Ebola Virus Antibodies in Fruit Bats, Bangladesh

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To determine geographic range for Ebola virus, we tested 276 bats in Bangladesh. Five (3.5%) bats were positive for antibodies against Ebola Zaire and Reston viruses; no virus was detected by PCR. These bats might be a reservoir for Ebola or Ebola-like viruses, and extend the range of filoviruses to mainland Asia.

Filoviruses are zoonotic pathogens that cause epidemic, lethal, hemorrhagic outbreaks among humans and nonhuman primates and case-fatality rates up to 80% (1). The family Filoviridae contains 2 genera: Marburgvirus, which contains Marburg virus (MARV), and Ebolavirus, which contains 4 viruses: Zaire Ebola virus (ZEBOV), Sudan Ebola virus, Reston Ebola virus (REBOV), and Côte d’Ivoire Ebola virus, and 2 tentative species (Bundibugyo Ebola virus and Lloviu Ebola virus) (2,3). Pathogenicity varies among Ebola viruses, from ZEBOV, which is highly lethal in humans, to REBOV, which causes disease in pigs and macaques but asymptomatically infects humans.

Despite their role in human disease, natural reservoirs of filoviruses have remained elusive for decades. Reports suggest that bats (Order Chiroptera) are the primary natural hosts, including Old World insectivorous bats (genera Rhinolophus and Miniopterus) and frugivorous bats (family Pteropodidae). Fruit bats of the genus Rousettus have been implicated as a reservoir of filoviruses in Africa (4–7) and REBOV in the Philippines (8). Lloviu Ebola virus was detected in Miniopterus schreibersii insectivorous bats from Spain and appears to cause pathologic changes in this species but is not known to infect humans (2). These studies point to a wide, and still poorly described, geographic distribution for viruses of the family Filoviridae in chiropteran hosts. We screened bats of several species from Bangladesh for Ebola virus infection to determine whether the geographic range of this virus extends to southern Asia.

The Study

We captured and sampled 276 bats (141 Rousettus leschenaultii bats, 75 Cynopterus spp. bats, 59 Megaderma lyra bats, and 1 Macroglossus sobrinus bat) during April 2010–March 2011 from the Faridpur, Rajbari, Lalmonirhat, and Comilla Districts in Bangladesh. All bats were identified to species in the field, except Cynopterus spp. bats, because of cryptic diversity in this group; we are awaiting genetic species confirmation. Bats were captured in mist nets near roosts or at feeding sites and were handled in accordance with the Tufts University (Medford, MA, USA) Institutional Animal Care and Use Committee protocol (no. G2011-106).

We collected 50–800 µL of blood from brachial or cephalic veins of each bat, and diluted it 1:4 with phosphate-buffered saline in the field before serum was separated, as described (9). We also collected throat, urine/urogenital, and fecal swab specimens, which were placed in 750 µL of NucliSERS lyss buffer (bioMérieux, Marcy l’Etoile, France). All samples were collected in cryovials, placed in liquid nitrogen in the field, and maintained at –80°C until testing. We recorded morphologic measurements, weight, sex, age, and body condition and collected a wing biopsy specimen before releasing animals at capture sites.

We screened serum samples for IgG against REBOV and ZEBOV by using ELISA and Western blotting at the Commonwealth Scientific and Industrial Research Organisation Australian Animal Health Laboratory Biocontainment Facility (Geelong, Victoria, Australia). To inactivate potentially infectious agents, serum samples were heated at 56°C for 20 min before shipment. All samples were screened by using a 1:1 mixture of purified recombinant nucleoproteins (0.2 mg/mL) of REBOV and ZEBOV (R + Z ELISA), which were expressed in an Escherichia coli vector that contained a histidine tag (10,11).

Potentially positive serum cutoff values were determined to be >0.454 for the R + Z ELISA by using maximum-likelihood estimation, gamma distribution, and 95% risk for error (7). Potentially positive serum samples were tested by ELISA against each nucleoprotein independently to confirm reactivity and by Western blotting against nucleoproteins of Reston and Zaire virus strains as described (10). Serum samples were tested at a dilution of 1:50. Endpoint titrations with an optical density >3× the background reading were determined for serum samples positive against REBOV and ZEBOV antigens individually.
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Our study provides evidence of Ebola virus infection in wildlife from mainland Asia and corroborates the observation that filoviruses are harbored across a much larger geographic range than assumed (2). Preferential reactivity to ZEBOV suggests exposure to an Ebola virus that is distinct from REBOV, the only filovirus currently found in Asia. We consider the likelihood of cross-reactivity with MARV as low because there is only a 35% aa identity between nucleoprotein genes of REBOV/ZEBOV and MARV. However, we cannot rule out co-infection with multiple filoviruses.

Seroprevalence found in this study is consistent with that found in another study (4). However, other studies of Rousettus spp. bats have reported higher values (e.g., 7%–20% and 8% of R. aegyptiacus bats seropositive for MARV and ZEBOV, respectively) (6,7), and 5 (31%) of 16 R. amplexicaudatus bats seropositive for REBOV (8). These differences might have been caused by poor specificity of the assay if this virus is novel, an artifact of low volume of blood collected, the potential that other species may have greater roles as reservoirs than Rousettus spp. in Bangladesh, or timing of sampling. R. leschenaultii bats have a large range (China to India) (73); and more detailed studies of virus ecology and diversity are warranted to better understand their role as a potential reservoir of zoonotic disease agents.

**Table 1. Ebola virus serologic assay results for bats, Bangladesh, 2010–2011**

| Year, specimen no. | Age of bat | Sex of bat | Species or control | ELISA OD (endpoint titration) | Western blot |
|--------------------|------------|------------|-------------------|------------------------------|--------------|
| 2010               | Rab691/d0  | ND         | Negative control  | 0.138 (50)                  | –            |
|                    | Rab691/EBVo-N | ND      | Positive control  | 2.303 (50)                  | ++           |
|                    | April 2010–001 | F       | Negative control (Rousettus leschenaultii) | 0.092 (50) | – |
| April 2010–002     | A          | F          | Negative control (R. leschenaultii) | 0.176 (80) | ++ |
| Monkey/EboV        | ND         | ND         | Positive control  | 1.753 (50)                  | NT           |
| April 2010–042     | A          | M          | R. leschenaultii  | 1.512 (50)                  | +            |
| April 2010–057     | A          | M          | R. leschenaultii  | 0.684 (50)                  | ++           |
| 2011               | Rab691/d0  | ND         | Negative control  | 0.165 (50)                  | –            |
|                    | SB0311–115 | ND         | Negative control  | 0.515 (50)                  | –            |
|                    | SB0311–117 | A          | Negative control  | 0.775 (50)                  | –            |
|                    | Rab691/EBVo-N | ND      | Positive control  | 1.598 (50)                  | ++           |
|                    | SB0311–001 | A          | R. leschenaultii  | 0.494 (50)                  | +            |
|                    | SB0311–004 | A          | R. leschenaultii  | 0.557 (50)                  | +            |
|                    | SB0311–059 | A          | R. leschenaultii  | 0.757 (50)                  | ++           |
|                    | SB0311–016 | A          | R. leschenaultii  | 0.542 (50)                  | ++           |
|                    | 66 additional negative | ND | R. leschenaultii  | <0.05 (50)                  | NT           |
|                    | 67 additional negative | ND | R. leschenaultii  | <0.05 (50)                  | NT           |
|                    | 73 additional negative | ND | M. lyra         | <0.05 (50)                  | NT           |
|                    | 75 negative | ND         | Cynopterus sp.   | <0.05 (50)                  | NT           |
|                    | 1 negative  | A          | Macroglossus sobrinus | <0.05 (50) | NT |

*Values in boldface are positive results. OD, optical density; R + Z, ELISA using a 1:1 mixture of recombinant nucleoproteins of Reston and Zaire Ebola viruses; R, Reston Ebola virus ELISA; Z, Zaire Ebola virus ELISA; ND, not determined; A, adult; –, negative; ++, strongly positive; NT, not tested; *, positive.
We demonstrated that serologic and virus surveys of bats can be informative for identifying potential virus hosts. Previous studies amplified ZEBOV nucleic acid from bat feces (14). We also screened bat feces to identify potential routes of virus excretion, which is useful when the route of exposure from bats to humans is known. A short interval for Ebola virus shedding by reservoir hosts and an inverse relationship between viremia and antivirus titer probably explain our negative PCR results for seropositive bats. Failure to detect filovirus nucleic acid might reflect our relatively small sample size, low virus prevalence, or use of a PCR that has low sensitivity for filoviruses circulated in Bangladesh.

In Bangladesh, human outbreaks of Nipah virus have been linked to drinking date palm sap contaminated with bat excreta, presumably from *Pteropus giganteus* bats (15). *R. leschenaultii* bats and other small fruit bat species visit date palm trees 10× more frequently than *Pteropus* spp. bats (15). This finding could indicate potential transmission of filoviruses or any other novel viruses that *R. leschenaultii* bats carry. It also highlights the need for more research to understand this ecologic system and for better implementation of low-cost barriers to reduce bat–human contact during periods of date palm harvesting (15).

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References

1. Sanchez A, Khan AS, Zaki SR, Nabel GJ, Ksiazek T, Peters C. Filoviridae: Marburg and Ebola viruses. In: Fields virology, 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 1279–304.
2. Negredo A, Palacios G, Vueaux-Moron S, Gonzalez F, Dopazo H, Molero F, et al. Discovery of an Ebolavirus-like filovirus in Europe. PLoS Pathog. 2011;7:e1002304. http://dx.doi.org/10.1371/journal.ppat.1002304
3. Towner JS, Sealy TK, Khristova ML, Albarino CG, Conlan S, Reed SA, et al. Newly discovered Ebola virus associated with hemorrhagic fever outbreak in Uganda. PLoS Pathog. 2008;4:e1000212. http://dx.doi.org/10.1371/journal.ppat.1000212
4. Leroy EM, Kumulungui B, Pourrut X, Rouquet P, Hassanin A, Yaba P, et al. Fruit bats as reservoirs of Ebola virus. Nature. 2005;438:575–6. http://dx.doi.org/10.1038/438575a
5. Towner JS, Amman BR, Sealy TK, Carroll SA, Comer JA, Kemp A, et al. Isolation of genetically diverse Marburg viruses from Egyptian fruit bats. PLoS Pathog. 2009;5:e1000536. http://dx.doi.org/10.1371/journal.ppat.1000536
6. Swanepoel R, Smit SB, Rollin PE, Formenty P, Leman PA, Kemp A, et al. Studies of reservoir hosts for Marburg virus. Emerg Infect Dis. 2007;13:1847–51. http://dx.doi.org/10.3201/eid1312.071115
7. Pourrut X, SOURIS M, Towner JS, Rollin PE, Nichol ST, Gonzalez JP, et al. Large serological survey showing cocirculation of Ebola and Marburg viruses in Gabonese bat populations, and a high seroprevalence of both viruses in Rousettus aegyptiacus. BMC Infect Dis. 2009;9:159. http://dx.doi.org/10.1186/1471-2334-9-159
8. Taniguchi S, Watanabe S, Masangkay JS, Omatu T, Ikekami T, Alviola P, et al. Reston Ebolavirus antibodies in bats, the Philippines. Emerg Infect Dis. 2011;17:1559–60.
9. Smith CS, De Jong C, Field H. Sampling small quantities of blood from microbats. Acta Chiropterologica. 2010;12:255–8. http://dx.doi.org/10.3161/1508111010X504752
10. Hayman DT, Emmerich P, Yu M, Wang LF, Sau-Ire R, Fooks AR, et al. Long-term survival of an urban fruit bat seropositive for Ebola and Lagos bat viruses. PLoS ONE. 2010;5:e1978. http://dx.doi.org/10.1371/journal.pone.0011978
11. Marsh GA, Haining J, Robinson R, Foord A, Yamada M, Barr JA, et al. Ebola Reston virus infection of pigs: clinical significance and transmission potential. J Infect Dis. 2011;204:S804–9. http://dx.doi.org/10.1093/infdis/jir300

Table 2. Bat specimen results for filovirus by PCR and Ebola virus by serologic analysis, Bangladesh, 2010–2011

| Bat species, sex, and sample type | No. positive/ no. tested |
|----------------------------------|-------------------------|
| **Cynopterus spp., n = 75, 43 M, 32 F** | | |
| Feces swab | 0/74 |
| Throat swab | 0/75 |
| Serum | 0/75 |
| Urine/urogenital swab | 0/39 |
| **Macroglossus sobrinus, n = 1, 1 M** | | |
| Feces swab | 0/1 |
| Throat swab | 0/1 |
| Serum | 0/1 |
| Urine/urogenital swab | 0/1 |
| **Megaderma lyra, n = 56, 23 M, 33 F** | | |
| Feces swab | 0/56 |
| Throat swab | 0/56 |
| Serum | 0/56 |
| Urine/urogenital swab | 0/50 |
| **Rousettus leschenaultii, n = 141, 106 M, 34 F, 1 ND** | | |
| Feces swab | 0/141 |
| Throat swab | 0/140 |
| Serum | 5/141 |
| Urine/urogenital swab | 0/58 |
| **Total** | 5/971 |

*ND, sex not determined.*
12. Zhai J, Palacios G, Towner JS, Jabado O, Kapoor V, Venter M, et al. Rapid molecular strategy for filovirus detection and characterization. J Clin Microbiol. 2007;45:224–6. http://dx.doi.org/10.1128/JCM.01893-06

13. Chen J, Rossiter SJ, Flanders JR, Sun YH, Hua PY, Miller-Butterworth C, et al. Contrasting genetic structure in two co-distributed species of Old World fruit bat. PLoS ONE. 2010;5:e13903. http://dx.doi.org/10.1371/journal.pone.0013903

14. Swanepoel R, Leman PA, Burt FJ, Zachariaides NA, Braack LE, Ksiazek TG, et al. Experimental inoculation of plants and animals with Ebola virus. Emerg Infect Dis. 1996;2:321–5. http://dx.doi.org/10.3201/eid0204.960407

15. Khan MS, Hossain J, Gurley ES, Nahar N, Sultana R, Luby SP. Use of infrared camera to understand bats’ access to date palm sap: implications for preventing Nipah virus transmission. EcoHealth. 2010;7:517–25. http://dx.doi.org/10.1007/s10393-010-0366-2

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