

African Primates: Likely Victims, Not Reservoirs, of Ebolaviruses

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(See the Major Article by Ayouba et al, on pages 1599–608)

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Ebola virus disease (EVD) kills almost half those people infected. Four different viruses from 4 *Ebolavirus* species have caused EVD in Africa: *Zaire ebolavirus* (EBOV), *Sudan ebolavirus* (SUDV), *Bundibugyo ebolavirus* (BDBV), and *Tai Forest ebolavirus* (TAFV), with all but TAFV causing fatal human disease. Outbreaks have been sporadic and unpredictable, but the frequency and size of outbreaks appear to be increasing [1, 2]. The ongoing outbreak in the Democratic Republic of the Congo (DRC) is the second largest on record, with >500 cases and >290 deaths reported from 15 health zones [3]. The largest EVD outbreak, originating in Guinea, West Africa, in 2013 [4], ended in 2016 after 28 616 cases and 11 310 deaths [5].

Preventing EVD outbreaks is challenging because the “reservoir hosts” of the viruses that cause the disease are not known. The weight of evidence suggests that fruit bats are the natural hosts, but this is uncertain [6]. The uncertainty is partially because most outbreaks in people are not directly linked to bats.

Circumstantial evidence linked bats to the 2013 West African outbreak [7], but index case exposure to bats has only once been reported with any confidence [8]. In contrast, hunting or butchering primates has been linked to several EVD index cases. In particular, Africa’s great apes, gorillas and chimpanzees, have been sources of human infection, and human EVD outbreaks have occurred concurrently with outbreaks in apes in Central and West Africa [9, 10]. High case fatality rates among apes [11–13], however, suggest they are not maintenance reservoir hosts [14].

Wildlife mortality events during EVD outbreaks have involved other mammals, including monkeys, pigs, and antelope [15]. Contact with monkeys has been reported in human outbreaks in Central Africa [16, 17] and chimpanzees in Ivory Coast [10]. Monkeys themselves appear to be susceptible to EBOV infection, at least experimentally [18]. Outside of Africa, *Reston ebolavirus* (RESTV) has been linked to monkeys, with macaques imported to the United States from the Philippines infected [19], but the mammals linked to RESTV in Asia are similar to Africa, with pigs, monkeys, and bats all implicated as hosts [20–22].

Serological data may be well suited for surveillance studies, because antibodies are longer lasting than viral infection and provide evidence of survival. Experimental evidence suggests that EBOV infection in bats may be acute, nonfatal, and short-lived, but induces

antibodies [23]. This experimental work is supported by field data from related Marburg viruses, first identified after African monkeys infected people in Europe [24], which apparently persist within large colonies of cave-dwelling Egyptian fruit bats, and RESTV in Asian bats. In both cases, viruses or viral RNA and antibodies were detected in apparently healthy bats [22, 25]. Just 1 study has detected EBOV RNA in bats, but anti-EBOV antibodies are widespread in African bats and the RNA-positive bats were, again, apparently healthy [11, 26–29]. In contrast, while anti-EBOV antibodies have been observed in African apes and monkeys [30, 31], suggesting that nonlethal infections might occur, the prevalence of antibodies is low (similar to those reported for RESTV in Asian macaques [21]), and EBOV RNA has been isolated from dead apes [32]. Thus, together the evidence for bats being the true reservoir host for EVD causing viruses is convincing, but relies on serological evidence of infection rather than virus detection, and the role of non-human primates as reservoirs remains uncertain.

The role of primates in EVD epidemiology has been unclear largely because study sample sizes have been small. Serology is further complicated by different methodologies and antibody-positive sera cross-reacting among different EVD-causing viruses. A report by Ayouba et al, in this issue of *The*

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Journal of Infectious Diseases, has taken a significant step toward addressing these problems [33]. The team utilized a large sample (N = 4649) of tissues from multiple species of African primates, collected from 1999 to 2016 from Ivory Coast in West Africa and DRC and Cameroon in Central Africa. The study more than triples the number of all previous primate samples reported and is similarly powered to some studies showing high seroprevalence of anti-EBOV antibodies in certain African fruit bat species [26, 27]. A single Luminex-based serological assay that included antigens from 4 viruses (EBOV, SUDV, BDBV, and RESTV) was used, and the team discovered that none of 2327 ape samples and only 1 of 2322 monkey samples met their seropositive criteria. The data strongly suggest that the primates sampled are unlikely reservoir hosts.

The work highlights the importance of multiyear, multisite empirical studies and archiving samples. Specimen collection in general has created some controversy in areas such as conservation biology [34], but for epidemiologists tissue archives may enable us to better understand the epidemiology of infectious diseases. Here, the primate samples were collected for lentivirus research (eg, human immunodeficiency virus [HIV] and its relatives), then repurposed for EVD research. In other systems, archived sample banks have helped identify Middle East respiratory syndrome coronavirus-seropositive camels in East Africa over 11-year (Kenya) and 30-year (Sudan and Somalia) periods, suggesting extensive virus circulation in camels prior to the first human outbreaks [35–38]. Some impressive examples of using archaeological samples have led to the sequencing of *Yersinia pestis* genomes from Black Death victims in London, England, dated to 1348–1350 [39], and Bronze Age hepatitis B viral DNA [40]. The instability of RNA viruses will prevent paleovirological studies on these timeframes, though gene sequencing from archived samples has helped identify HIV type 1 (HIV-1) sequences predating the

first AIDS diagnosis, with HIV sequences from 1959 and 1960 in DRC informing our understanding of pandemic HIV-1 origins and evolution [41, 42].

Ideally, EVD-causing viruses themselves will be isolated in space and time through wildlife surveillance to understand viral transmission dynamics. Phylogenetic models that estimate the relationship between genetic sequences have been used with sample location data to place the first 1976 case from DRC near the root of the EBOV phylogenetic tree, suggesting that all other known outbreaks descended from a closely related virus [43]. Although the analysis contained just a few viral fragments, it suggested that later outbreaks were epidemiologically linked and occurred in a wave-like pattern, spreading at approximately 50 km per year. Once EBOV RNA fragments were discovered in bats, the same team used similar models to reconstruct the ancestry of EBOV, including fragments of viral RNA from bats [44]. Their analyses suggested that all of the genetic variation present in EBOV, including from fruit bats, was the product of mutations accumulated within a 30-year time period, supporting the ancestry of EBOV in bat reservoirs and the role of bats in EBOV epidemiology.

The absence of robust data on Ebola virus reservoirs makes forecasting when and where outbreaks may occur difficult, limiting preventive measures [45, 46]. The lack of data relating to bats themselves led researchers to characterize the traits of all filovirus-seropositive and virus-positive bat species to predict potential undetected bat species [47]. Putative bat hosts have been included in models to predict the spatial risk of human outbreaks [48]. Similar modeling approaches have been used to model the spatial and temporal risk of human and ape EVD, finding the greatest risk during wet to dry season transitions in sparsely populated regions of tropical Africa [49], supporting previous work [50]. All of these studies are limited by data, but Ayoubia et al's comprehensive study supports the assumption that bats, not primates, are likely reservoir

hosts and that nonhuman primates may be viewed as both sentinels for human infection and victims of EVD [9, 15, 33, 51]. These are important findings because they can inform field and surveillance studies, which are costly and difficult in most areas where EVD outbreaks occur and for the species linked to EVD. To really manage and prevent EVD, however, we also need to understand why outbreaks appear to be increasing in frequency. Recent analyses of forest fragmentation and EVD emergence suggest there may be links [52, 53]. If so, there may be management options that can be implemented alongside human and wildlife surveillance and public health interventions to reduce the risk of human and, potentially, primate EVD emergence in the first place.

Notes

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