therapy. Sequence analysis of the viral protein 1 gene revealed that both of these viruses clustered with the major outbreak strain from the United States. Partial gene sequences of viral protein 1 were deposited in the GenBank database under accession nos. KP247599 and KP247600.

We next used CLART PneumoVir to retest samples that had been positive for enterovirus/rhinovirus by FilmArray during September–October 2013 and 2014. The number of overall samples tested for respiratory viruses did not increase from 2013 to 2014 (227 and 218, respectively), but the percentage of enteroviruses detected increased strikingly (from 2.6% to 14.6%). We then compared clinical characteristics and their frequency of occurrence among enterovirus-positive patients hospitalized during September–October 2013 and September–October 2014. The clinical features of 24 enterovirus-positive patients hospitalized during 2014 differed from those of 5 enterovirus-positive children hospitalized during 2013. Hospitalization in 2014 was mostly for asthmatic crisis in children 2.5 to 7 years of age; this pattern is less clear for the few patients hospitalized in 2013 (Table). A substantial proportion of patients hospitalized in 2014 required oxygen support and admission to the pediatric intensive care unit.

In conclusion, we report 2 confirmed cases of EV-D68 in a Southern Hemisphere country during the 2014 outbreak reported in the United States. That these cases are virologically and clinically related to those reported in the United States documents that the virus had been introduced to the Southern Hemisphere during the spring of 2014. A substantial increase in enterovirus cases displaying a notably similar clinical pattern (asthmatic crisis in children) strongly suggests that EV-D68 infections are increasingly rapidly. This virus has been previously identified in the region (*3*) but only sporadically. The virus could spread to other areas in Santiago and to other cities, and similar situations could occur in other Latin American countries, especially those with many residents who travel to the United States. Public health officials need to be notified of this potential, and appropriate surveillance and treatment strategies need to be implemented.

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# Bat Coronavirus in Brazil Related to Appalachian Ridge and Porcine Epidemic Diarrhea Viruses

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**To the Editor:** *Tadarida brasiliensis* (I. Geoffroy, 1824) is a species of free-tailed bat that has resident and migratory populations in Brazil (*1*). This species has adapted to urban areas, occupying roofs, ceilings, and other human constructions, and often coexists with other bat species and humans (*2*), enabling epidemiologic risks (*3*). In recent studies, an alphacoronavirus has been detected in urban bat species *Molossus molossus*, *M. rufus*, and *Tadarida brasiliensi* in Brazil (*4*,*5*). Evidence suggests that alphacoronaviruses may use bats as hosts to spread human coronavirus (HCoV) NL63, which originated by evolution of Appalachian Ridge CoV strain 2 (ARCoV.2) (*6*).

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In this study, a total of 20 anal and tracheal swab samples from 10 bats (*T. brasiliensis*) were collected at the Jequitibás Wood, in Campinas, São Paulo State, Brazil (22°54′31.34″S 47°02′58.01″W). We extracted viral genetic material using the RNA Extraction Mini Kit (QIAGEN, Hilden, Germany) and synthesized cDNA using random primers from the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA), following the manufacturer's protocol.

Samples were analyzed by conventional reverse transcription PCR assays using panCoV primers targeting a 215-bp replicase fragment as previously described (*7*) with slight modifications to include more cycles and less extension time in order to obtain more PCR products. Sequencing reactions on a Pancoronavirus-positive anal swab sample (*7*) were performed at Central Laboratory of High Performance Technologies in Life Sciences (LaCTAD) at UNICAMP (http://www.lactad.unicamp.br) using an automated sequencer (3730xl DNA Analyzer; Applied Biosystems).

The chromatograms were edited using the program UGENE version 1.14 (UGENE, http://ugene.unipro.ru/forum/YaBB.pl?num=1407749393) and evaluated using Phred scores for base calling. Alignment was made with ClustalW v.2.1 software (http://www.clustal.org> implemented on Linux command line interface, and a similarity matrix was generated with sequences retrieved from the GenBank database. A 144-nt fragment of the replicase gene was obtained after editing, and phylogenetic analysis was performed after determining the best evolution model by using the jModelTest2 software (https://code.google.com/p/jmodeltest2/). Different CoV sequences were included to represent the genera *Alpha-*, *Beta-*, *Gamma-*, and *Deltacoronavirus*. Clustering with the ARCoV.2 and porcine epidemic diarrhea virus (PEDV) was obtained using the maximum-likelihood (ML) method after 1,000 Shimodaira-Hasegawa–like support values with the general time-reversible model and category approximation in 20 rates category in a gamma distribution (online Technical Appendix Figure, panel A, http://wwwnc.cdc.gov/EID/article/21/4/14-1783-Techapp1.pdf) and neighbor-joining methods under Kimura-2-parameter and 1,000 replicates of bootstrap (online Technical Appendix Figure, panel B).

Subsequently, metagenomic analysis was made by creating a pool of the 10 bat samples. Samples were resuspended in Dulbecco Modified Eagle Medium (Life Technologies-GIBCO, Grand Island, NY, USA) and filtered through 0.22 μm. The recovered sample was then treated with DNase (Invitrogen, Carlsbad, CA, USA) to remove contaminating DNA and with Proteinase K (Invitrogen) to eliminate inhibitors and to disrupt viral capsids. Samples were then subjected to RNA extraction (QIAGEN) and sent to the sequencing core facility. Sequencing was performed on Illumina HiSeq2500 instrument by using the 2×100 bp kit according to manufacturer's instructions.

Through these analyses, we obtained 34,409,110 reads, of which 76.47% had quality index ≥30. The contigs were assembled by de novo genome assembly (blastx E-value $\leq 1^{-5}$) (*8*) generating 10.742 scaffolds: 35 matches for coronaviruses (using the Coronavirus Database, http://covdb.microbiology.hku.hk), 3 matches for PEDV, and 2 matches for HCoV-NL63 (both using the UniProt database, http://www.uniprot.org) (online Technical Appendix). The sequences obtained had 87.5% (126/144) nucleotide identity with ARCoV.2, an unclassified alphacoronavirus (GenBank accession no. JX537912) for which a zoonotic role has been suggested (*6*). Preliminary analysis indicated good coverage of the polymerase region of the ARCoV.2 reference sequence by the reads (quality index >40) by using reference assembly against CoV complete genomes. This finding reinforces the hypothesis of this viral agent in the specimens analyzed. Moreover, molecular assays are under way in our laboratory to elucidate the alternative hypothesis of PEDV presence in bats in Brazil.

In summary, we found that a CoV detected in *T. brasiliensis* bats in Brazil has close phylogenetic relationships to ARCoV.2 and PEDV. Considering the zoonotic impact of these viral agents on the emergence of new diseases in animal and human populations, we believe that both results may strongly contribute to a better understanding of the molecular eco-epidemiology of these alphacoronaviruses. The reconstruction of their evolutionary history to trace their occurrence in humans and in bat populations as well as in other animals is being conducted to clarify their evolutionary pathway.

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# Tandem Repeat Insertion in African Swine Fever Virus, Russia, 2012

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**To the Editor:** The recent introduction of African swine fever virus (ASFV) into the European Union (http://www.oie.int/animal-health-in-the-world/the-world-animal-health-information-system/data-after-2004-wahid/) has caused serious concern in pig industries in Europe and their trade partners. African swine fever is one of the most feared infections that can affect pig industries because no vaccine is available and the socioeconomic effect of an outbreak would be serious (*1*). Therefore, early detection and coordinated countermeasures are urgently needed. For these countermeasures, information on disease dynamics and evolution is mandatory. In this respect, molecular epidemiology can be used to trace virus spread and transmission pattern.

Because it is a DNA virus, ASFV evolves rather slowly, and the use of routine genome fragments (variable region of the B646L gene and parts of the E183L gene) for partial sequencing has so far shown 100% identity among strains found in Russia (*2*) and the neighboring countries (*3*). Thus, the resolution power of these approaches is too low for in-detail analyses, which depend on information regarding larger genome fragments or whole genomes.

In 2014, an insertion of a tandem repeat sequence (TRS) in the intergenic region between the I73R and the I329L protein genes was found in ASFV strains from Poland and Lithuania (*3*). This TRS insertion was also found in ASFV strains from Ukraine in July 2012 and from Belarus in June 2013, but not in strains from Russia, Georgia, or Azerbaijan. Gallardo et al. (*3*) concluded that ASFV strains in Lithuania and Poland most likely originated from Belarus. However, these authors indicated that for a full understanding of evolution and spread, additional sequence analyses would be needed, especially from regions of Russia bordering Belarus and Ukraine. We report information for 3 additional sequences from ASFV strains from Russia that were analyzed for the previously-mentioned TRS insertion on the basis of full-genome sequences.

These ASFV strains originated from domestic pigs from the Tulskaya oblast (Tula06/2012), the Tverskaya oblast Kashinskiy district (Kashinskiy 09/2012), and the Tverskaya oblast (Tver06/2012) in 2012. Genome sequences were obtained by using a primer-walking method that was adapted from Portugal et al. (*4*). Resulting PCR products were subjected to next-generation sequencing by using the MiSeq platform (Illumina, San Diego, CA, USA). Raw sequence data were analyzed and assembled by using Genome Sequencer software version 2.6 (Roche, Mannheim, Germany). Additional sequences of the intergenic region of 17 virus isolates from domestic pigs and wild boar from Russia were obtained by using conventional PCR, and amplicons were directly sequenced by using a 3130x1 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's recommendations.

Chromatograms were manually edited and assembled by using CAP3 (http://pbil.univ-lyon1.fr/cap3.php). All nucleotide sequences of ASFV isolates obtained in this study were deposited in GenBank under accession nos. KP137625–KP137644. In the alignment, other published sequences available in GenBank from Poland, Lithuania, Belarus, Ukraine, Armenia, Azerbaijan, Russia, and Georgia were included (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/4/14-1792-Techapp1.pdf). Sequence alignment was performed by using the ClustalW algorithm (http://www.clustal.org) as implemented in Geneious version 7.1.7 (Biomatters Ltd., Auckland, New Zealand).

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