Novel Bat-borne Hantavirus, Vietnam

To the Editor: Compelling evidence of genetically distinct hantaviruses (family Bunyaviridae) in multiple species of shrews and moles (order Soricomorpha, families Soricidae and Talpidae) across 4 continents (1–7) suggests that soricomorphs, rather than rodents (order Rodentia, families Muridae and Cricetidae), might be the primordial hosts (6,7). Recently, the host range of hantaviruses has been further expanded by the discovery that insectivorous bats (order Chiroptera) also serve as reservoirs (8,9). Conjecturing that Mouyassué virus in the banana pipistrelle (Neoromicia nanus) in Côte d’Ivoire (8) and Magboi virus (MBGV) in the hairy split-faced bat (Nycteris hispida) in Sierra Leone (9) also serve as reservoirs (9), we analyzed tissues from bats captured in Mongolia and Vietnam.

Total RNA was extracted from 51 lung tissues, collected in RNA later Stabilization Reagent (QIAGEN, Valencia, CA, USA), from insectivorous bats, representing 7 genera and 12 species, captured in Mongolia and Vietnam. cDNA was then prepared by using PrimeScript II 1st strand cDNA Synthesis Kit (Takara Bio, Otsu, Shiga, Japan) in the hairy split-faced bat (Nycteris hispida) in Sierra Leone (9). In 1 of 5 Pomona roundleaf bats (Hipposideros pomona), a nested small (S)–segment primer set (outer: OS-M55F, 5′-TAGTAGTAGACTCC3′, and XSV-S6R, 5′-AGTCTGGRTC-CATRCTCIC3′; inner: Cro-2F, 5′-AGYCCGTATGRGW-GTIRTYYG3′, and JUVS-1233R, 5′-TCACMAGRTGRAAGT- GRTCIAC-3′). The bat was captured during July 2012 in Xuan Son National Park, a nature reserve in Thanh Son District, Phu Tho Province, ≈100 km west of Hanoi (21°07′26.75″N, 104°57′29.98″E).

For confirmation, RNA extraction and RT-PCR were performed independently in a laboratory in which hantaviruses had never been handled. After initial detection, the L-segment sequence was extended by using another primer set (PHL-173F: 5′-GAT- WAAGCATGAYTGTTGCTGA3′; and TNL-5084R: 5′-GATCTGAARTAC- ATGTTGTGG3′). To calculate the number of virus copies in tissues by real-time RT-PCR, we used a virus-specific primer set (XSV-F: 5′-GTTGACACAGTCTGGTATTG3′; and XSV-R: 5′-TTAGCACCCAAACCTCAAG3′) and probe (XSV-Probe: 5′-ACAGCTCCTGGCATTGGAATTCCTCC3′).

Pairwise alignment and comparison (with ClustalW, www.clustal.org) of a 4,582-nt (1,527 aa) region of the RNA-dependent RNA polymerase–encoding L segment indicated sequence similarities of 71.4%–71.5% and 75.9%–78.7% at the nucleotide and amino acid levels, respectively, between XSV and Mouyassué virus and MGBV. Sequence analysis of a 499-nt (166 aa) region of the nucleocapsid-encoding S segment showed that XSV differed by 42.8%–58.3% from representative hantaviruses harbored by rodents and most soricomorphs. XSV sequences were identical in lung, liver, kidney, and spleen; and the highest number of virus copies (7.6 × 10¹⁰) was in lung tissue, determined by real-time RT-PCR. No additional hantavirus-infected Pomona roundleaf bats were found by RT-PCR that used XSV-specific primers.

Phylogenetic analyses was performed with maximum-likelihood and Bayesian methods, and we used the GTR+I+Γ model of evolution, as selected by the hierarchical likelihood-ratio test in MrModel-test version 2.3 and jModelTest version 0.1 (10), partitioned by codon position. Results indicated 4 distinct phylogroups, with XSV sharing a common ancestry with MGBV (Figure). Similar topologies, supported by high bootstrap (>70%) and posterior node (>0.70) probabilities, were consistently derived when various algorithms and different taxa and combinations of taxa were used. Moreover, as we reported previously, the incongruence between some hantaviruses and their reservoir hosts might be indicative of host-switching events (5–7).

The striking sequence divergence of XSV presented considerable challenges for designing suitable primers for RT-PCR and sequencing. Also, sequencing efforts were constrained by the limited availability of tissues and concurrent virus isolation attempts. Consequently, we were unable to obtain the full-length sequence of XSV. Similarly, the inability to detect hantavirus RNA in tissues from other species of bats in this study might be attributed to several factors, including the highly focal nature of hantavirus infection, small sample sizes of bats of any given species, primer mismatches, and suboptimal cycling conditions.

Bats of the genus Hipposideros, family Hipposideridae, are among the most speciose insectivorous bats; ≈70 species are distributed across Africa, Europe, Asia, and Australia. Pomona roundleaf bats are frequently found in or near limestone or sandstone caves. Their colony sizes vary from few to many hundreds of individuals. The vast geographic distribution of the Pomona roundleaf bat throughout...
Vietnam and in Bangladesh, Cambodia, China, India, Laos, Malaysia, Myanmar, Nepal, and Thailand, provides opportunities to ascertain the genetic diversity and phylogeography of XSV and XSV-related hantaviruses. In this regard, although hantavirus RNA was not detected in archival tissues from bats of 20 genera, including several other Hipposideros species, many more genetically divergent hantavirus species are probably harbored by insectivorous bats. Not all orphan viruses warrant intensive study at the time of their discovery. However, insights into the ecology and transmission dynamics of newfound bat-borne hantaviruses might prepare us to more rapidly diagnose future outbreaks caused by emerging hantaviruses.

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Possible Cause of Liver Failure in Patient with Dengue Shock Syndrome

To the Editor: We report a rare hepatic ultrasonograph finding for a patient with liver failure associated with dengue virus (DENV) infection. This finding might shed light on the pathogenesis of liver involvement in this disease.

In March 2006, a 10-year-old previously healthy boy was hospitalized for a 3-day history of fever, headache, and nausea/vomiting. Fever subsided on the day of admission, but the patient was in shock (blood pressure 80/40 mm Hg) and had gastrointestinal bleeding and hematuria. Physical examination showed an obese, confused patient with generalized petechiae and hepatomegaly. The initial diagnosis was dengue shock syndrome (DSS). The patient was intubated and received intravenous fluid infusion, packed red blood cells, ceftriaxone, sodium bicarbonate, and ranitidine before being transferred to King Chulalongkorn Memorial Hospital in Bangkok. The patient’s blood pressure increased to 130/90 mm Hg after the initial fluid resuscitation (28 mL/kg free flow), and systolic pressure remained at ≥130 mm Hg until transfer.

Laboratory examinations found 14,930 leukocytes/mm³, hemoglobin 16.4 g/dL, hematocrit 48.2%, platelet 18,000/mm³, blood urea nitrogen 33 mg/dL, creatinine 1 mg/dL, sodium 128 mEq/L, potassium 6.2 mEq/L, chloride 91 mEq/L, carbon dioxide 28.7 s). Blood and urine cultures showed negative results. Serum was positive for IgM against DENV. Unfortunately, we did not investigate other viral causes of liver failure.

DSS with liver failure was diagnosed and treated with intravenous fluid, sodium bicarbonate, omeprazole, fresh frozen plasma, platelet transfusion, vitamin K, and recombinant factor VIIa concentrate (NovoSeven; Novo Nordisk, Bagsvaerd, Denmark). Despite stable blood pressure over the next 6 days, liver enzymes continued to rise with progressive jaundice (online Technical Appendix, wwwnc.cdc.gov/EID/article/19/7/12-1820-Techapp1.pdf). Hepatic ultrasonograph on the second