Henipavirus-related Sequences in Fruit Bat Bushmeat, Republic of Congo

To the Editor: Bats are hosts for various emerging viruses, including the zoonotic paramyxoviruses Hendra virus and Nipah virus, which occur in Australia and Southeast Asia, respectively, and cause severe disease outbreaks among humans and livestock (*1*). Antibodies and henipavirus-related RNA have also been found in the straw-colored fruit bat, *Eidolon helvum*, in Ghana, West Africa (*2*,*3*). These bats are a chief protein source for humans in sub-Saharan Africa and are therefore targeted by hunters (*4*,*5*). This practice raises special concern about the risk for virus transmission from bats to humans.

To investigate the risk of zoonotic disease emergence through hunting and preparation and consumption of bats, in October 2009, we obtained animals from local hunters. This meat was destined to be sold at markets in downtown Brazzaville, Republic of Congo. All bats were *E. helvum*, one of the most frequently hunted and traded fruit bat species in Africa (*4*,*5*). According to hunters, bats were captured with nets in an area near the capital (4°22′40″S, 15°06′27″E) during the night and collected in the morning. Animals were maintained in cages until they were sold alive in the market. For this study, living bats were brought to the National Laboratory in Brazzaville.

All animals appeared clinically healthy on arrival at the laboratory. Animals were euthanized, and samples were stored immediately in RNA or later in liquid nitrogen; additional organ samples were transferred into a 10% buffered formalin solution. Neither macroscopic pathologic changes nor histopathologic evidence for viral infection was found. A total of 339 samples collected from 42 bats were tested for paramyxovirus RNA by PCR targeting L-gene sequences of respirovirus, morbillivirus, and henipavirus (*6*). Fifteen samples from 11 individual bats yielded a product of the expected size of 494 bp. These amplicons were cloned and underwent Sanger sequencing. Virus load in tissue samples, as determined by use of specific real-time PCR, ranged from 1.1×10^2 to 3.4×10^4 copies per piece (≈0.3 cm^3). Four samples could not be quantified, probably because copy numbers were too low. Virus load in urine was 1.8×10^6 per mL. For 4 of the 14 positive samples, we gathered additional sequence information by using pan-*Paramyxovirinae* primers targeting the most conserved genomic region (*6*). Sequencing of the cloned urine sample resulted in 2 distinct sequences for each fragment. Details regarding positive samples and dataset composition are found in the Technical Appendix (wwwnc.cdc.gov/EID/pdfs/11-1607-Techapp.pdf).

In a phylogenetic tree, *Eidolon* paramyxovirus (EPMV) sequences are shown to form at least 3 distinct groups in the *Paramyxoviridae* family (Figure, Appendix, panel A, wwwnc.cdc.gov/EID/article/18/9/11-1607-F1.htm) and seem to be highly diverse compared with other paramyxovirus genera. At least 1 bat appeared to be infected with 2 different strains. Despite a geographic distance of >2,000 km among bats sampled, no spatial distinction was found between sequences from bats from Ghana and bats from the Republic of Congo. The same result can be seen when phylogenetic trees are built on the basis of the *Paromyxovirinae* fragment (Figure, Appendix, panel B). In both trees, henipaviruses cluster in between EPMV sequences. Because EPMV and henipaviruses originate from fruit bats, this finding is not surprising. All animals in this study originate from a single locality just outside Brazzaville, the capital of the Republic of Congo. *E. helvum* bats are one of the most abundant species of fruit bats in sub-Saharan Africa; they roost in large colonies comprising up to 1 million animals. Bats in this species migrate up to 2,500 km per year, probably following seasonal changes in food availability (*7*). The diversity of distinct EPMV lineages recovered by this study at a single site, and the variable clustering with sequences retrieved from animals in Ghana, demonstrate that different strains are exchanged over large distances by migratory *E. helvum* bats.

Humans are exposed to these paramyxoviruses primarily by 2 mechanisms: 1) through bushmeat hunting (using nets or shotguns), handling, and consumption; and 2) through environmental contamination with bat excretions and saliva. *E. helvum* bats frequently roost in the middle of cities, and viral load in bat urine has been shown to be high. In Bangladesh, humans became infected with Nipah virus after consuming palm sap contaminated by bat urine and saliva (*8*). Infection of domestic pigs in Ghana (*9*) might also be a result of contact with bat excreta, which is especially troubling because pigs have acted as amplifying hosts in previous Nipah virus outbreaks in humans (*10*).

Despite the substantial exposure suggested by this study, to our knowledge, no human infection associated with bat paramyxoviruses has been reported in Africa, and elevated numbers of deaths have not been observed in bat hunters. Nevertheless, the existence of isolated cases cannot be excluded because underreporting is widespread, and many cases are undiagnosed. Additional studies on virus-host ecology, along with clinical surveys of exposed persons (hunters, vendors, cooks, etc.), are required to assess the zoonotic risk of these viruses and, ultimately, diminish the threat of a novel paramyxovirus entering and spreading in human populations.

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Severe Measles, Vitamin A Deficiency, and the Roma Community in Europe

To the Editor: The Roma community in Europe is a subgroup of the Romani people, whose origins are in northern India and who have been known in English-speaking countries as "gypsies." Measles outbreaks, including severe cases, were reported in the European Roma community during 2008-2010 (*1*,*2*). We describe the potential roles of malnutrition and vitamin A deficiency as risk factors for severe measles in adults from this community.

In Europe, >25,000 cases of measles, more than half of which occurred in France, were reported during a 2011 outbreak (*3*). The exact proportion of measles cases occurring among the Roma community in France during the outbreak are not available (*2*). Measles epidemiology has changed; the disease now mainly affects children <1 year old and young adults, the latter of whom are mostly unvaccinated or have unknown vaccination status (*2*,*4*). Roma people in Europe experience some of the worst health conditions in the industrialized world: they live in overcrowded conditions and have limited access to prevention programs and to healthcare services (*2*,*5*). In such populations, deficiencies of vitamins, such as A, C, and E, have been reported (*6*). Vitamin A deficiency has been associated with severe cases of measles in children in developing countries (*7*,*8*). To date, we did not find published data associating vitamin A deficiency with severe measles among adults. We describe 6 adults from the Roma community in Marseille, France, who had measles and low levels of vitamin A; 2 of these persons had severe measles.

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Technical Appendix

Details Regarding Positive Samples and Dataset Composition

Animals obtained for this study were euthanized under anesthesia with xylazine and ketamine by cardiac exsanguination. Tissue samples were extracted by using the NucleoSpin RNA II Kit (Macherey-Nagel, Düren, Germany); throat swab specimens were dissolved in 500 µL of phosphate-buffered saline and extracted by using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). The same kit was utilized for RNA extraction from urine. Fecal samples were extracted with the Stool DNA Extraction Kit (ROBOKLON, Berlin, Germany) in the presence of carrier RNA. RNA was reverse transcribed with random hexamer primers. The 339 samples, from 42 bats that were tested, included major organs of all animals (kidney, spleen, lung, small intestine). Liver samples were only available from 41, blood samples from 39, salivary gland of 1, throat swab specimens from 28, fecal samples from 31, and urine samples from 21 individual animals. Fifteen samples that tested positive with respirovirus, morbillivirus, and henipavirus (RES-MOR-HEN) PCR included 10 spleens, 3 kidneys, 1 liver, and 1 urine sample. Only 1 organ from each animal tested positive, except for 1 animal (RC09_Eid_239) which had viral sequences found in kidney, liver, and spleen. In 1 animal (RC09_Eid_236), spleen and urine samples tested positive. Five sequence-specific real-time PCRs were designed and used to re-screen spleen, kidney, and urine samples. Two spleen samples and 1 urine sample displayed additional positive results with very low copy numbers in 1 of 2 independent experiments. Because of low copy numbers and limitation of material, no sequences were derived for these samples. *Paramyxovirinae* (PAR) sequences were obtained for the positive liver and urine samples and for 2 positive spleen samples.

L-gene sequences for phylogenetic trees were downloaded from GenBank for all paramyxoviruses available on September 21, 2011, and reduced to unique sequences by using

the FaBox program (version 1.40) (*1*). From those, we assembled separate datasets for all paramyxovirus genera, which were aligned at the amino acid level by using the muscle algorithm as implemented in MEGA (version 5) (*2*). The best-fit model of nucleotide substitution was determined for all datasets using maximum likelihood–based Akaike information criterion scores (*2*). Maximum likelihood (ML) trees were generated with MEGA and, for each genus, the 2 sequences exhibiting the maximum patristic distances were determined with PATRISTIC (version 1) (*3*). Resulting sequences were used to build up final datasets, which additionally included all available paramyxovirus sequences from *Eidolon* bats and those not assigned to an existing genus. Final datasets were thus comprised of 74 and 59 sequences (RES-MOR-HEN and PAR fragment, respectively). These datasets were aligned at the amino acid level using the muscle algorithm as implemented in SeaView, version 4.2.12. (*4*). To increase alignment quality. we selected conserved blocks from protein alignments using the Gblocks server (*5*). Blocks were reported on the corresponding nucleotide alignments, which were manually edited for final refinement. Model selection on both resulting alignments was performed using the jModeltest program (version 0.1.1) (*6*) and resulted in selecting a general time reversible model with gamma site heterogeneity (+G) and invariant sites (+I) for both fragments.

Phylogenetic analyses were performed in both ML and Bayesian frameworks. ML analyses were performed using PhyML version 3.0 (*7*), and nearest-neighbor interchange (NNI) and subtree pruning and regrafting algorithms were applied for tree search. Branch robustness was assessed by performing nonparametric bootstrapping (500 pseudo-replicates). Bayesian analyses were performed using BEAST (version 1.7.1) (*8*) under the assumption of a relaxed, uncorrelated lognormal clock and the Yule process speciation model. Two independent runs of 62,000,000 generations were performed for the RES-MOR-HEN; 15,000,000 generations were performed for the PAR fragment. Trees and numerical values taken by all parameters were sampled every 1,000 generations. The Tracer program (version 1.5) (*9*) was used to check that individual runs had reached convergence, that independent runs converged on the same zones of parameter spaces, and that chain mixing was satisfactory (global effective sample size values >100 and >200 for RES-MOR-HEN and PAR, respectively). After removal of a visually conservative 10% burn-in period, tree samples were gathered into a single file using the LogCombiner program (version 1.7.1; distributed with BEAST), and the information was summarized onto the maximum clade credibility trees using the TreeAnnotator program (version

1.7.1; distributed with BEAST). Posterior probabilities were taken as a measure of branch robustness.

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