Novel Paramyxoviruses in Bats from Sub-Saharan Africa, 2007–2012

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As part of a larger survey for detection of pathogens among wildlife in sub-Saharan Africa conducted during 2007–2012, multiple diverse paramyxovirus sequences were detected in renal tissues of bats. Phylogenetic analysis supports the presence of at least 2 major viral lineages and suggests that paramyxoviruses are strongly associated with several bat genera.

Members of the Paramyxoviridae family are enveloped negative-sense RNA viruses, further classified into either the Pneumovirinae or Paramyxovirinae subfamily (1). The Paramyxovirinae subfamily has increasingly been associated with bat species across the globe. The Henipavirus genus is 1 of 7 genera in this subfamily and contains the first recorded zoonotic paramyxoviruses, Hendra virus and Nipah virus. These 2 viruses are associated with severe respiratory and neurologic syndromes, and regular spillover from Pteropus spp. bats causes infections in humans and domestic animals (2).

Enhanced surveillance for bat-associated pathogens has led to the discovery of numerous novel paramyxoviruses (3–5). Henipavirus-related viruses were identified in another pteropodid species, Eidolon helvum, sampled in Ghana, West Africa. This finding suggests an extension of the geographic and host ranges of the members of this virus genus (6). Subsequent studies demonstrated a high diversity of paramyxoviruses in E. helvum bat population in Africa, as well as in other bat species from different continents. This finding suggests that bats may have a global role as potential paramyxovirus reservoirs (3,4). To contribute toward the knowledge of bat-associated paramyxovirus diversity and distribution, we sampled multiple bat species from several sub-Saharan African countries.

The Study

During 2007–2012, we sampled 1,220 bats representing at least 48 species from multiple locations in selected countries in Africa (Table 1). Bats were anesthetized with the use of ketamine (0.05–0.1 mg/g body mass) and exsanguinated by cardiac puncture. Voucher specimens were identified through morphologic characterization (7) or, alternatively, through genetic barcoding. Approximately 30–100 mg of renal tissue was used for RNA extraction. A heminested primer set targeting the conserved polymerase (large) gene of Respirovirus, Morbillivirus, and Henipivirus was used for sample screening through reverse transcription PCR (8). A total of 103 samples (8.4%) tested positive, and the obtained amplicons of ≈490 bp were sequenced (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/20/10/14-1368-Techapp1.pdf). For phylogenetic analysis, representative paramyxovirus sequences available from GenBank were included (online Technical Appendix Table 2), and Bayesian analysis was performed by using BEAST version 1.7.4 software (http://beast.bio.ed.ac.uk/) (Figure; http://wwwnc.cdc.gov/EID/article/21/10/14-1368-F1.htm).

Several samples from bat species not previously implicated as paramyxovirus reservoirs tested positive in our study. Some of these implicated species are known to roost in peridomestic environments. Sequence analysis of paramyxovirus sequences showed a clear bifurcation of the phylogenetic tree, segregating paramyxoviruses detected in pteropodid bats (Pteropodidae) from paramyxoviruses detected in bats of other families (Figure). The former contained henipaviruses and related viruses. Two viral sequences detected in Rousettus aegyptiacus bats grouped within this cluster as part of a sister clade to the henipaviruses. The second cluster contained sequences derived from nonpteropodid bats. Some of these sequences grouped with the sequences from the Morbillivirus and proposed Jeilongvirus genera, whereas others could not be included in any of the other paramyxovirus genera.

We observed a strong association of several viral lineages to particular bat genera for paramyxoviruses identified in Hipposideros, Miniopterus,Coleura,Myotis, and Pipistrellus bats, although the bats were sampled from geographically distant locations. In contrast to the sequences of

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Table 1. African bat species sampled and the number of paramyxovirus sequences detected in sub-Saharan Africa, by country, 2007–2012

| Country                        | Bat Species                                                                 | Paramyxovirus Sequences Detected |
|--------------------------------|------------------------------------------------------------------------------|----------------------------------|
| **South Africa**               |                                                                              |                                  |
| Chaerephon ansorgei (2/0)       | Neoromicia nana (7/2)                                                       | Rhinolophus sp. (1/0)            |
| Chaerephon pumilus (8/0)        | Neoromicia rupePELLi (1/0)                                                  | Rousettus aegyptiacus (18/0)     |
| Epomophorus gambianus (2/0)     | Neoromicia zulimensis (1/0)                                                 | Sauromys petrophilus (1/0)       |
| Epomophorus wahlbergi (15/0)    | Nycteris thebaica (12/1)                                                    | Scotophilus sp. (12/0)           |
| Eptesicus hottentotus (2/1)     | Nycticeinops schlieffeni (9/0)                                              | Scotophilus dingani (26/0)       |
| Glauconycteris variegata (5/0)  | Pipistrellus hesperidus (5/0)                                               | Scotophilus leucogaster (2/0)     |
| Hipposideros caffer (6/2)       | Pipistrellus rusticus (5/0)                                                 | Scotophilus nigrita (1/0)        |
| Kerivoula argentata (1/1)       | Pipistrellus sp. (5/0)                                                      | Scotophilus viridis (3/0)         |
| Miniopterus natalensis (5/0)    | Rhinolophus darlingi (5/0)                                                  | Tadarida aegyptiaca (5/0)        |
| Miniopterus sp. (37/0)          | Rhinolophus denti (3/2)                                                     | Taphozous mauritianus (2/0)      |
| Mops condylurus (7/0)           | Rhinolophus fumigatus (2/0)                                                 |                                  |
| Neoromicia capensis (16/0)      | Rhinolophus landeri (1/1)                                                   |                                  |
| Neoromicia helios (6/0)         | Rhinolophus simulator (2/0)                                                 |                                  |
| Swaziland                      | Nycteris thebaica (4/0)                                                     |                                  |
| **Eastern Africa**              |                                                                              |                                  |
| Kenya                          |                                                                              |                                  |
| Eidolon helvum (27/10)          | Miniopterus natalensis (15/0)                                               | Rousettus aegyptiacus (84/2)     |
| Epomophorus labiatus (6/0)      | Neoromicia sp. (25/0)                                                       | Scotophilus dingani (2/0)        |
| Epomophorus wahlbergi (2/0)     | Nycteris sp. (2/1)                                                          | Taphozous sp. (1/0)              |
| Hipposideros vittatus (71/0)    | Otomops martiensseni (40/9)                                                 | Triaenops afer (16/12)           |
| Hipposideros sp. (8/1)          | Rhinolophus landeri (12/0)                                                  |                                  |
| Miniopterus minor (151/14)      | Rhinolophus sp. (14/0)                                                      |                                  |
| **Central Africa**              |                                                                              |                                  |
| Cameroon                       |                                                                              |                                  |
| Chaerephon sp. (32/0)           | Hipposideros sp. (39/1)                                                     | Taphozous sp. (12/3)             |
| Eidolon helvum (15/0)           | Rhinolophus sp. (9/1)                                                       |                                  |
| Epomophorus sp. (1/0)           | Scotophilus dingani (1/0)                                                   |                                  |
| Democratic Republic of the Congo|                                                                              |                                  |
| Chaerephon pumilus (25/0)       | Hypsognathus monstrosus (2/0)                                               | Myonectes torquata (8/0)         |
| Chaerephon sp. (22/0)           | Megaloglossus woermanni (10/0)                                              | Myotis sp. (3/0)                 |
| Eidolon helvum (22/0)           | Microteropus pusillus (1/0)                                                 | Neoromicia sp. (1/0)             |
| Glauconycteris argentata (1/0)  | Micromyrus moloneyi (1/0)                                                   | Pipistrellus sp. (40/20)         |
| Hipposideros fuliginosus (21/3) | Miniopterus sp. (41/2)                                                      | Rhinolophus sp. (1/0)            |
| Hipposideros gigas (2/0)        | Mops condylurus (33/0)                                                      | Scotophilus dingani (2/0)        |
| **Western Africa**              |                                                                              |                                  |
| Nigeria                        |                                                                              |                                  |
| Eidolon helvum (20/0)           | Hipposideros sp. (3/1)                                                      | Rousettus aegyptiacus (21/0)     |
| Hipposideros vittatus (8/0)     | Lissonycteris angolensis (8/0)                                               |                                  |

European and South American origin, for which geographic clustering was observed, no such clustering was found among the sequences from African bats.

The incidence and diversity of viral sequences varied according to bat species. For example, nearly identical sequences were detected in 50% of Pipistrellus spp. sampled from a single colony in the Democratic Republic of the Congo (n = 40). In other cases, several distinct viral sequences were detected in different individual bats of 1 species, such as Miniopterus minor bats sampled from a single colony in Kenya (n = 53), which harbored 6 distinct viral sequences. Some of the sequences were found more frequently than others. In contrast to a previous study which did not identify paramyxoviruses in Coleura afra bats sampled in Ghana (n = 71) (4), we detected a substantial paramyxovirus incidence (37%, n = 27) in the same bat species sampled in Kenya (Table 2).

Conclusions

The henipaviruses were the first bat paramyxoviruses directly linked to human disease; however, most aspects of pathogenicity and the host ranges of the increasingly detected novel bat paramyxoviruses remain to be investigated. Here we report information regarding paramyxovirus distribution through molecular evidence of bat-associated paramyxoviruses in Cameroon, Nigeria, and South Africa, as well as evidence of paramyxoviruses in nonpteropodid bats from the Democratic Republic of the Congo. Our results suggest that 2 separate lineages were established during the evolution of bat-associated paramyxoviruses:
the pteropid bats potentially harbor 1 lineage, and the nonpteropid bats potentially harbor the other. In contrast to the proposed chiropteran classification, which supports a sister-taxon relationship between Rhinolophoidae and Pteropodidae on the suborder level, paramyxovirus divergence appears to correlate with traditional bat taxonomy. The evolution behind this divergence might be a result of multiple evolutionary origins or a single origin with subsequent divergence. As with the evolution of echolocation, this question remains to be answered (11). More extensive bat sampling and molecular dating of the paramyxovirus phylogeny may help resolve this question.

Intensified anthropogenic transformations have facilitated closer contact between humans, domestic animal populations, and wildlife. Our study demonstrates that some bat species, adapted to peridomestic roosting, can have a substantial incidence of diverse paramyxoviruses. The variation in incidence and viral diversity observed in several bat species may suggest that some species are the true reservoirs, whereas others are mere incidental hosts. Given the observed virus diversity, implications for public health and veterinary medicine should be taken into account, especially considering the known likelihood of direct bat-to-human and human-to-human transmission of Nipah virus (12). Enhanced surveillance in bats and other animals will be useful for detecting possible spillover events and host shifts. Clearly, systematic longitudinal studies are needed to elucidate critical factors of paramyxovirus circulation within bat communities (13), and further research is needed to clarify the pathobiology, tissue tropism, and excretion pathways of these novel paramyxoviruses because these factors can be directly related to their zoonotic potential.

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Table 2. Paramyxovirus incidence in selected bat species from various African countries

| Species | Country | Tissue type† | No. sampled | No. positive | Incidence, % | Reference |
|---------|---------|--------------|-------------|--------------|--------------|-----------|
| Coleura afra | Ghana | Kidney | 71 | 0 | 0.0 | (4) |
| | Kenya | Kidney | 27 | 10 | 37.0 | § |
| | Central Africa‡ | Spleen | 25 | 1 | 4.0 | (4) |
| Eidolon helvum | Cameroon | Kidney | 15 | 0 | 0.0 | § |
| | DRC | Kidney | 22 | 0 | 0.0 | § |
| | Ghana | All solid organs, blood | 673 | 67 | 10.0 | (4) |
| | Kenya | Kidney | 15 | 0 | 0.0 | § |
| | Central Africa | Spleen | 49 | 17 | 34.5 | (4) |
| | Nigeria | Kidney | 20 | 0 | 0.0 | § |
| | Republic of Congo | All solid organs, blood, salivary gland, throat swab, feces, urine | 42 | 11 | 26.2 | (9) |
| Epomophorus gambianus | Central Africa | Spleen | 48 | 3 | 6.3 | (4) |
| | South Africa | Kidney | 2 | 0 | 0.0 | § |
| | Ghana | ‡ | 20 | 1 | 5.0 | (4) |
| Hipposideros caffer | Central Africa | Spleen | 337 | 3 | 0.9 | (4) |
| | South Africa | Kidney | 6 | 2 | 33.3 | § |
| | DRC | Kidney | 2 | 0 | 0.0 | § |
| Hipposideros gigas | Gabon | Spleen | 196 | 3 | 1.5 | (4) |
| | DRC | Kidney | 2 | 0 | 0.0 | § |
| Hypsignathus monstrosus | Central Africa | Spleen | 53 | 4 | 7.5 | (4) |
| | DRC | Kidney | 10 | 0 | 0.0 | § |
| Megaloglossus woermannii | Central Africa | Spleen | 34 | 1 | 2.9 | (4) |
| | DRC | Kidney | 8 | 0 | 0.0 | § |
| Myonycteris torquata | Central Africa | Spleen | 111 | 3 | 2.7 | (4) |
| | Ghana | ‡ | 1 | 0 | 0.0 | (4) |
| Rhinolophus lendri | Kenya | Kidney | 12 | 0 | 0.0 | § |
| | South Africa | Kidney | 1 | 1 | 100.0 | § |
| | Ghana | ‡ | 30 | 0 | 0.0 | (4) |
| Rousettus aegyptiacus | Kenya | Kidney | 84 | 2 | 2.4 | § |
| | Central Africa | Spleen | 183 | 18 | 9.8 | (4) |
| | Nigeria | Kidney | 21 | 0 | 0.0 | § |
| | South Africa | Kidney | 18 | 0 | 0.0 | § |

*DRC, Democratic Republic of the Congo.
†Tissue type stated for positive samples only and may not indicate all tissues sampled.
‡Information not available.
§Species and countries sampled during this study.
††Central Africa refers to Gabon/Republic of Congo/DRC/Republic of Central Africa.
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