Expanded Host Diversity and Geographic Distribution of Hantaviruses in Sub-Saharan Africa

Hae Ji Kang, a William T. Stanley, b Jacob A. Esselstyn, c Se Hun Gu, a Richard Yanagihara a

Departments of Pediatrics and Tropical Medicine, Medical Microbiology, and Pharmacology, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, Hawaii, USA; a Science and Education, Field Museum of Natural History, Chicago, Illinois, USA; b Department of Biological Sciences and Museum of Natural Science, Louisiana State University, Baton Rouge, Louisiana, USA

The recent discovery of hantaviruses in shrews and bats in West Africa suggests that other genetically distinct hantaviruses exist in East Africa. Genetic and phylogenetic analyses of newfound hantaviruses, detected in archival tissues from the Geata mouse shrew (Myosorex geata) and Kilimanjaro mouse shrew (Myosorex zinki) captured in Tanzania, expands the host diversity and geographic distribution of hantaviruses and suggests that ancestral shrews and/or bats may have served as the original mammalian hosts of primordial hantaviruses.

While myriad disease-causing RNA viruses were first discovered in sub-Saharan Africa, hantaviruses have been notable exceptions until recently, when Sangassou virus was detected in the African wood mouse (Hylomyscus simus) (1) and Tanganya virus (TGNV) in the Therese’s shrew (Crocidura theresaee) (2), captured in Guinea. More recently, genetically distinct hantaviruses, designated Azangy virus (AZGV) and Bowé virus (BOWV), have been found in the West African pygmy shrew (Crocidura obscurior) in Côte d’Ivoire (3) and in the Doucet’s musk shrew (Crocidura douceti) in Guinea (4), respectively. Here, we report the genetic and phylogenetic analyses of two novel hantaviruses harbored by myosoricine shrews in East Africa.

In accordance with guidelines approved by the American Society of Mammalogists (5), shrews were collected using pitfall traps during faunal surveys of montane forests in Tanzania (6, 7). Frozen liver tissues from 19 Geata mouse shrews (Myosorex geata) (Fig. 1A), 13 Kilimanjaro mouse shrews (Myosorex zinki) (Fig. 1B), 25 Kihaule’s mouse shrews (Myosorex kihaulei) (7), 8 climbing shrews (Suncus megalurus), 10 Grant’s forest shrews (Sylvisorex granti), and 10 Howell’s forest shrews (Sylvisorex howelli), captured between August 1995 and August 2002, were analyzed for hantavirus RNA by reverse transcription (RT)-PCR (8). Within minutes after the shrews were sacrificed, tissues were collected and placed in liquid nitrogen and then shipped and stored in liquid nitrogen until testing. The use of archival tissues was exempt from protocol review by the University of Hawaii Institutional Animal Care and Use Committee.

Kilimanjaro virus (KJMV strain FMNH174124) was found in a male Kilimanjaro mouse shrew, captured at 3,475 m elevation in Mt. Kilimanjaro National Park, 13.5 km N and 4 km W of Maua, in the Moshi District, Kilimanjaro Province, on 7 August 2002, and Uluguru virus (ULUV strain FMNH158302) was detected in a male Geata mouse shrew, captured at 1,535 m elevation in Uluguru North Forest Reserve, Uluguru Mountains, 5.1 km W and 2.3 km N of Tegetero, in the Morogoro District, Morogoro Province, on 16 August 1996 (Fig. 1C). Identification of the hantavirus-infected mouse shrews was verified by analysis of the complete 1,140-nucleotide mitochondrial DNA (mtDNA) cytochrome b gene (GenBank JX193701 and JX193702). Despite repeated RT-PCR attempts, hantavirus RNA was not detected in tissues of the other shrew species.

The full-length S and L segments and partial M segment of KJMV and the entire L segment and partial S and M segments of ULUV were sequenced and compared with those of representative rodent- and soricomorph-borne hantaviruses (Table 1).

The full-length 6,604- and 6,568-nucleotide L segments of KJMV and the entire L segment and partial S and M segments of ULUV were sequenced and compared with those of representative rodent- and soricomorph-borne hantaviruses (Table 1).

Received 29 January 2014 Accepted 7 April 2014 Published ahead of print 16 April 2014
the S, M, and L segments and generated by maximum likelihood and Bayesian methods, were implemented with the RAxML Blackbox Web server (11) and MrBayes 3.1 (12), under the best-fit GTR+1+Γ model of evolution in MrModeltest v2.3 (13) and jModelTest version 0.1 (14). In trees based on each genomic segment, KMJV and ULUV were distinct from newfound hantaviruses harbored by insectivorous bats, and they shared a common ancestry with Asian crocidurine shrew-borne hantaviruses, namely, Thottapalayam virus (TPMV) (15) and Imjin virus (MJNV) (10) (Fig. 2).

In the S and L trees, which were based on full-length and/or nearly full-length sequences, KMJV and ULUV were monophyletic, whereas in the M tree, which was based on less than half of the entire M-segment sequence, ULUV was basal to KMJV, TPMV, and MJNV (Fig. 2). The high bootstrap values and posterior node probabilities for ULUV and KMJV in the M segment tree suggested that the disparate topologies based on the M and S/L segments were probably not the result of missing sequences. Instead, this might indicate that the evolutionary history of the M segment differed from that of the S and L segments. Specifically, given that the M segment encodes the envelope glycoprotein, the ULUV M segment may have been under greater selection pressure or the KMJV M segment may have been under negative selection pressure after the two viruses diverged from a shared common ancestor. Alternatively or additionally, genetic recombination or genetic drift may be responsible. The full-length M genomic sequence and future phylogeographic studies may help to explain the discrepant trees.

The Myosorex genus comprises 15 extant species which exist in the forested highlands of central, eastern, and southern Africa (16). As the only endemic mammalian species on Mt. Kilimanjaro, Myosorex zinki is found in forests, heaths, and moorlands and near the edge of the alpine desert, along an elevation gradient, ranging from 2,470 to 4,000 m (17). While Myosorex geata is also endemic in moist montane forests in Tanzania, the true distribution of this species is unclear, particularly at higher altitudes undisturbed by frequent human activities (17).

On the basis of albeit meager fossil records and assuming equally probable and bidirectional exchanges between Eurasia and Africa, the family Soricidae likely originated in Eurasia (18). Shrews of the Myosorex genus are believed to have originated in the tropical forests of central Africa during the Middle Miocene, approximately 12 to 15 million years before the present (16, 18). In reconstructing the biogeographic history of the Soricidae, Dubey and colleagues proposed three equally parsimonious scenarios, based on the premise of two independent origins of the Crocidura genus (18). Viewed within this context, the phylogenetic positions of KMJV and ULUV in relation to TPMV and MJNV, which are hosted by Asian crocidurine shrews, and the clade comprising African crocidurine shrew-borne hantaviruses (BOWV, TGNV, and AZGV) and Jeju virus (JJUV) (19), which is hosted by the Asian lesser white-toothed shrew (Crocidura shantungensis) in Korea, supports a scenario in which the first diversification of the monophyletic Crocidurinae into Crocidurini and Myosoricini tribes occurred in Eurasia rather than Africa.

The discovery of hantaviruses in myosoricine shrew species endemic in Tanzania expands the host diversity and geographic distribution of non-rodent-borne hantaviruses and suggests that other genetically distinct hantaviruses may be widespread elsewhere in Africa. Compared to tissues from rodents, fewer tissues from either shrews or bats in Africa have been examined for hantavirus RNA by RT-PCR. Yet, many more hantaviruses have been found in shrews and bats. That is, of the nine hantaviruses detected thus far in sub-Saharan Africa, five are from shrews (2–4;
### Table 1: Sequence similarities of the S, M, and L segments of ULUV strain FMNH158302 in *M. geata* and KMJV strain FMNH174124 in *M. zinki* from Tanzania, compared to other rodent- and soricomorph-borne hantaviruses

<table>
<thead>
<tr>
<th>Virus and strain</th>
<th>S segment</th>
<th>M segment</th>
<th>L segment</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTNV</td>
<td>76-118</td>
<td>48.8</td>
<td>46.1</td>
</tr>
<tr>
<td>SOOV SOO-1</td>
<td>48.3</td>
<td>46.3</td>
<td>54.2</td>
</tr>
<tr>
<td>DOBV Greece</td>
<td>47.7</td>
<td>47.1</td>
<td>52.2</td>
</tr>
<tr>
<td>NVAV MSB95703</td>
<td>60.6</td>
<td>47.6</td>
<td>53.4</td>
</tr>
<tr>
<td>ULUV FMNH158302</td>
<td>71.2</td>
<td>73.7</td>
<td>59.6</td>
</tr>
<tr>
<td>KMJV FMNH174124</td>
<td>71.2</td>
<td>73.7</td>
<td>59.6</td>
</tr>
</tbody>
</table>

**Abbreviations:** ANDV, Andes virus; ARRV, Ash River virus; ASAV, Asama virus; AZGV, Azagny virus; BOGV, Boginia virus; BOWV, Bowé virus; CBNV, Cao Bang virus; DOBV, Dobrava virus; HTNV, Hantaan virus; JJUV, Jeju virus; JMSV, Jemez Spring virus; KKMV, Kenkeme virus; KMJV, Kilimanjaro virus; MJNV, Imjin virus; NVAV, Nova virus; OXBV, Oxbow virus; PHV, Prospect Hill virus; PUUV, Puumala virus; QHSV, Qian Hu Shan virus; RKPV, Rockport virus; SANGV, Sangassou virus; SEOV, Seoul virus; SNV, Sin Nombre virus; SOOV, Soochong virus; SWSV, Seewis virus; TGNV, Tanganya virus; TPMV, Thottapalayam virus; TULV, Tula virus; ULUV, Uluguru virus. **nt,** nucleotides; **aa,** amino acids.
Springs virus (JMSV MSB144475), virus (OXBV Ng1453, virus sequences in shrew, Guinea. Emerg. Infect. Dis. doi.org/10.3201/eid1205.051487


REFERENCES


