya. *J Clin Microbiol* **46**, 1451–1461.

Kuzmin, I.V., Niezgoda, M., Franka, R., Agwanda, B., Markotter, W., Beagley, J.C., Urazova, O.Y., Breiman, R.F., Rupprecht, C.E. (2008b). Possible emergence of West Caucasian bat virus in Africa. *Emerg Inf Dis* **14**, 1887 – 1889.

Kuzmin, I.V., Novella, I.S., Dietzgen, R.G., Padhi, A., & Rupprecht, C.E. (2009). The rhabdoviruses: Biodiversity, phylogenetics, and evolution. *Inf Gen Evol* **9**, 541—553.

Kuzmin, I.V., Mayer, A.E., Niezgoda, M., Markotter, W., Agwanda, B., Breiman, R.F., &Rupprecht, C.E. (2010a). Shimoni bat virus, a new representative of the Lyssavirus genus. *Virus Res* 149, 197– 210.

Kuzmin, I.V., Niezgoda, M., Franka, R., Agwanda, B., Markotter, W., Breiman, R.F., Shieh, W-J., Zaki, S.R., & Rupprecht, C.E. (2010b). Marburg virus in fruit bat, Kenya. *Emerg Inf Dis* **16**,352–354.

Leroy, E.M., Kumulungui ,B., Pourrut, X., Rouquet, P., Hassanin, A., Yaba, P., Délicat, A., Paweska, J.T., Gonzalez, J.P., & other authors. (2005). Fruit bats as reservoirs of Ebola virus. *Nature* **438**, 575—576.

Leroy, E.M., Epelboin, A. Mondonge, V., Pourrut, X., Gonzalez, J-P, Muyembe-Tamfum, J-J, & Formenty, P. (2009). Human Ebola Outbreak Resulting from Direct Exposure to Fruit Bats in Luebo, Democratic Republic of Congo, 2007. *Vector-Borne and Zoonotic Dis* **9**, 723—728.

Li, W., Shi, Z., Yu, M., Ren, W., Smith, C., Epstein, J.H., Wang, H., Crameri, G., Hu, Z., & other authors. (2005). Bats are natural reservoirs of SARS-like coronaviruses. *Science* **310**, 676–679.

Markotter, W., Van Eeden, C., Kuzmin, I.V., Rupprecht, C.E., Paweska, J.T., Swanepoel, R., Fooks, A.R., Sabeta, C.T., Cliquet, F., & other authors.(2008). Epidemiology and pathogenicity of African bat lyssaviruses. *Dev Biol (Basel)* **131**, 317—325.

Metselaar, D., Williams, M.C., Simpson, D.I.H., West, R., & Mutere, F.A. (1969). Mount Elgon bat virus: A hitherto undescribed virus from *Rhinolophus hildebrandtii eloquens* (K. Anderson). *Arch Gesamte Virusforch*, **26**,183 – 193.

Miller, B.R., Mitchell, C.J., & Ballinger, M.E. (1989). Replication, tissue tropisms and transmission of yellow fever virus in *Aedes albopictus*. *Trans R Soc Trop Med Hyg* **83**, 252–255.

Murphy, F.A., & Bernard, B.N. (1967). Kern Canyon virus: electron microscopic and immunological studies. *Virol* **33**, 625—637.

Murphy, F.A., Shope, R.E., Metselaar, D., & Simpson, D.I.H. (1970). Characterization of Mount Elgon bat virus, a new member of the Rhabdovirus group. *Virol* **40**, 288—297.

Ndiaye, M., Saluzzo, J.F., Digoutte, J.P., & Mattei, X. (1987). Identification du virus Nkolbisson par microscopie electronique. *Ann Inst Pasteur Virol* **138**, 517—521.

Negredo, A., Palacios, G., Vázquez-Morón, S., González, F., Dopazo, H., Molero, F., Juste, J., Quetglas, J., Savji, N., & other authors. (2011). Discovery of an ebolavirus-like filovirus in Europe. *PLoS Pathog* **7,** e1002304**.**

Noda, M., Inaba, Y., Banjo, M., Kubo, M. (1992). Isolation of Fukuoka virus, a member of the Kern Canyon serogroup viruses of the family Rhabdoviridae, from cattle. *Vet Microbiol* **32**, 267—271.

Quan, P.L., Firth, C., Conte, J.M., Williams, S.H., Zambrana-Torrelio, C.M., Anthony, S.J., Ellison, J.A., Gilbert, A.T., Kuzmin, I.V., Niezgoda, M., Osinubi, M.O., & other authors. (2013). Bats are a major natural reservoir for hepaciviruses and pegiviruses. *Proc Natl Acad Sci U S A*. **110**, 8194—8199.

Rupprecht, C.E., Turmelle, A., Kuzmin, I.V. (2011). A perspective on lyssavirus emergence and perpetuation. *Curr Opin Virol* **1**, 662—670.

Sang, R., Onyango C., Gachoya, J., Mabinda, E., Konongoi, S., Ofula, V., Dunster, L., Okoth, F., Coldren, R., & other authors (2006). Tickborne Arbovirus Surveillance in Market Livestock, Nairobi, Kenya. *Emerg Infect Dis* **12,** 1074—1080.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei , M., & Kumar S., (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Mol Biol Evol* **28**, 2731–2739.

Tao, Y., Tang, K., Shi, M., Conrardy, C., Li, K.S., Lau, S.K., Anderson, L.J., & Tong, S. (2012). Genomic characterization of seven distinct bat coronaviruses in Kenya. *Virus Res 167,* 67—73.

Tao, Y., Mang, S., Conrardy, C., Kuzmin, I.V., Recuenco, S., Agwanda, B., Alvarez, D.A., Ellison, J.A., Gilbert, A.T., & other authors. (2012). Discovery of Diverse Polyomaviruses in Bats and the Evolutionary History of the Polyomaviridae. *J GenVirol* doi:10.1099/vir.0.047928-0.

Tong, S., Conrardy, C., Ruone, S., Kuzmin, I., Guo, X., Tao, Y., Niezgoda, M., Haynes, L., Agwanda, B., & other authors. (2009). Detection of novel SARS-like and other coronaviruses in bats from Kenya. *Emerg Inf Dis* **15**, 482—485.

Towner, J.S., Pourrut, X., Albariño, C.G., Nkogue, C.N., Bird, B.H., Grard, G., Ksiazek, T.G., Gonzalez, J.P., Nichol, S.T., & Leroy, E.M. (2007). Marburg virus infection detected in a common African bat. *PLoS One* 2, e764.

Towner, J.S., Amman, B.R., Sealy, T.K., Carroll, S.A.R., Comer, J.A., Kemp, A., Swanepoel, R., Paddock, C.D., Balinandi, S., & other authors (2009). Isolation of Genetically Diverse Marburg Viruses from Egyptian Fruit Bats. *PLoS Pathog* **5**, e1000536.

van Thiel, P.P., de Bie, R.M., Eftimov, F., Tepaske, R., Zaaijer, H.L., van Doornum, G.J., Schutten, M., Osterhaus, A.D., Majoie, C.B., & other authors (2009). Fatal human rabies due to Duvenhage virus from a bat in Kenya: failure of treatment with coma-induction, ketamine, and antiviral drugs. *PLoS Negl Trop Dis* **28**,e428.

Woolhouse, M., Gaunt, E. (2007). Ecological origins of novel human pathogens. *Critical Rev Microbiol* **33**, 231–242.

Young, P., Field, H., & Halpin, K. (1996). Identification of likely natural hosts for equine morbillivirus. *Communicable Diseases Intelligence* **20**, 476.

Supplementary Tables.

Table S1. Primers used for RT-PCR amplification of Fikirini
bat rhabdovirus genome fragments

Primer	Position	Sequence (5'→3')
KEN352-p1Fb	00044	TGAGCTTCACAGGCAAGCG
KEN352-p1R	02935	TCACGGTCTTTCGACTGGC
KEN352-p2F	02660	CCATCTTGGCGATTTCTCG
KEN352-p2R	04858	GCAGTCCGTGTCTCTTACG
KEN352-p3F	04713	ACCCAGCCTTTGTAGAATGC
KEN352-p3R	06918	GTTGAGGTACCATTTCCAGC
KEN352-p4F	06714	GATTGCATAGTCTCATCCTC
KEN352-p4R	09253	ATACGGAGAGAAGCTAGGG
KEN352-p5F	09015	GGACAACTCCCGATACACC
KEN352-p5Ra	10739	ACCACACAAGGTACCTCC

Position designations are approximate, based on Next Generation sequencing
results. Forward/reverse designations are based on positive-sense RNA.

Table S2. Primers used for sequencing Fikirini bat rhabdovirus

Primer	Sequence (5'→3')	Primer	Sequence (5'→3')
KEN352-00002F	CGTGAGCTTCACAGGCAA	KEN352-10473R	CTGATGTGATGTTGATTA
KEN352-00601F	ATACTGCATCCTCAGTAC	KEN352-09873R	GTGGTCTGCTAGCCTAGA
KEN352-01201F	GGATTCCAGCTTCCTGAA	KEN352-09273R	ATGAGGTGGACAACTCCC
KEN352-01801F	TATATCGTCTCTTGGACT	KEN352-08673R	TCTCTGCGACGGAAACAG
KEN352-02401F	AGTTCAGTCAAGTATTGA	KEN352-08073R	AAGTAAGCTGTATATGGA
KEN352-03001F	GCTCCGTTCTATGAACCT	KEN352-07473R	ATTGGGTTTTTCAAGAAG
KEN352-03601F	CACGCCGAATTATCACTA	KEN352-06273R	TAGCAATGCAAACTTGGG
KEN352-04201F	TCCTATTCGATACCTGCT	KEN352-05673R	ACTTCTTTCTCAACACAA
KEN352-04833F	ACGTCACTTTCCCGGTGG	KEN352-05073R	CATCTTGAATCCCAGTAT
KEN352-05401F	CCCAATTTTGAGCGACAT	KEN352-04473R	CCATATGAAATCATCAGG
KEN352-06001F	CCACCAGGTGAATGAGAC	KEN352-03873R	TCCTCCATGTCGTATATG
KEN352-06601F	GTGATCAGAACTCTAAGT	KEN352-03273R	CTCCTTGGCAAAATACCA
KEN352-07201F	GGAAAATACCTGAAGGAT	KEN352-02673R	TAAGAGACACGGACTGCT
KEN352-07801F	GTTGGGCACATACAGGTA	KEN352-02073R	CTTCCTGATTTGTTCTTC
KEN352-08401F	GGTGGAGAAAATTATGAC	KEN352-01473R	GACTCAGCTGTTTCGAAG
KEN352-09004F	TTGATCAACTCACCACAC	KEN352-00872R	TAGGCCACGTAGGAGTCT
KEN352-09601F	GCATAACCTACTCTGACT	KEN352-00273R	GAATACCTTGTACAGCAC
KEN352-10202F	TGCGATCAAAATATGTTG		
KEN352-10806F	CAGGGCTGTGTGCTGAAT		

Position designations are approximate, based on Next Generation sequencing results. Forward/reverse designations are
based on positive-sense RNA.