Filoviruses and bats



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While Reston and Lloviu viruses have never been associated with human disease, the other filoviruses cause outbreaks of hemorrhagic fever characterised by person-to-person transmission and high case fatality ratios. Cumulative evidence suggests that bats are the most likely reservoir hosts of the filoviruses. Ecological investigations following Marburg virus disease outbreaks associated with entry into caves inhabited by *Rousettus aegyptiacus* bats led to the identification of this bat species as the natural reservoir host of the marburgviruses. Experimental infection of *R. aegyptiacus* with Marburg virus has provided insight into the natural history of filovirus infection in bats that may help guide the search for the reservoir hosts of the ebolaviruses.

Filovirus history and geographic range

The phylogeny illustrates the genetic relationships between the filoviruses and the associated map shows the known range of filovirus circulation according to virus (Figure 1). Marburg virus (MARV) was first described in 1967 following two successive filovirus hemorrhagic fever (FHF) outbreaks among German and former-Yugoslavian laboratory workers that had handled primates imported from Uganda[1]. Ravn virus (RAVV), also a marburgvirus, was initially isolated from a 1987 fatal case in Kenya[2]. Nearly simultaneous FHF outbreaks in present-day South Sudan and the Democratic Republic of the Congo (DRC), led to the identification of Sudan virus (SUDV)[3] and Ebola virus (EBOV)[4], respectively. Reston virus was discovered in 1989 following an epizootic of FHF among macaques exported to the United States from the Philippines[5]. Taï Forest virus has been isolated once only from a nonfatal case that became ill following the necropsy of a chimpanzee that died from a hemorrhagic disease in Côte d'Ivoire in 1994[6]. Bundibugyo virus was initially isolated during a FHF outbreak in Uganda in 2007[7]. Lloviu virus was identified during the investigation of a die-off of *Miniopterus schreibersii* bats in Spain in 2002[8]. A partial genomic sequence recovered from a *Rousettus leschenaultii* bat captured in China in 2013 likely represents a novel filovirus[9]. Ecological niche modelling has confirmed the known range of filovirus circulation and has predicted additional areas throughout sub-Saharan Africa and Southeast Asia that are suitable for zoonotic transmission of filoviruses[10–13].

Evidence suggests that bats are natural reservoir hosts of the filoviruses

Although contact with non-human primate or duiker tissue has been linked to FHF outbreaks[1,14–16], the high mortality caused by filoviruses in these animals indicate that they are only incidental hosts. However, FHF outbreak investigations have revealed that many of the index cases had entered environments inhabited by bats prior to disease onset. In 1975, MARV disease occurred in a tourist that had stayed in two hotels populated with bats and visited Chinhoyi Caves in present-day Zimbabwe 8–9 days prior to disease onset[17]. The index case in the 1976 outbreak of SUDV disease worked at a cotton factory containing *Mops trevori*[18] and the index case in the 1979 SUDV disease outbreak worked at the same factory[19]. Fifteen days before becoming ill, the index case in the

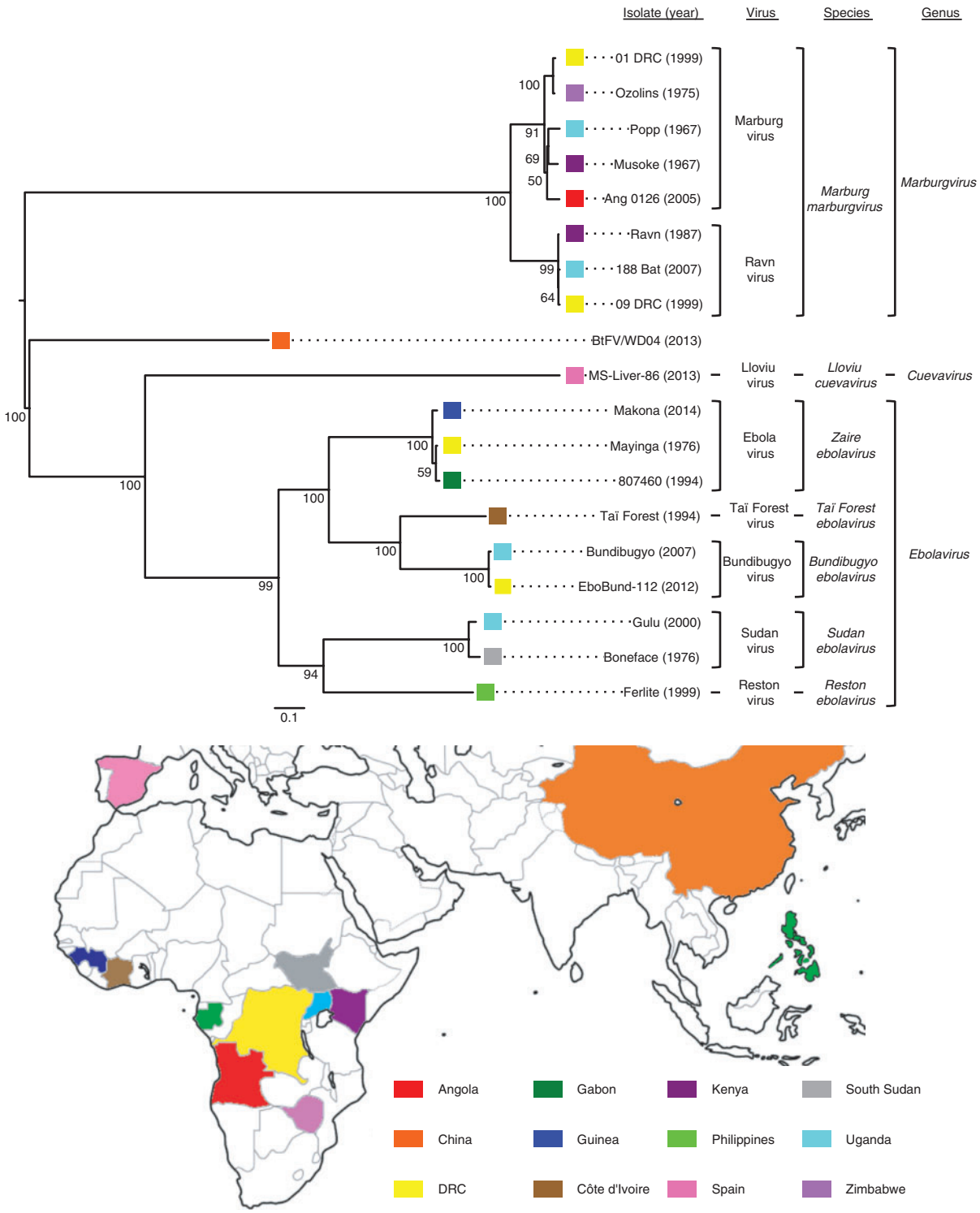

Figure 1. Filovirus maximum-likelihood phylogeny and geographic distribution. The phylogeny was derived from concatenated partial nucleoprotein, viral protein 35 and RNA-dependent RNA polymerase filovirus gene sequences. A single representative sequence from each country in which filovirus zoonotic spillover has been detected or spillover into humans has occurred was selected to capture the geographic range of virus circulation. Sequences are coloured according to the sampling location and the colours correspond to those used in the associated map and legend. The numbers to the lower-left of the nodes are bootstrap percentages based on 1000 replicates. Horizontal branch lengths are proportional to the genetic distance between sequences and the scale underneath the phylogeny indicates the number of nucleotide substitutions per site.

1980 MARV disease outbreak had entered Kenya's bat-populated Elgon Caves[2] and the 1987-isolated case of RAVV disease had visited Kenya's Kitum Cave prior to becoming ill[20]. After the large 1995 epidemic of EBOV disease in present-day DRC, 24 plant and 19 vertebrate and invertebrate native species were experimentally inoculated with EBOV[21]. Three bat species (*Mops condylurus*, *Chaerephon pumilus* and *Epomophorus wahlbergi*) supported EBOV replication and seroconverted in the absence of overt clinical disease, while the remaining animal and plant species were refractory to virus infection. These findings supported the accumulating

number of links between FHF index cases and prior exposure to environments inhabited by bats. This linkage became stronger when it was discovered that 52% of the 154 cases in a series of MARV disease outbreaks in the DRC between 1998 and 2000 worked in the underground Goroumbwa Mine known to house hundreds of thousands of bats[22]. In 2007, an EBOV disease outbreak followed a reported annual migration of *Hypsignathus monstrosus* and *Epomops franqueti* and the putative index case had purchased bats for consumption[23]. The index cases in a series of MARV and RAVV disease outbreaks in 2007 worked in Kitaka Mine, Uganda[24] and two cases of MARV disease were found in tourists that had separately visited nearby-Python Cave in 2008[25,26].

Rousettus aegyptiacus identified as a natural reservoir host for the marburgviruses

Ecological investigations following the 2007–2008 MARV and RAVV disease outbreaks in Uganda revealed that Kitaka Mine and Python Cave were inhabited by large numbers of *R. aegyptiacus*[24,27]. Follow-up longitudinal studies of *R. aegyptiacus* populations at these sites revealed a consistent prevalence of both MARV and RAVV infection in 2–5% of the bats. Genetically diverse marburgviruses were isolated from bat tissues that were genetically similar to those sequences generated from outbreak cases. Further, the studies found a temporal association between marburgvirus spillover events, biannual pulses of active MARV infection in juvenile bats and the biannual birthing season. These studies provided the evidence needed to definitively identify *R. aegyptiacus* as a natural reservoir host of the marburgviruses and a source of spillover into the human population.

Natural history of MARV infection in *R. aegyptiacus*

Following the discovery of *R. aegyptiacus* as the natural reservoir host for the marburgviruses, experimental studies were initiated to investigate the natural history of virus infection in this bat species. The first published study by Paweska *et al*. found that bats inoculated by the intraperitoneal and subcutaneous routes with a Vero cell-adapted, human-derived MARV strain exhibited viral replication in multiple tissues in the absence of overt illness followed by seroconversion, while bats dually inoculated by the oral and nasal routes showed no evidence of infection within the 21-day study period[28]. A second study by Amman *et al*. found that bats subcutaneously inoculated with a low-passage, bat-derived MARV strain shed virus in their oral secretions up to 11 days following infection and led to the hypothesis that the virus may be horizontally transmitted between bats through direct and/or indirect contact with infectious oral secretions or biting[29]. To investigate the mechanisms of bat-to-bat MARV transmission, a third study by Paweska *et al*. housed groups of donor bats inoculated with a human MARV strain with naïve contact bats in direct, indirect or airborne contact and monitored for evidence of infection for 42 days[30]. No evidence of infection was detected in the contact bats; however, the inoculated bats shed little to no MARV in their bodily fluids and were serially sacrificed as the study progressed. The possibility that hematophagous ectoparasitic argasid ticks (*Ornithodoros faini*) found in large colonies of *R. aegyptiacus* might facilitate marburgvirus transmission was ruled-out when >3000 *O. faini* ticks collected from Python Cave tested negative for marburgvirus RNA[31]. Further studies are needed to determine how MARV is maintained in its natural reservoir host.

Search for the natural reservoir hosts of the ebolaviruses

Although the index cases of ebolavirus disease outbreaks have been linked to bats, they have never been associated with a particular environment, such as caves, like the index cases of marburgvirus disease outbreaks. Therefore, the search for the reservoir hosts of the ebolaviruses has involved testing a wide-range of wild-caught, forest-dwelling bats for evidence of ebolavirus infection. Serological reactivity of bat sera with ebolavirus antigen has been detected in 307 bats representing at least 17 species throughout sub-Saharan Africa and Asia[32–40]. Evidence of active ebolavirus infection has been found in seven bat species – EBOV RNA has been detected in three solitary, forest-dwelling frugivorous species (*E. franqueti*, *H. monstrosus* and *Myonycteris torquata*) captured in Gabon and the Republic of Congo[32] and RESTV RNA has been detected in four diverse species (*Chaerephon plicatus*, *Cynopterus brachyotis*, *Miniopterus australis* and *M. schreibersii*) captured in the Philippines[39]. However, infectious ebolavirus has never been isolated from any of these bat species. Consequently, it is unknown whether they are primary reservoir hosts of the virus, secondary reservoir hosts that play a minor role in virus maintenance or incidental dead-end hosts that are susceptible to infection, but do not shed infectious virus. It is interesting to note that MARV RNA in the absence of infectious virus has been detected in *Miniopterus inflatus*, *Rhinolophus eloquens* and *Hipposideros sp.* bats that roost with *R. aegyptiacus*[24,41]. Similarly, investigations examining the susceptibility of *R. aegyptiacus* bats to experimental infection with each of the five ebolaviruses demonstrated very limited replication and no viral shedding followed by seroconversion[42,43]. These findings suggest that sporadic detection of filovirus RNA or IgG antibodies from wild-caught bats may only represent virus spillover resulting from contact with a primary reservoir host.

Expectations of a filovirus natural reservoir host

Based on what we have learned about marburgvirus infection in *R. aegyptiacus*, we would expect the reservoir hosts of the ebolaviruses to have a consistent prevalence of both active and past infection, shed sufficiently high levels of infectious virus to maintain virus circulation in the population and exhibit host population dynamics conducive to virus transmission. Host population-level virus persistence is highly dependent on host population dynamics, particularly community size and annual fluctuations in age-structure from births and deaths. Mathematical modelling of marburgvirus transmission in a closed population of *R. aegyptiacus* revealed that the virus was only able to persist if the model included: (1) a biannual breeding component that provided a twice-yearly influx of susceptible juveniles; (2) a latent period of ≥21 days; and (3) a host population size ≥20 000[44]. This suggests that if the natural reservoirs of the ebolaviruses are a solitary bat species that only congregates during the breeding season(s), host population-level virus maintenance may depend on other mechanisms such as persistent infection with intermittent shedding, as has been observed with other bat-borne viruses[45–49]. The large number of bat species within the geographical range of ebolavirus circulation complicates the search for the natural reservoir host of these viruses. In an effort to guide field sampling efforts, Peterson *et al*. used a series of biological principles to develop a priority list of mammalian clades that coincided with past filovirus disease outbreaks[50] and Han *et al*. used a machine learning algorithm to identify potential filovirus-positive bat species based on intrinsic trait similarity with known filovirus RNA-, isolation- and antibody- positive bat species[51].

For more information on filoviruses and bats, we would like to direct readers to recent overviews published by Olival and Hayman[52], Wood *et al*.[53], Leendertz *et al*.[54] and Amman *et al*[55].

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