Novel Henipavirus, Moijiang Paramyxovirus, in Rats, China, 2012

To the Editor: The genus Henipavirus (family Paramyxoviridae) contains 3 established species (Hendra virus, Nipah virus, and Cedar virus) and 19 newly identified species, including 1 full-length sequenced virus, Bat Paramyxovirus Eidngh/GH-74a/GHA/2009 (1,2). The zoonotic pathogens Hendra virus and Nipah virus have been associated with lethal neurologic and respiratory diseases in humans, horses, and pigs (3–5). The known natural reservoirs of henipaviruses are fruit bats (1,3); these viruses have not been reported in other wild animals. We report on a novel henipavirus-like virus, Moijiang paramyxovirus (MoJV), in rats (Rattus flaviceps) in China.

In June 2012, in Moijiang Hani Autonomous County, Yunnan Province, China, severe pneumonia without a known cause was diagnosed in 3 persons who had been working in an abandoned mine; all 3 patients died. Half a year later, we investigated the presence of novel zoonotic pathogens in natural hosts in this cave. For the investigation, we collected anal swab samples from 20 bats (Rhinolophus ferrumequinum), 9 rats (R. flaviceps), and 5 musk shrews (Crocidura dracula) from the mine for virome analysis.

All samples were processed by using a virus particle–protected nucleic acid purification method, followed by sequence-independent PCR amplification of extracted RNA and DNA (6). The amplified viral nucleic acid libraries were then sequenced by using an Illumina Genome Analyzer II (Illumina Trading, Beijing, China) for a single read of 81 bp. All raw reads were then aligned to the nonredundant protein database of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/RefSeq/) by using BLASTx (http://blast.ncbi.nlm.nih.gov/blast.cgi) and BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The resulting alignments were used to generate putative Henipavirus-like consensus sequences, which were aligned to representative sequences of known Henipaviridae virus species using ClustalW (http://www.ebi.ac.uk/Tools/clustalw2/index.html). The results of the alignment were analyzed with the EINheim tool of the ReviVac system (http://www.revivac.de) to classify the novel virus with unknown characteristics.

The blast results showed that the unknown Henipavirus-like virus was closest to the genus Henipavirus (Hendra virus, Nipah virus, and Cedar virus) with the following hits: 84% identity with Hendra virus (Genbank: KC565861), 81% with Nipah virus (Genbank: AY149033), and 79% with Cedar virus (Genbank: AY149034). These results are consistent with the classification of the unknown virus as a novel Henipavirus-like virus (MoJV). This is the first report of a Henipavirus-like virus associated with disease in China.

The henipaviruses have also been reported in Europe (7,8). However, the clinical signs of Hendra and Nipah virus infections are usually not detectable in the natural hosts because the viruses are transmitted to humans and domestic animals through contact with infected ruminants. This is the first report of a novel Henipavirus-like virus in the wild in China, and the transmission route of this virus is unknown. There is a potential risk of disease transmission to domestic animals and humans, and we emphasize the following points:

1. The novel Henipavirus-like virus is a zoonotic pathogen, and the natural hosts and transmission route of this virus are unknown.
2. This virus poses a potential risk of disease transmission to domestic animals and humans.
3. Further investigations are required to determine the natural hosts and transmission route of this virus.

Address for correspondence: Oscar Cabezón, Servei d’Ecopatologia de Fauna Salvatge, Wildlife Diseases Research Group, Departament de Medicina i Cirurgia Animals, Edifici V, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona), Spain; email: oscar.cabezon@cresa.uab.cat

Find emerging infectious disease information on Facebook

http://www.facebook.com
nih.gov/Blast.cgi) after filtering reads as described (6). The taxonomy of the aligned reads was parsed by using the MEGAN4 MetaGenome Analyzer (7).

On the basis of the nonredundant protein alignment results, we identified 38 sequence reads that were classified as Henipavirus spp. However, the sequences shared low nucleotide and amino acid identities with known henipaviruses. The reads were then used for reads-based PCR to identify the partial genome of this virus. The remaining genomic sequences were determined by using genome walking. The 5' and 3' untranslated regions were obtained by nested PCR with combined specific primers and henipavirus-specific degenerate primers as described (8), and the exact sequences of the 5' and 3' genome termini were determined by rapid amplification of cDNA ends.

MojV shares similar features with known henipaviruses. The virus has a genome length of 18,404 nt (submitted to GenBank under accession no. KF278639), and has the characteristic henipavirus gene order: 3'-nucleocapsid (N) protein (539 aa); P/V/W/C proteins (phosphoprotein; 694 aa, 464 aa, 434 aa, 177 aa); matrix protein (340 aa); fusion protein (545 aa); attachment glycoprotein (625 aa); and large (L) protein (2,277 aa)-5' (online Technical Appendix Figure, wwwnc.cdc.gov/EID/article/20/6/13-1022-Techapp1.pdf). The predicted conserved sequences between genes showed features characteristic of henipaviruses (online Technical Appendix Table). The central domain of the N protein contains 3 conserved motifs common in all paramyxoviruses: QXW [I/V] X, K [A/C] XT, FX, [I/L] [R/K] [Φ/G/A] [L/I/V] XT, and FX, Φ, S Φ, AMG, where Φ

Figure. Phylogenetic trees based on the nucleocapsid proteins (A) and large proteins (B) of Mojiang paramyxovirus (MojV) and other previously reported paramyxoviruses. Bold font indicates MojV and Henipavirus spp. Scale bars indicate nucleotide substitutions per site.

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 20, No. 6, June 2014 1065
is an aromatic amino acid (9). In addition, the RNA editing site (AAAA GG) for the processing of V and W proteins conserved in the phosphoprotein gene sequences of Hendra virus and Nipah virus was found, and 6 conserved domains within the L proteins of the order Mononegavirales (8) were found in the MojV L protein.

The nucleotide identities of predicted MojV genes exhibited similarity with genes of known henipaviruses: N (53.0%–57.0% identity), phosphoprotein (37.8%–43.0% identity), matrix (59.5%–63.4% identity), fusion (47.5%–51.4% identity), attachment glycoprotein (36.6%–41.8% identity), and L (55.9%–58.6% identity) genes. Using MEGA5 (10), we used the phylogenetic trees based on N and L proteins to describe the evolutionary relationships between MojV and members of the family Paramyxoviridae (Figure). MojV clustered with the 4 members of the genus Henipavirus and was distant from other clusters. Thus, considering the similar genome features between MojV and other henipaviruses, we confirmed that MojV could be classified as a new species closely related to Henipavirus spp.

Specific nested primer sets targeting the L gene of MojV were designed to separately re-evaluate the 34 anal swab samples and some tissue samples. Of 9 anal swab samples from the R. flaviepectus rats, 3 were positive for MojV, and a tissue sample from 1 of the 3 MojV-positive rats was also MojV positive (tissue was not collected from the other 2 rats). All 20 samples from R. ferrumequinum bats and all 5 samples from C. dracula musk shrews were MojV negative. The 3 MojV-positive anal swab samples were cultured in Vero E6, Hep2, and BHK21 cells for virus isolation; no cytopathic effects or viral replication was detected after 2 blind subculture passages.

Our study showed the presence of a rodent-origin, henipa-like virus, MojV, in China. R. flaviepectus rats are the natural reservoir of MojV. This finding and its context indicate that Henipavirus spp. viruses might infect more mammalian hosts than previously thought and that bats may not be the only hosts of henipaviruses.

This work was supported by a National S&T Major Project (China Mega-Project for Infectious Disease; grant no. 2011ZX10004-001) from the People’s Republic of China, and by a Basic Research and Operating Expenses grant (no. 2013IPB301) from the Institute of Pathogen Biology, Chinese Academy of Medical Sciences and Peking Union Medical College.

Zhiqiang Wu,1 Li Yang,1 Fan Yang,1 Xianwen Ren,1 Jinyong Jiang, Jie Dong, Lilian Sun, Yafang Zhu, Hongning Zhou, and Qi Jin
Author affiliations: Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China (Z. Wu, L. Yang, F. Yang, X. Ren, J. Dong, L. Sun, Y. Zhu, Q. Jin); Institute of Pathogen Biology, Beijing (Z. Wu, L. Yang, F. Yang, X. Ren, J. Dong, L. Sun, Y. Zhu, Q. Jin); and Yunnan Institute of Parasitic Diseases, Puer, China (J. Jiang, H. Zhou)
DOI: http://dx.doi.org/10.3201/eid2006.131022

References

Address for correspondence: Qi Jin, No. 6 Rongjing East St, Yizhuang, Beijing, 100176, China; email: zdysy@vip.sina.com

The Public Health Image Library (PHIL)
PHIL, Centers for Disease Control and Prevention, contains thousands of public health-related images, including high-resolution (print quality) photographs, illustrations, and videos.

PHIL collections illustrate current events and articles, supply visual content for health promotion brochures, document the effects of disease, and enhance instructional media.

PHIL Images, accessible to PC and Macintosh users, are in the public domain and available without charge.

Visit PHIL at http://phil.cdc.gov/phil
Novel Henipa-like Virus, Mojiang Paramyxovirus, in Rats, China, 2012

Technical Appendix

Figure. Genomic organization of Mojiang paramyxovirus. N, nucleocapsid protein; L, large protein; P/V/W/C, phosphoprotein; M, matrix protein; F, fusion protein; G, attachment glycoprotein.

Table. Conserved nucleotide sequences for the gene start, intergenic region, and gene stop of Mojiang paramyxovirus and henipaviruses*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Gene stop</th>
<th>Intergenic region</th>
<th>Gene start</th>
</tr>
</thead>
<tbody>
<tr>
<td>MojV, gene /N</td>
<td>TTAAACAAAAA</td>
<td>CTT</td>
<td>AGGAATCCAGG</td>
</tr>
<tr>
<td>N/P</td>
<td>TCATAAAAAA</td>
<td>CTT</td>
<td>AGGAATCCAGG</td>
</tr>
<tr>
<td>P/M</td>
<td>ATATAAAAAA</td>
<td>CTT</td>
<td>AGGTGTCAGG</td>
</tr>
<tr>
<td>M/F</td>
<td>TTAATAAAAA</td>
<td>CTT</td>
<td>AGGAATCCAGG</td>
</tr>
<tr>
<td>F/G</td>
<td>TTACAAAAAA</td>
<td>CTT</td>
<td>AGGATTMCAGG</td>
</tr>
<tr>
<td>G/L</td>
<td>TWAHRAAAAAA</td>
<td>CTT</td>
<td>AGGATMCARG</td>
</tr>
</tbody>
</table>

* N, nucleocapsid; P, phosphoprotein (P/V/W/C); M, matrix; F, fusion; G, glycoprotein; L, large protein.