

PCR/ESI-MS of BAL fluid followed by EV-D68-specific PCR testing of 1 nasopharyngeal swab and 2 BAL fluid samples confirmed our clinical suspicion of ARDS secondary to EV-D68 in an adult. The patient's history of contact with sick family members and clinical signs (non-productive cough, nausea, and vomiting) were suggestive of a viral infection. Lessons learned from the emergence of swine-origin influenza A(H1N1)pdm09 virus and recognition (in the midst of the pandemic) that younger age and obesity were risk factors for severe disease were also suggestive of a viral respiratory infection.

We are developing a specific rapid molecular assay for EV-D68, which should help clinicians recognize when EV-D68 is present in the community. During those times, EV-D68 infection should be included in the differential diagnosis of severe respiratory infection. Documentation of EV-D68 infection may help with clinical management for individual patients and minimize unnecessary use of antimicrobial drugs within communities.

#### Acknowledgments

We thank the staff at Washington University in St. Louis, MO, USA, particularly Anthony Orvedahl, for assistance with EV-D68-specific PCR.

#### References

- Centers for Disease Control and Prevention. Nonpolio enterovirus and human parechovirus surveillance—United States, 2006–2008. *MMWR Morb Mortal Wkly Rep.* 2010;59:1577–80.
- Centers for Disease Control and Prevention. Non-polio enterovirus: enterovirus D68 in the United States, 2014 [cited 2015 Jan 8]. <http://www.cdc.gov/non-polio-enterovirus/outbreaks/EV-D68-outbreaks.html>
- Midgley CM, Jackson MA, Selvarangan R, Turabelidze G, Obringer E, Johnson D, et al. Severe respiratory illness associated with enterovirus D68—Missouri and Illinois. *MMWR Morb Mortal Wkly Rep.* 2014;63:798–9.
- Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, et al. The American-European Consensus Conference on ARDS: definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med.* 1994;149:818–24. <http://dx.doi.org/10.1164/ajrccm.149.3.7509706>
- Couturier MR, Barney T, Alger G, Hymas WC, Stevenson JB, Hillyard D, et al. Evaluation of the FilmArray® Respiratory Panel for clinical use in a large children's hospital. *J Clin Lab Anal.* 2013;27:148–54. <http://dx.doi.org/10.1002/jcla.21576>
- Wylie KM, Wylie TN, Orvedahl A, Buller RS, Herter BN, Magrini V, et al. Genome sequence of enterovirus D68 from St. Louis, Missouri, USA. *Emerg Infect Dis.* 2015;21:184–6. <http://dx.doi.org/10.3201/eid2101.141605>
- Farrell JJ, Sampath R, Ecker DJ, Bonomo RA. 'Salvage microbiology': detection of bacteria directly from clinical specimens following initiation of antimicrobial treatment. *PLoS ONE.* 2013;8:e66349. <http://dx.doi.org/10.1371/journal.pone.0066349>
- Metzgar D, Baynes D, Myers CA, Kammerer P, Unabia M, Faix DJ, et al. Initial identification and characterization of an emerging zoonotic influenza virus prior to pandemic spread. *J Clin Microbiol.* 2010;48:4228–34. <http://dx.doi.org/10.1128/JCM.01336-10>
- Metzgar D, Lovari R, Ray K, Baynes D, Drapp D, Frinder M, et al. Analytical characterization of an assay designed to detect and identify diverse agents of disseminated viral infection. *J Clin Virol.* 2014;59:177–83. <http://dx.doi.org/10.1016/j.jcv.2013.12.005>

Address for correspondence: John J. Farrell, University of Illinois School of Medicine, Division of Infectious Disease, 723 NE Glen Oak Ave, Peoria, IL 61604, USA; email: [jjf@uic.edu](mailto:jjf@uic.edu)

## Enterovirus D68–Associated Severe Pneumonia, China, 2014

Tiegang Zhang, Lili Ren, Ming Luo, Aihua Li, Cheng Gong, Meng Chen, Xiali Yu, Jiang Wu, Ying Deng, Fang Huang

Author affiliations: Beijing Center for Disease Prevention and Control, Beijing, China (T. Zhang, M. Luo, A. Li, C. Gong, M. Chen, X. Yu, J. Wu, Y. Deng, F. Huang); Institute of Pathogen Biology, Beijing (L. Ren)

DOI: <http://dx.doi.org/10.3201/eid2105.150036>

**To the Editor:** Over the past 4 years, outbreaks caused by enterovirus type D68 (EV-D68) infection have occurred in many parts of the world (1); this virus can cause severe respiratory tract infections (RTIs) in children. This public health concern has been boosted by the recent outbreaks of EV-D68 infection in the United States (<http://www.cdc.gov/non-polio-enterovirus/outbreaks/EV-D68-outbreaks.html>). Outbreaks associated with novel EV-D68 have also been reported during 2006–2012 in China (2,3). However, since 2012, no EV-D68 infections in China have been reported. Whether the EV-D68 outbreaks in the United States affected those in China is unclear. Continuous characterization of EV-D68 epidemics is therefore necessary for purposes of early alert and for facilitating control measure decisions.

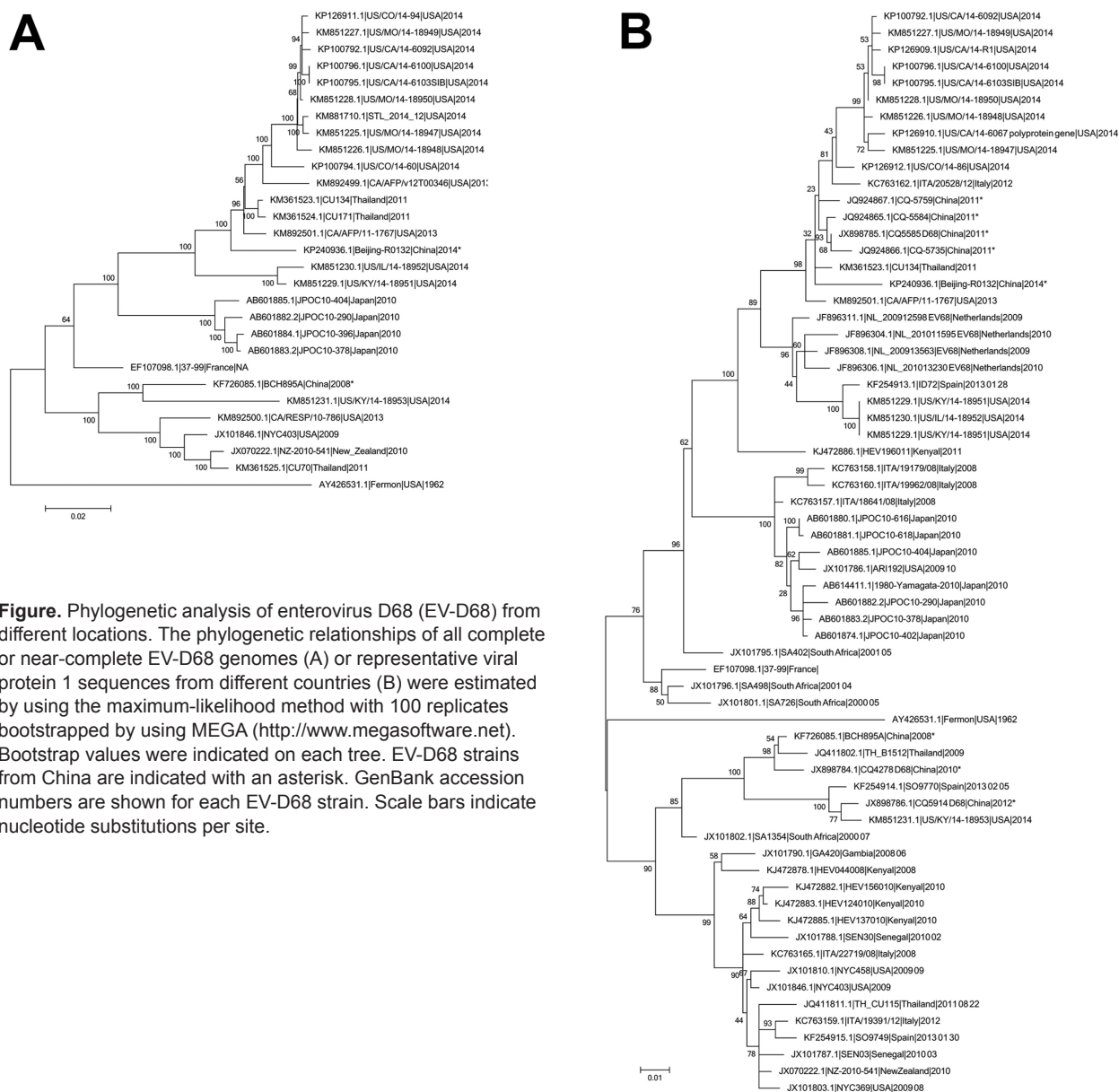
To determine EV-D68 prevalence in China, we screened for EV-D68 infections in 2014 in Beijing, China. We tested patients with RTI during August–November 2014, reported by the Respiratory Virus Surveillance System, established by Beijing Center for Disease Prevention and Control. The System covers 30 sentinel hospitals in all 16 districts of Beijing. We obtained 1,478 clinical specimens (1,034 nasopharyngeal swab and 444 sputum). Patient ages ranged from 8 months to 93 years (median 33.5 years, mean 37.9 years). Enteroviruses and other known respiratory viruses were detected by real-time PCR (4). A total of 70 enterovirus-positive samples were identified. Other respiratory viruses detected were 89 rhinoviruses, 87

influenza viruses, 70 human parainfluenza viruses (types 1–4), 43 human coronaviruses, 26 respiratory syncytial viruses, 29 adenoviruses, 9 bocaviruses, and 2 metapneumoviruses. Among the EV-positive samples, 1 was positive for EV-D68 according to PCR amplification of the viral protein 1 (VP1) gene (5); no other respiratory viruses were detected in this patient.

The EV-D68-positive patient was a 5-year-old girl with no underlying disease. On August 5, 2014, she had fever (highest temperature 40°C), cough, breathing difficulty, abdominal pain, diarrhea, and vomiting. Her condition deteriorated, and on August 11, she was hospitalized in Beijing Children's Hospital with a diagnosis of severe

pneumonia. A nasopharyngeal swab sample was collected at the time of admission. She was released on August 16, after receiving symptomatic supportive treatment in the general ward; she had not required mechanical ventilation. She and her family had no history of travel in the months before she became ill.

To further characterize this virus strain, Beijing-R0132, we amplified the genome sequence directly from a nasopharyngeal swab sample by using overlapping primers designed according to the reference sequence (GenBank accession no. KM892501; online Technical Appendix, <http://wwwnc.cdc.gov/EID/article/21/5/15-0036-Techapp1.pdf>). The termini of the genome were independently



**Figure.** Phylogenetic analysis of enterovirus D68 (EV-D68) from different locations. The phylogenetic relationships of all complete or near-complete EV-D68 genomes (A) or representative viral protein 1 sequences from different countries (B) were estimated by using the maximum-likelihood method with 100 bootstrapped replicates by using MEGA (<http://www.megasoftware.net>). Bootstrap values were indicated on each tree. EV-D68 strains from China are indicated with an asterisk. GenBank accession numbers are shown for each EV-D68 strain. Scale bars indicate nucleotide substitutions per site.

determined by using the RACE System (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Each amplicon was sequenced 4 times by using the Sanger method, and the forward and reverse sequences agreed well. Sequences were assembled by using DNA-Star software (Lasergene, Madison, WI, USA) and deposited in GenBank (accession no. KP240936). The genome of Beijing-R0132 was 7,334 nt long, including 699 nt in 5'-untranslated regions (5'-UTRs), 6,567 nt in open reading frame (ORF), and 68 nt in 3'-UTR. Beijing-R0132 shared 96% nt sequence identity with the virus circulating in the United States in 2014, US/CO/14-60. In contrast to the prototype EV-D68 (Fermon strain, AY426531), deletions of the CTCAAAACCTCCAGTACATAACA sequence in the 5'-UTR and TTATTTATAACA sequence in the front of the ORF of Beijing-R0132 were observed, corresponding with nt 682–704, and nt 721–732 of the Fermon strain, which were similar to those identified in the United States in 2014.

We then used MEGA software version 6.06 (<http://www.megasoftware.net>) to analyze the phylogeny of the whole genome and the VP1 gene with EV-D68 sequences available in GenBank (Figure). Beijing-R0132 was clustered with most of the EV-D68 strains that circulated throughout the United States during 2014. The strains identified from Beijing in 2008, represented by BCH895A/2008, belong to another distinct lineage according to genome phylogeny (Figure, panel A). Similar relationships were observed in the phylogenetic tree of the VP1 gene (Figure, panel B). Beijing-R0132 and some EV-D68 strains from China identified in 2011 grouped with most of the strains obtained from the United States in 2014. These findings demonstrate that the EV-D68 strain circulating in Beijing was closely related to strains circulating in the United States.

The origin of the 2014 EV-D68 outbreaks in the United States is unclear. This Beijing-R0132 genome sequence provides information for tracking EV-D68 as it spreads throughout the world and for evaluating the sequence diversity in circulating EV-D68 strains. In contrast to EV-D68

detection during the outbreaks in the United States, positive detection of EV-D68 in this study was limited, although the circulating virus strains were closely related. The reason for this disparity warrants further investigation. However, the severe pneumonia caused by EV-D68 reported here underlies the need for intensive attention to surveillance and control of EV-D68 in vulnerable populations, such as young children. In addition, for a megacity with very high population density and mobility, such as Beijing, the city-wide implementation of the Respiratory Virus Surveillance System is critical for monitoring the epidemic or potential outbreaks of EV-D68 and other respiratory viruses and for enabling early warning.

#### Acknowledgment

We thank sentinel hospitals of the Respiratory Virus Surveillance System in Beijing for collecting samples and investigating cases.

This work was supported by the National Major Science and Technology Project for Control and Prevention of Major Infectious Diseases of China (2012ZX10004-206).

#### References

1. Renois F, Bouin A, Andreoletti L. Enterovirus 68 in pediatric patients hospitalized for acute airway diseases. *J Clin Microbiol.* 2013;51:640–3. <http://dx.doi.org/10.1128/JCM.02640-12>
2. Xiang Z, Gonzalez R, Wang Z, Ren L, Xiao Y, Li J, et al. Coxsackievirus A21, enterovirus 68, and acute respiratory tract infection, China. *Emerg Infect Dis.* 2012;18:821–4. <http://dx.doi.org/10.3201/eid1805.111376>
3. Lu QB, Wo Y, Wang HY, Wei MT, Zhang L, Yang H, et al. Detection of enterovirus 68 as one of the commonest types of enterovirus found in patients with acute respiratory tract infection in China. *J Med Microbiol.* 2014;63:408–14. <http://dx.doi.org/10.1099/jmm.0.068247-0>
4. Li AiH, Zhang TG, Shi WX, Cui SJ, Chen M, Wu J, et al. A comparison of three methods for detecting respiratory viruses [in Chinese]. *Chinese Journal of Microbiology and Immunology.* 2013;33:541–4.
5. Tokarz R, Firth C, Madhi SA, Howie SR, Wu W, Sall AA, et al. Worldwide emergence of multiple clades of enterovirus 68. *J Gen Virol.* 2012;93:1952–8. <http://dx.doi.org/10.1099/vir.0.043935-0>

Address for correspondence: Fang Huang, No. 16 Hepingli Middle Ave, Dongcheng District, Beijing 100013, China; email: hhhfxdd@126.com

## Correction: Vol. 21, No. 4

The Continuing Medical Education quizzes for 2 articles were inadvertently omitted from the April issue. The quizzes are printed at the back of this issue, and

the complete articles and quizzes are available online ([http://wwwnc.cdc.gov/eid/article/21/4/14-1479\\_intro](http://wwwnc.cdc.gov/eid/article/21/4/14-1479_intro) and [http://wwwnc.cdc.gov/eid/article/21/4/14-1033\\_intro](http://wwwnc.cdc.gov/eid/article/21/4/14-1033_intro)).

# Enterovirus D68–Associated Severe Pneumonia, China, 2014

## Technical Appendix

Technical Appendix Table. Primers used for amplification and sequencing of the genome of enterovirus D68 strain Beijing-R0132

Primer	Sequences, 5'→3'	Position*	Amplicons
P-EV68-1-F	TTAAAACAGCCTTGGGGTTG	1–20	833 bp
P-EV68-1-R	TGGCTGAAGCCGCATAACTA	833–814	
P-EV68-2-F	AACATTGCCACAAATGGATC	758–777	917 bp
P-EV68-2-R	GTATGTTGGGACACCTTGAGTAA	1675–1653	
P-EV68-3-F	TACGCGTACAATGTCAAGTATG	1570–1591	899 bp
P-EV68-3-R	ACCACACCAAGTTCAGCGTTA	2469–2449	
P-EV68-4-F	CCTTGATAGGGTTCATAGCAGC	2292–2313	1007 bp
P-EV68-4-R	CTGGACCAGTGGTCACTA	3299–3282	
P-EV68-5-F	CACCACGAACCCTGCCATA	3159–3177	871 bp
P-EV68-5-R	CTGTCTAGGTACATAAGGAATGCC	4030–4007	
P-EV68-6-F	ACACTAGCATTGTTGGGATGC	3938–3958	817 bp
P-EV68-6-R	AATCTGCGTGACAAAGCCT	4755–4737	
P-EV68-7-F	GGAActCTGTACACCAGTCCAT	4658–4679	893 bp
P-EV68-7-R	AGTCAAATCTCTAAGCGCACAC	5551–5530	
P-EV68-8-F	TATGATAGGGTAGCGGTCATC	5444–5464	819 bp
P-EV68-8-R	TCCCTTGTAGTAAGTAAGGGAAT	6263–6241	
P-EV68-9-F	TCAGTGTGGATCCCATACC	6159–6177	591 bp
P-EV68-9-R	GGCATCCCACCATTAActATATAT	6750–6727	
P-EV68-10-F	ATGCTAGTTTGTCAACAGTGTG	6603–6624	740 bp
P-EV68-10-R	GCCCCAAGTGACCAAAATT	7343–7324	
P-EV68-5-RACE-R	GTCCAGACTCATCGACC	333–317	5'-UTR†
P-EV68-3-RACE-F	GATATTTCAAGCAGATGATC	7017–7037	3'-UTR

\*Nucleotide positions indicated are those corresponding to GenBank accession no. KM892501.

†UTR, untranslated region.