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## Respiratory Infection with Enterovirus Genotype C117, China and Mongolia

**To the Editor:** Enteroviruses (EVs) are small, nonenveloped viruses of the family *Picornaviridae* (1). EVs are classified into 12 species according to the molecular and antigenic properties of their viral capsid protein (VP1). To date, 7 species are known

to infect humans, including EV-A to EV-D and rhinovirus A, B, and C ([www.picornastudygroup.com/taxa/species/species.htm](http://www.picornastudygroup.com/taxa/species/species.htm))

EV-C117 was a newly found EV-C genotype. It was identified in a nasopharyngeal sample from a hospitalized child, 3 years and 9 months of age, with community-acquired pneumonia in Lithuania in 2012 (2,3). However, aside from this case, little is known about the prevalence and clinical significance of EV-C117. Here, we report the detection of EV-C117 in children in China and Mongolia with respiratory tract infections (RTIs).

During March 2007–March 2013, we screened for EV-C117 in respiratory samples from patients with RTIs in China and Mongolia, including nasopharyngeal aspirates collected from 3,108 children in China who had lower respiratory tract infections when they were admitted to Beijing Children's Hospital (4) and swab samples from 2,516 patients in Mongolia with influenza-like illness (online Technical Appendix Table 1, [wwwnc.cdc.gov/EID/article/20/6/13-1596-Techapp1.pdf](http://wwwnc.cdc.gov/EID/article/20/6/13-1596-Techapp1.pdf)). Respiratory viruses in samples from China were screened by using multiplex PCR and single PCR assays as described (4). Samples from Mongolia were screened by using the FTD Respiratory Pathogens Multiplex Assay Kit (Fast-track Diagnostics, Luxembourg City, Luxembourg). EV-positive samples were further genotyped by using reverse transcription PCR (RT-PCR) and primers sequentially targeting the VP1 region (5), the 5'-untranslated region (5'-UTR)/VP4/VP2 region (6) and the 5'-UTR (7). A 394-nt amplicon corresponding to the 5'-UTR of EVs was obtained from 10 children in China; a 598-nt amplicon corresponding to the 5'-UTR/VP4/VP2 region was obtained by RT-PCR from 5 children in Mongolia. Blastn analysis ([www.blast.ncbi.nlm.nih.gov/Blast.cgi](http://www.blast.ncbi.nlm.nih.gov/Blast.cgi)) of PCR amplicons showed that only amplicons detected in 2 children from China

(patients BCH096A and BCH104A) and 2 children from Mongolia (patients MGL126 and MGL208) had the highest similarity (95%–98%) to the EV-C117 prototype strain LIT22.

To further confirm that these 4 strains belong to EV-C117, we attempted to amplify the full-length viral genome sequences. However, we only obtained full-length viral genome sequences from the 2 strains found in patients from China (GenBank accession nos. JX560527 [patient BCH096A], and JX560528 [patient BCH104A], respectively). For the remaining 2 strains from Mongolia, MGL126 (5'UTR/VP4/VP2: KF726102; VP1: KF726100) and MGL208 (5'UTR/VP4/VP2: KF726103; VP1: KF726101), we obtained the sequence of the 5'-UTR/VP4/VP2 region and VP1 gene. Phylogenetic analysis of these sequences showed that they all belonged to genotype EV-C117 (Figure, panels A and B).

Virus isolation for EV-C117 by using Vero and H1-HeLa cells was unsuccessful. Through blastn and phylogenetic analyses, we also found that the previously identified EV-C strain HC90835 (EU697831, from Nepal) (8), EV-C104 strain CL-C22 (EU840734, EU840744, and EU840749, from Switzerland) (9) and a rhinovirus strain SE-10-028 (JQ417886, from South Korea), also belong to EV-C117 (Figure, panel A), indicating that EV-C117 is widely distributed geographically. Because a large proportion of EV infections are subclinical or mild (1), the prevalence of EV-C117 should be further estimated by using serologic investigations in general populations.

The VP1 sequences of the EV-C117 strains isolated in China and Mongolia were 89.9%–95.6% (nt) and 95.2%–98.3% (aa) identical to the EV-C117 prototype strain LIT22 (patient JX262382). Alignment analysis of amino acid sequences showed differences between strains isolated in this study and LIT22, i.e., Ser<sup>15</sup> (strains

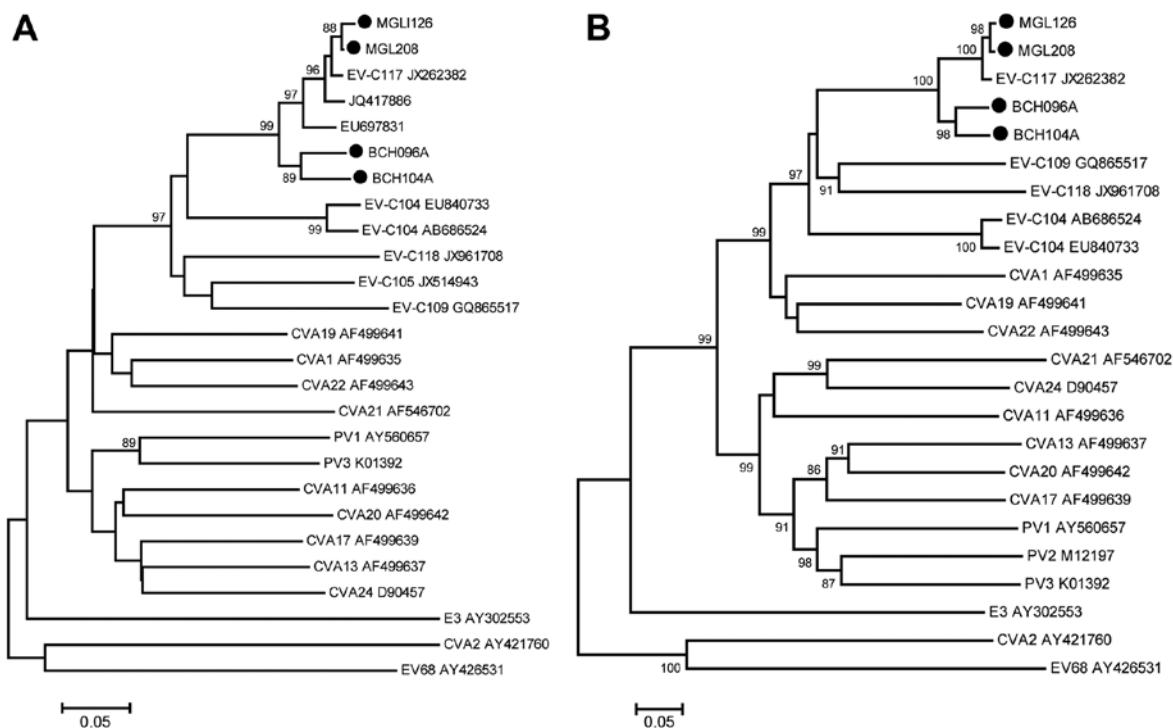


Figure. Phylogenetic analysis of enterovirus genotype C117 (EV-C117) based on nucleotide sequences. Phylogenetic trees were generated with 1,000 bootstrap replicates. Neighbor-joining analysis of the targeted nucleotide sequence was performed by using the Kimura 2-parameter model with Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0 ([www.megasoftware.net](http://www.megasoftware.net)). The EV-C117 strains identified in this study are indicated by black circles. Enterovirus 68, cocksackievirus (CV) A2, and echovirus (E) 3 (GenBank accession nos. AY426531, AY421760, and AY302553) were used as outgroups. PV, poliovirus. A) Phylogenetic analysis of the VP 4/VP2 region (399 nt, corresponding to nt 673–1,071 of EV-C117 prototype strain LIT22 [JX262382]). B) Phylogenetic analysis of the viral protein 1 region (888 nt, corresponding to nt 2416–3303, numbered according to the sequence of LIT22). Scale bars represent nucleotide substitutions per site.

in this study) versus Asn<sup>15</sup> (LIT22). In addition, we found that the strains from patients in China contain Lys<sup>63</sup> and Ala<sup>90</sup>, and those from Mongolia have Thr<sup>93</sup>, Asn<sup>97</sup>, and Ser<sup>276</sup>. The biological significance of these mutations is unknown.

Of these 4 EV-C117-positive children, 3 were hospitalized with respiratory disease (online Technical Appendix Table 2); the nonhospitalized child (MGL208) had a sore throat, but no other signs or symptoms. The viral loads of EV-C117 and co-detected viruses were quantified by using real-time PCR (methods available upon request), with a median EV-C117 load of  $2.9 \times 10^5$  RNA copies/mL (range  $1.1\text{--}4.8 \times 10^5$  RNA copies/mL [Technical Appendix Table 2]). EV-C117 was the only virus detected in patients

BCH104A and MGL126. Respiratory syncytial virus ( $5.0 \times 10^6$  copies/mL) and rhinovirus ( $1.5 \times 10^5$  copies/mL) were detected in patient BCH096A, and influenza virus A (IFVA, H3N2;  $5.1 \times 10^{10}$  copies/mL) and human bocavirus ( $3.7 \times 10^2$  copies/mL) were detected in patient MGL208.

The co-detection of viruses in 2 of the EV-C117-positive patients raises the question of what role EV-C117 plays in RTIs. However, it is notable that EV-C117 was the only virus detected in the other 2 patients. This finding indicates that, at least in patients with low resistance (patient BCH104A had severe bacterial infection before EV-C117 was detected and patient MGL126 had congenital heart disease), EV-C117 might be associated with RTIs. In addition, the strain

isolated in Nepal and the strain isolated in Switzerland, EV-C117, were both detected in specimens collected from patients with RTIs (8,9). Collectively, these data indicate the respiratory tropism of EV-C117. Additional epidemiologic and virologic studies on EV-C117 may be warranted to establish its role in RTIs.

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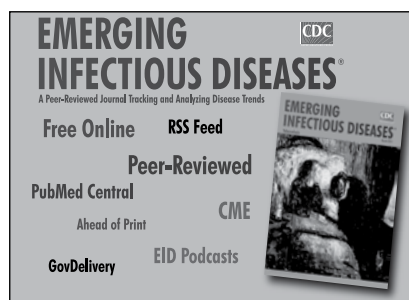
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## Bufavirus in Feces of Patients with Gastroenteritis, Finland

**To the Editor:** For nearly 3 decades, human parvovirus B19 (B19V) was considered to be the only pathogenic parvovirus found in humans. Since 2005, several new human parvoviruses have been found, including human bocaviruses 1–4 and human parvovirus 4 (PARV4) (1–5), and during 2012, metagenomic analysis of fecal samples from children in Burkina Faso with acute diarrhea showed a highly divergent parvovirus, which was named bufavirus (BuV) (6). Its sequence in the coding region showed <31% similarity with known parvoviruses, the closest genera being *Protoparvovirus* and *Amdoparvovirus*. Subsequent studies, on the basis of PCR results, showed that 4% of fecal samples from Burkina Faso (n = 98) and 1.6% from Tunisia (n = 63) harbored either of 2 genotypes of this new virus, which belongs to the species *Primate protoparvovirus 1* of the genus *Protoparvovirus* (6,7; <http://ictvonline.org>).

To assess the occurrence of BuV in northern Europe, we analyzed 629 fecal samples from patients of all ages (median 51.5 years, range 0–99) in Finland who had gastroenteritis. To gain a more complete representation of BuV occurrence, we obtained samples retrospectively from routine diagnostics for bacterial and viral gastroenteritis-inducing pathogens (HUSLAB, Helsinki University Central Hospital Laboratory Division, Helsinki, Finland) and analyzed all samples available during the collection periods.

The samples originally sent to HUSLAB for bacterial diagnosis (bacterial cohort, n = 243) had been analyzed during October 2012–March 2013 for *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Yersinia*

<sup>1</sup>These authors contributed equally to this work.

# Respiratory Infection with Enterovirus Genotype C117, China and Mongolia

## Technical Appendix tables

Technical Appendix Table 1. Molecular diagnosis and characteristics of patients screened for EV-C117, China and Mongolia\*

Characteristics	China	Mongolia
Patients, no.	3,108†	2,516
Type of sample	Nasopharyngeal aspirates	Swab
Period of sample collection	Mar. 2007–Feb. 2012	Nov. 2008–Mar. 2013
Male	1,963	1, 244
Female	1,145	1,272
Age range	9 d–14 y	0–86 y
Median age	1 y	2 y
Total no. of EV positive	37 (24 male, 13 female)†	55 (35 male, 20 female)
Total no. of EV-C117 positive	2	2

\*EV-C117, enterovirus C117.

†This data has been reported in a previous study (4).

Technical Appendix Table 2. Characteristics of 4 patients in whom EV-C117 was detected

Patient identification no.	BCH096A	BCH104A	MGL126	MGL208
Age (mo)/sex	3/F	23/M	27/F	36/F
Date of admission	Jul. 2007	Jul. 2007	Nov. 2011	Jan. 2013
Diagnosis or clinical symptoms	Asthmatic bronchitis, rhinorrhea, sneezing, expectoration	Pneumonia, lung abscess, purulent pleurisy, fever (39.3°C), cough	Lobar pneumonia, cough, fever (39.5°C), expectoration	Sore throat
Comorbid conditions	None	Bacterial infection	Congenital ventricular septal defect	None
Virus and viral load (RNA or DNA copies/mL)	EV-C117: $1.1 \times 10^5$ RSVA: $5.0 \times 10^6$ RV: $1.5 \times 10^5$	EV-C117: $4.4 \times 10^5$	EV-C117: $1.3 \times 10^5$	EV-C117: $4.8 \times 10^5$ IFVA: $5.1 \times 10^{10}$ HBoV: $3.7 \times 10^2$
GenBank no.	Full length: JX560527	Full length: JX560528	VP1: KF726100 5 KF726102	VP1: KF726101 5 KF726103

\*EV-C117, enterovirus C117; RSV, respiratory syncytial virus; RV, human rhinovirus; IFVA, human influenza virus A; HBoV, human bocavirus.