Human Enterovirus Genotype C104, China

To the Editor: Human enteroviruses (EVs) are small, nonenveloped RNA viruses belonging to the family *Picornaviridae*. Approximately 100 EV genotypes have been identified. Recently, EV68 epidemics in respiratory tract infections (RTIs) have been reported worldwide (*1,2*). Moreover, rarely detected EVs (e.g., EV-C104 and EV-C109) have been increasingly identified in patients with RTIs (*3–7*), indicating a possible association of EVs with respiratory syndromes.

Little is known about the role of EV-C104 in RTIs. EV-C104 has been reported in 3 countries: Switzerland (7 children with pneumonia or otitis media) (*3*), Italy (3 adults and 4 children with RTIs) (*4,7),* and Japan (1 adult with an upper RTI [URTI]) (*5*). We report additional EV-C104 strains in 4 children with lower RTIs and in 1 adult with a URTI in China.

To identify EV infections, we collected nasopharyngeal aspirates from 3,108 children (1,963 boys) ≤14 years of age (median age 12 months; age range 0.3–168 months) who had lower RTIs at admission to Beijing Children's Hospital during March 2007–February 2012. Throat and nasal swab specimens were also collected from 9,232 adults (4,140 men) ≥15 years of age (median age 35.3 years; age range 15–97 years) with acute RTIs who received treatment at Peking Union Medical College Hospital during August 2006–February 2012. All samples were screened for influenza virus, parainfluenza virus type 1–4, respiratory syncytial virus (RSV), coronaviruses (229E, NL63, HKU1, and OC43), metapneumovirus, adenovirus, rhinovirus, bocavirus, and EVs (*8*). Overall, 37 (1.2%) children and 158 (1.7%) adults were positive for EV.

Because we could not amplify EV-C104 by using primers specific for the viral protein (VP) 1 region (*9*), we used a reverse transcription PCR to amplify the 5′ -untranslated region/VP4/VP2 gene (*10*). Amplicons of ≈600 bp were obtained for samples from 5 patients: 4 boys 2–11 months of age (BCH2859A, BCH2892A, BCH2894A, and BCH3034A) and a 30-year-old man (PUMCH12286). BLAST analysis (www.ncbi.nlm.nih.gov) of sequences of these amplicons showed that the 590-nt sequences had 94.0% identity with that of the EV-C104 prototype strain CL-12310945

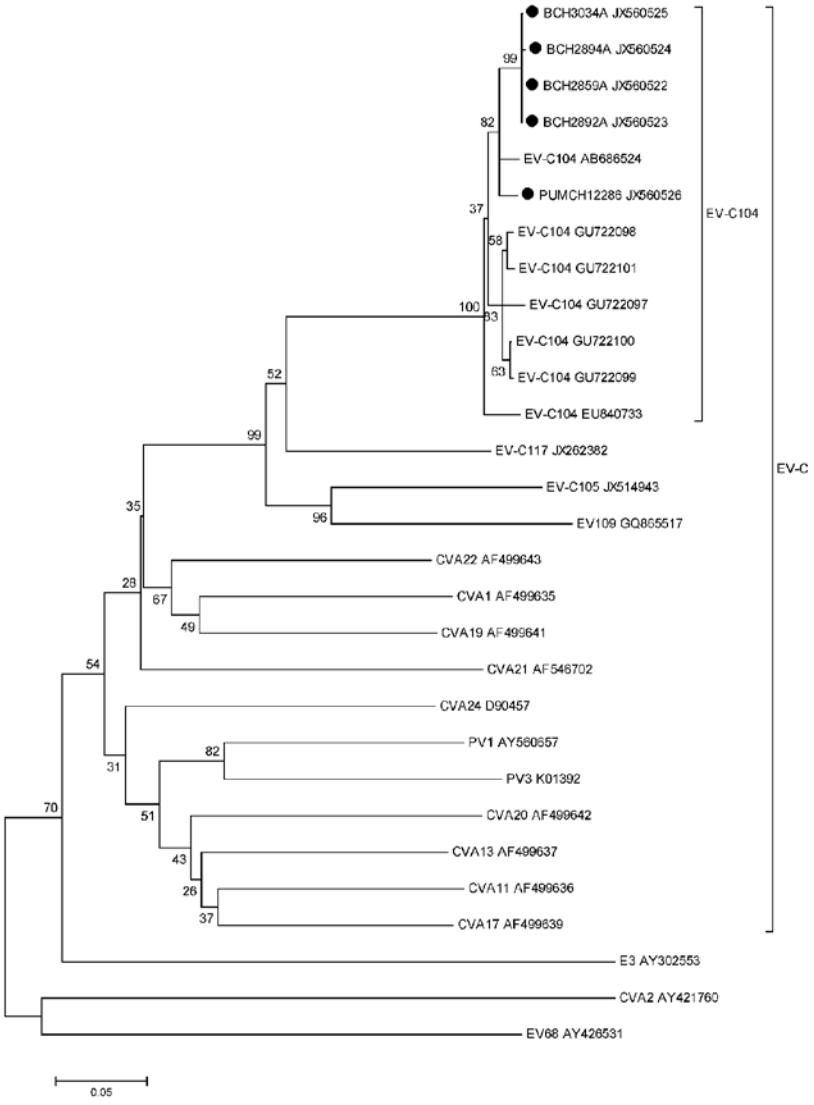

Figure. Phylogenetic tree of human enteroviruses (EVs) for nucleotide sequences of the viral protein (VP) 4/VP2 gene region (435 nt, corresponding to nt positions 654–1,088 of EV-C104 prototype strain CL-12310945 [EU840733]), People's Republic of China, March 2007–February 2012. The tree was generated with 1,000 bootstrap replicates. Neighbor-joining analysis of targeted nucleotide sequence was performed by using the Kimura 2-parameter model with the Molecular Evolutionary Genetics Analysis software version 4.0 (www.megasoftware.net/). Each strain detected in this study is indicated by a black circle and a specific identification code (BCH/PUMCH), followed by the patient number. Enterovirus 68, cocksackievirus (CV) A2, and echovirus (E) 3 (GenBank accession nos. AY426531, AY421760, and AY302553) were used as outgroups. PV, poliovirus. EV-C denotes the EV species to which EV-C104 belongs. Scale bar indicates evolutionary distance.

(EU840733). The amplicon contained 155 nt in the 5′-untranslated region, 207 nt in VP4, and 228 nt in VP2. Phylogenetic analysis of VP4/VP2 sequences showed that the 5 sequences obtained in this study (GenBank accession nos. JX560522–JX560526) belonged to genotype EV-C104 within the EV-C species (Figure).

Virus isolation for EV-C104 with Vero and H1-HeLa cells was unsuccessful. Although we screened 591, 797, 459, 664 and 597 samples from children for 5 consecutive years and 1,765, 1,978, 1,350, 1,562, 1,573, and 1,004 samples from adults for 6 consecutive years, we did not detect EV-C104 strains until November 2011–February 2012.

Nucleotide identity of the EV-C104 sequences from this study was 99.5%–100% among BCH strains and 97.7%–98.0% between the PUMCH strain and the BCH strains. Deduced amino acid sequences in VP4/VP2 among the BCH strains were identical, albeit for 1 aa difference for the PUMCH strain (BCH strains had Pro^{110}, but the PUMCH strain had Leu^{110}, which was consistent with strains detected in children and adults in Italy). Deduced amino acid sequences for all 5 strains isolated in this study had 97.9%–100.0% identity with those from Switzerland, Italy, and Japan. BCH strains were community acquired because these 4 patients came from different cities and were admitted to different wards on different dates.

The 4 EV-C104–positive boys all had fever and cough for >10 days before their hospitalization. Chest radiographs showed increased lung markings or patchy shadows diagnosed as pneumonia or bronchopneumonia. RSV or adenovirus was also detected in 3 of the boys. The fourth boy was positive for parainfluenza virus type 1, adenovirus, and bocavirus. Clinical outcomes for all 4 children were favorable. The EV-C104–positive man had fever, chills, pantalgia, and expectoration for 1 day before a URTI was diagnosed. EV-C104 was the only virus detected in this patient.

We compared relative viral loads for all viruses in the 5 patients and quantified viral load of EV-C104 and other viruses by using real-time PCR (methods available upon request). Median viral load in the 5 patients was 2.4×10^6 RNA copies/mL (range 5.6×10^4–7.0×10^6 copies RNA/mL (Table, Appendix, wwwnc.cdc.gov/EID/article/19/4/12-1435-T1.htm).

Overall, we found few (5/12,340) EV-C104–positive specimens. All EV-C104–positive children were co-infected with RSV or adenoviruses (high viral loads) in our study. The role of EV-C104 in RTIs needs to be further studied. Nevertheless, the finding of EV-C104–positive adults with high viral loads in China (3.9×10^6 RNA copies/mL) and Italy (2.0×10^6 RNA copies/mL) (*7*) indicates a possible association between EV-C104 with RTIs. Our data also confirm a wide distribution of EV-C104.

This study was supported by the National Major Science and Technology Project for the Control and Prevention of Major Infectious Diseases in China (2012ZX10004-206), the National Science Foundation for Outstanding Young Scientists (81225014), and the Fondation Mérieux.

Zichun Xiang, Zhengde Xie, Zhong Wang, Lili Ren, Yan Xiao, Linlin Li, Guy Vernet, Gláucia Paranhos-Baccalà, Kunling Shen, and Jianwei Wang

Author affiliations: Ministry of Health Key Laboratory of Systems Biology of Pathogens, Beijing, People's Republic of China (Z. Xiang, L. Ren, L. Li, J. Wang); Institute of Pathogen Biology, Beijing (Z. Xiang, L. Ren, Y. Xiao, L. Li, J. Wang); Beijing Children's Hospital, Beijing (Z. Xie, K. Shen); Peking Union Medical College Hospital, Beijing (Z. Wang); and Fondation Mérieux, Lyon, France (G. Vernet, G. Paranhos-Baccalà)

DOI: http://dx.doi.org/10.3201/eid1904.121435

References

1. Centers for Disease Control and Prevention. Clusters of acute respiratory illness associated with human enterovirus 68—Asia, Europe, and United States, 2008–2010. MMWR Morb Mortal Wkly Rep. 2011;60:1301–4.
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. Tapparel C, Junier T, Gerlach D, Van-Belle S, Turin L, Cordey S, et al. New respiratory enterovirus and recombinant rhinoviruses among circulating picornaviruses. Emerg Infect Dis. 2009;15:719–26. http://dx.doi.org/10.3201/eid1505.081286
4. Piralla A, Rovida F, Baldanti F, Gerna G. Enterovirus genotype EV-104 in humans, Italy, 2008–2009. Emerg Infect Dis. 2010;16:1018–21. http://dx.doi.org/10.3201/eid1606.091533
5. Kaida A, Kubo H, Sekiguchi J, Hase A, Iritani N. Enterovirus 104 infection in adult, Japan, 2011. Emerg Infect Dis. 2012;18:882–3. http://dx.doi.org/10.3201/eid1805.111890
6. Yozwiak NL, Skewes-Cox P, Gordon A, Saborio S, Kuan G, Balmaseda A, et al. Human enterovirus 109: a novel interspecies recombinant enterovirus isolated from a case of acute pediatric respiratory illness in Nicaragua. J Virol. 2010;84:9047–58. http://dx.doi.org/10.1128/JVI.00698-10
7. Piralla A, Lilleri D, Sarasini A, Marchi A, Zecca M, Stronati M, et al. Human rhinovirus and human respiratory enterovirus (EV68 and EV104) infections in hospitalized patients in Italy, 2008–2009. Diagn Microbiol Infect Dis. 2012;73:162–7. http://dx.doi.org/10.1016/j.diagmicrobio.2012.02.019
8. Ren L, Gonzalez R, Wang Z, Xiang Z, Wang Y, Zhou H, et al. Prevalence of human respiratory viruses in adults with acute respiratory tract infections in Beijing, 2005–2007. Clin Microbiol Infect. 2009;15:1146–53. http://dx.doi.org/10.1111/j.1469-0691.2009.02746.x
9. Nix WA, Oberste MS, Pallansch MA. Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens. J Clin Microbiol. 2006;44:2698–704. http://dx.doi.org/10.1128/JCM.00542-06

10. Savolainen C, Blomqvist S, Mulders MN, Hovi T. Genetic clustering of all 102 human rhinovirus prototype strains: serotype 87 is close to human enterovirus 70. J Gen Virol. 2002;83:333–40.

Address for correspondence: Jianwei Wang, 9 Dong Dan San Tiao, Dongcheng District, Beijing 100730, People's Republic of China; email: wangjw28@163.com

Monkey Bites among US Military Members, Afghanistan, 2011

To the Editor: We take serious issue with the dispatch by Mease and Baker on monkey bites among US military members in Afghanistan during 2011 (*1*). In particular, we are troubled by the first paragraph. The dispatch opens by listing bites from rhesus macaques (*Macaca mulatta*) as one of the many risks faced by military personnel deployed to Afghanistan. Although technically a true statement, it is misleading in its perspective. Since 2001, ≈2,000 US soldiers have died in Afghanistan and another ≈18,000 have been wounded in action (*2*). The authors juxtapose this toll with minor injuries incurred by 10 soldiers who flouted explicit rules prohibiting contact with pet monkeys.

None of the bitten soldiers were reported to have sequelae. Furthermore, the first paragraph leaves the impression that a US Army soldier who died of rabies while serving in eastern Afghanistan may have contracted the disease from a macaque. This finding would be an extremely unlikely occurrence.

We have yet to see a single credible report of macaque-to-human transmission of rabies. In fact, we have yet to see a report of naturally acquired rabies infection in a macaque. Similarly, although antiviral prophylaxis is routinely prescribed to persons bitten by rhesus monkeys, there is not a single report of herpes B virus infection in a human outside the laboratory/zoo context, although thousands of persons are likely bitten by macaques in Asia every year (*3*,*4*).

In contrast, zoonotic transmission of simian foamy virus, a retrovirus ubiquitous in nonhuman primates, has been shown to occur from macaques to humans, probably through monkey bites, although this virus has not been shown to cause disease in humans (*5*). Although it is advisable to avoid contact with monkeys, risk for disease transmission should be placed in proper perspective. Exaggerating risks of bites has, in the past, led to irrational culling of entire populations of macaques (*6*).

Gregory A. Engel, Agustin Fuentes, Benjamin P.Y.-H. Lee, Michael A. Schillaci, and Lisa Jones-Engel

Author affiliations: Swedish Family Medicine, Seattle, Washington, USA (G.A. Engel); University of Notre Dame, South Bend, Indiana, USA (A. Fuentes); National Parks Board, Singapore (B.P.Y.-H. Lee); University of Toronto Scarborough, Toronto, Ontario, Canada (M.A. Schillaci); University of Washington, Seattle (G.A. Engel, L. Jones-Engel)

DOI: http://dx.doi.org/10.3201/eid1904.121505

References

1. Mease LE, Baker KA. Monkey bites among US military members, Afghanistan, 2011. Emerg Infect Dis. 2012;18:1647–9. http://dx.doi.org/10.3201/eid1810.120419
2. US Department of Defense. Casualty status: Operation Iraqi Freedom; Operation New Dawn; Operation Enduring Freedom. November 6, 2012 [cited 2012 Nov 7]. http://www.defense.gov/news/casualty.pdf
3. Engel GA, Jones-Engel L, Schillaci MA, Suaryana KG, Putra A, Fuentes A. Human exposure to herpesvirus B–seropositive macaques, Bali, Indonesia. Emerg Infect Dis. 2002;8:789–95. http://dx.doi.org/10.3201/eid0808.010467
4. Fuentes A, Gamerl S. Disproportionate participation by age/sex classes in aggressive interactions between long-tailed macaques (*Macaca fascicularis*) and human tourists at Padangtegal Monkey Forest, Bali, Indonesia. Am J Primatol. 2005;66:197–204. http://dx.doi.org/10.1002/ajp.20138
5. Jones-Engel L, Engel GA, Schillaci MA, Aida Rompis A, Putra A, Suaryana KG, et al. Primate-to-human retroviral transmission in Asia. Emerg Infect Dis. 2005;11:1028–35. http://dx.doi.org/10.3201/eid1107.040957
6. Monkeys with herpes B virus culled at a safari park. Commun Dis Rep CDR Wkly. 2000;10:99,102.

Address for correspondence: Lisa Jones-Engel, University of Washington–National Primate Research Center, 1705 Pacific St NE, HSB I-039, Seattle, WA 98195, USA; email: jonesengel@wanprc.org

In Response: In response to the letter by Engel et al. (*1*), we concur that combat-related deaths and illness are a greater risk than monkey bites for deployed military personnel. Furthermore, we agree that risk for monkey bites should be considered in perspective with other risks faced by deployed personnel. We also believe that action taken to decrease macaque populations in response to risks mentioned would be irrational and inappropriate; in a country affected by war, wildlife conservation efforts are needed. We did not intend to imply that the rabies-associated death mentioned in our article was caused by contact with a macaque (*2*). As reported elsewhere, the patient likely contracted rabies from a dog bite (*3*).

Nonetheless, we believe that risk for monkey bites deserves the attention of deployed medical providers. Risks for bacterial infection and major local trauma are critical for any macaque bite. We acknowledge that risk for contracting viral disease (rabies or B virus infection) from macaques in