

Our study has several limitations. For example, information on clinical features and outcomes of patients, except for death, was unavailable, and we could not determine prognostic factors for recent JE cases. Because details of each patient's travel history was not identified, we could not clearly understand the mechanism of JEV transmission. In addition, we do not explore the possible cause of JE re-emergence. Moreover, although JE incidence was detected by the national surveillance system, incidence might be underestimated because the database identified only serologically confirmed cases.

JE vaccination is presumed to have failed to induce lifelong immunity so that older age groups become susceptible again. Further research is warranted to determine the long-term protection against JEV after primary vaccination. Moreover, future studies should address the need for booster vaccination for adults to maintain immunity against JEV.

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## Recombinant Enterovirus A71 Subgenogroup C1 Strains, Germany, 2015

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**To the Editor:** Enterovirus A71 (EV-A71) strains circulate worldwide, and numerous outbreaks have been reported from Asia, Australia, Europe, and America (1). Symptomatic infections range from mild febrile illness or characteristic diseases such as hand, foot and mouth disease to severe neurologic disorders such as meningitis/encephalitis and acute flaccid paralysis. EV-A71 infections are usually asymptomatic and self-limiting but can also result in life-threatening complications such as pulmonary edema and cause death, predominantly in children <5 years of age. On the basis of viral protein 1 (VP1) sequences, 3 genogroups (A, B, C), including different subgenogroups (B0–B5, C1–C5), have been defined (2,3). Additional genogroups (D, E, F, G) have been proposed (4,5). In Europe, C1 and C2 strains have circulated predominantly within the past 2 decades, and recent introduction of C4 strains has been reported (6,7). Within subgenogroup C1, a lineage is replaced by the subsequent lineage over time (8).

National enterovirus surveillance (EVSURV) in Germany monitors polio-free status by testing fecal or cerebrospinal fluid (CSF) samples from hospitalized patients with suspected meningitis/encephalitis or acute flaccid paralysis. Enterovirus typing, using molecular and virologic methods, is performed within a laboratory network for enterovirus diagnostics. Since 2006, ≈2,500 samples have been tested annually; 25%–30% were enterovirus positive. Of the typed strains, 0.8%–12.7% were identified as EV-A71 (2006, 0.8%; 2007, 6.8%; 2008, 0.9%; 2009, 3.4%; 2010, 12.7%; 2011, 2.3%; 2012, 2.8%; 2013, 8.6%; 2014, 2.7%), indicating peaks with increased EV-A71 detection rates. Molecular characterization based on the VP1 region of a subset of EV-A71–positive samples revealed that C2 was the predominant subgenogroup in Germany from 2006 to 2014. Subgenogroups B5, C1, and C4 have also been identified, but less frequently (online Technical Appendix Table 3, <http://wwwnc.cdc.gov/EID/article/22/10/16-0357-Techapp1.pdf>).

In 2015, a total of 419 samples tested enterovirus positive within EVSURV. Of these, 43 fecal specimens and

<sup>1</sup>Contributing members of the Laboratory Network for Enterovirus Diagnostics are listed at the end of this article.



**Figure.** Phylogenetic tree based on complete viral protein 1 (VP1) nucleotide sequences of the strains identified within the German enterovirus surveillance (bold) and a representative set of enterovirus A71 strains available from GenBank (891 bases, corresponding to nucleotide positions 2439–3329 in the prototype BrCr ETU22521). The tree was constructed by using the neighbor-joining method (Kimura 2-parameter model) with 1,000 replicates through MEGA 6.06 (<http://www.megasoftware.net/>). Coxsackievirus A16 prototype (CAU05876) was used as the outgroup. Only bootstrap values >70 are shown. Genogroup and subgenogroup assignment, GenBank accession number, country and year of isolation are provided in the virus names. The enlarged subtree includes enterovirus A71 C1-like strains detected in Germany in 2015. Virus names contain strain number, abbreviation of federal state, country, and year of isolation. BE, Berlin; BB, Brandenburg; DE, Germany; MV, Mecklenburg Western Pomerania; NI, Lower Saxony; NW, Northrhine-Westphalia; RP, Rhineland Palatinate; TH, Thuringia. Scale bars indicate nucleotide substitution per site.

1 CSF specimen tested EV-A71 positive (11.2% of the typed enteroviruses); these samples were obtained from patients with signs of meningitis/encephalitis hospitalized in 25 secondary and tertiary care hospitals from 13 of 16 federal states of Germany. Thirty-six strains were further characterized at the National Reference Centre for Poliomyelitis and Enteroviruses (online Technical Appendix Table 2). Seventeen strains were identified as C2 by using the RIVM Enterovirus Genotyping Tool Version 1.0 (<http://www.rivm.nl/mpf/enterovirus/typingtool>) based on the VP1 region sequences (9). Sequence analyses of the remaining 19 strains revealed highest nucleotide identity (90%–93%) with recently circulating C1 strains from GenBank. Phylogenetic analysis that used the neighbor-joining tree algorithm showed separate clustering of these strains within the C1 subgenogroup (Figure). In contrast to the VP1 tree, phylogenetic analyses based on the 5' untranslated region (UTR) and the P2 and P3 regions revealed different clustering of the German 2015 EV-A71 C1-like group (online Technical Appendix Figure). In line with these phylogenetic tree topologies, we found highest nucleotide identities to subgenogroup B3 and C2-like strains for 5' UTR (both 90%), whereas P2 and P3 regions showed highest nucleotide identity of 82% and 84%, respectively, with C4 strains identified in China. We found no specific amino acid changes within the conserved major antigenic sites of the capsid proteins. However, we observed a V16M change in VP1. Few EV-A71 strains (all B1) also carry a methionine at this position, including the outbreak strains from Bulgaria (1975) and Hungary (1978). Also, we identified a valine residue at position 262 in VP1. Tee et al. have proposed that toggling of amino acids isoleucine and valine at this position in recent C1 lineages generates antigenic novelty (8). Within the 5' UTR, a C526U change has been proposed to affect replication efficiency (10). All isolates belonging to the new EV-A71 C1 variant carried uracil at this position. In addition, we found a 2-nucleotide deletion within the spacer 2 region between the internal ribosome entry site and the coding region, similar to the EV-A71 prototype BrCr and the C2-like strains (GenBank accession nos. HM622392, HM622391, JQ280307) (data available on request).

Our findings highlight the need for molecular surveillance of enteroviruses to identify new variant strains with potential for increased virulence and pathogenicity. One limitation of the EVSurv is the lack of detailed clinical data because the request form deliberately asks for only basic cardinal symptoms justifying the clinical suspicion of meningitis/encephalitis or acute flaccid paralysis. Nevertheless, all patients had been hospitalized, suggesting severe disease. Besides characteristic symptoms (including nuchal rigidity, headache, fever, and vomiting), cerebral seizures, myoclonia, ataxia, petechiae, and stomatitis

were also mentioned for some patients tested for the new variant C1-like strains described here. All but 1 patient was <5 years of age (online Technical Appendix Table 1). Therefore, pediatricians, in particular, should be aware of this new recombinant, potentially more pathogenic, strain and intensify diagnostic work-ups to better monitor EV-A71 circulation. In addition to CSF samples, fecal samples, throat swab specimens, and samples related to other clinical prodromes (e.g., vesicle fluids in cases of hand, foot and mouth disease) should be obtained. Particular attention should be paid to measures in daycare centers to prevent large outbreaks of enterovirus-associated meningitis/encephalitis.

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## Cerebral Syphilitic Gumma within 5 Months of Syphilis in HIV-Infected Patient

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**To the Editor:** Tertiary syphilis, including cerebral syphilitic gumma, usually occurs >10 years after contracting syphilis (1) and is a rare manifestation since the introduction of penicillin (2). However, progression of syphilis is reported to be faster in HIV-infected patients than in those without such infections (3). We report a case of cerebral syphilitic gumma in an HIV-1-infected patient for whom serum samples obtained as recently as 5 months earlier showed negative results for syphilis.

A 21-year-old man infected with HIV came to the AIDS Clinical Center, National Center for Global Health and Medicine (Tokyo, Japan), because of a 2-hour loss of consciousness. He reported an uncomfortable feeling at the back of his head and neck and eye fatigue that lasted for 1 week. His HIV-1 infection was well-controlled with an antiretroviral combination of tenofovir, emtricitabine, and dolutegravir. The patient had a CD4 count of 565 cells/mL and a viremia level below detectable limits (<20 copies/mL). He was not using any other medications.

At examination, his vital signs were within reference ranges. Apart from a tongue bite, physical and neurologic examinations showed no abnormal findings. Results for chest radiograph, Holter electrocardiogram, and electroencephalogram were unremarkable. There were no abnormal ophthalmologic findings. Computed tomography of the brain showed a hypodense lesion at the left frontal lobe (Figure, panel A). Subsequent magnetic resonance imaging showed that the lesion (mass) was hypointense by gadolinium-enhanced, axial, T1-weighted imaging (Figure, panel B), hyperintense by T2-weighted imaging, and surrounded by extensive cerebral edema (Figure, panel C).

Symptomatic epilepsy caused by the mass was suspected to have caused the loss of consciousness. This conclusion was based on the intracranial mass, long duration of loss of consciousness, increase in creatine kinase level (471 U/L), and tongue bite.

When the HIV-1 infection was diagnosed in the patient 15 months earlier, results of serum rapid plasma reagin (RPR) and *Treponema pallidum* hemagglutination test (TPHA) were negative. However, during this examination, serum RPR and TPHA titers were 1:32 and 1:10,240, respectively. Results of cerebrospinal fluid (CSF) analysis were compatible with neurosyphilis (4,5) and showed a leukocyte count of 35 cells/mL (2 neutrophils/mL, 33 lymphocytes/mL), a total protein level of 30 mg/dL, a glucose level of 59 mg/dL (serum glucose level 92 mg/dL), an RPR titer of 1:<1, a TPHA titer of 1:160, and a fluorescent treponemal antibody-absorption titer of 1:32.

Cerebral syphilitic gumma was suspected on the basis of neurosyphilis and compatible imaging findings (6) and because other conditions, such as meningioma, primary central nervous system lymphoma, toxoplasmosis,

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## Technical Appendix

### Clinical Specimens

All samples were collected from hospitalized patients with symptoms of meningitis/encephalitis within the German enterovirus surveillance network. Specimens were sent to a network laboratory that was participating in the Laboratory Network for Enterovirus Diagnostics (LaNED), including the National Reference Centre for Poliomyelitis and Enteroviruses (Berlin, Germany). All patient data were analyzed pseudonymously.

RNA was isolated directly from fecal samples or from cell culture supernatant by using the QIAGEN RNA Viral Mini Kit (QIAGEN, Hilden, Germany). Amplification was performed for all PCRs by using the One-Step-RT-PCR Kit (QIAGEN), followed by nested PCR using HotStarTaq-Mastermix (QIAGEN), according to the manufacturer's protocol. Reverse transcription PCR (RT-PCR) and nested PCR was done with 600 nM of forward and reverse primers as mentioned in Technical Appendix Table 1. The temperature profile for the VP1 region was the following: 10 min 22°C, 45 min 45°C, 15 min 95°C for RT, followed by 40 cycles of 30 s 94°C, 30 s 46°C, 90 s 72°C, and final elongation for 10 min at 72°C. The second PCR was carried out by using a touchdown protocol with 10 cycles of 30 s 94°C, 30 s 60°C, 90 s 72°C, with a decrease of 1°C of the annealing temperature per cycle, followed by 30 cycles of 30 s 94°C, 30 s 50°C, 90 s 72°C, and final elongation for 10 min at 72°C. Amplification of the 5' untranslated region (UTR) was done as described (*1*). Temperature profile for all other PCRs was as follows: 30 min 50°C, 15 min 95°C for RT, followed by 35 cycles of 30 s 94°C, 30 s 50°C, 90 s 72°C, and final elongation 10 min at 72°C. Nested PCR was done in 35 cycles of 30 s 94°C, 30 s 50°C, 90 s 72°C, followed by final elongation of 10 min 72°C. The resulting products were sequenced with primers used for amplification (BigDye 3.1 kit, Applied Biosystems,

Weiterstadt, Germany). PCR products of VP3 and 3ABC PCR were extracted from the agarose gel using QIAGEN Mini Elute Gel Kit (QIAGEN), according to the manufacturer's protocol.

Sequences were assembled with Sequencher software version 5.2.4 (<https://www.genecodes.com/>). Alignments were performed by using MAFFT (2). Phylogenetic relationships among strains circulating in Germany and representative strains taken from GenBank were estimated with the neighbor joining method based on the Kimura 2-parameter model conducted in MEGA6 by using a bootstrap procedure with 1,000 replicates (3).

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**Technical Appendix Table 1.** Overview and accession numbers of enterovirus A71 strains described in this study

Strain	GenBank accession no.	Collection date	Federal state	Age, y	Sex	Diagnosis
45841/BE/DE 2015	KU641500	2015 Jun	Berlin	Newborn	F	Meningitis
45893/BE/DE 2015	KU641491	2015 Jul	Berlin	4 mo	M	Meningitis
38474/BE/DE 2015	KU641492	2015 Jul	Berlin	2	F	Meningitis
45903/BE/DE 2015	KU641493	2015 Jul	Berlin	2	F	Meningitis
46440/BE/DE 2015	KU641494	2015 Jul	Berlin	3	F	Meningitis
45900/BE/DE 2015	KU641495	201 Jul	Berlin	3	F	Meningitis
43232/BB/DE 2015	KU641490	2015 Jul	Brandenburg	3	F	Meningitis
45849/BE/DE 2015	KU641502	2015 Jul	Berlin	2	F	Meningitis
45894/BE/DE 2015	KU641503	2015 Jul	Berlin	3	M	Meningitis
992990/NI/DE 2015	KU641496	2015 Aug	Lower Saxony	4	F	Meningitis
37507/TH/DE 2015	KU641501	2015 Aug	Thuringia	4	M	Meningitis
44930/BE/DE 2015	KU641504	2015 Aug	Berlin	11 mo	F	Meningitis
43538/MV/DE 2015	KU641505	2015 Aug	Mecklenburg Western Pomerania	3	M	Meningitis
44932/BE/DE 2015	KU641506	2015 Aug	Berlin	1	M	Meningitis
44172/RP/DE 2015	KU641507	2015 Sep	Rhineland Palatinate	1	F	Meningitis
45906/BE/DE 2015	KU641497	2015 Sep	Berlin	17	M	Meningitis
992879/NI/DE 2015	KU641498	2015 Oct	Lower Saxony	2 mo	F	Meningitis
46411/NW/DE 2015	KU641508	2015 Oct	Northrhine-Westphalia	5	F	Meningitis
47159/BE/DE 2015	KU641499	2015 Nov	Berlin	1 mo	F	Meningitis

**Technical Appendix Table 2.** Primers used for amplification of enterovirus A71\*

Primer	Region amplified	Sequence 5'–3'	Orientation	Location†	Reference
NRZ 1	5'UTR	CAA GCA CTT CTG TTT CCC CGG	Sense	167–187	(1)
NRZ 2	5'UTR	ATTGTCACCATAAGCAGCCA	Antisense	602–585	(1)
NRZ 5	5'UTR	TAC TTC GAG AAR CCY AGT A	Sense	248–266	(1)
NRZ80	5'UTR	AAC ACG GAC ACC CAA AGT A	Antisense	565–547	(1)
NRZ 338	VP4	TGG CGG CCT GCC YAT GG	Sense	369–385	This study
NRZ 339	VP4	TCC TCC GGC CCC TGA ATG C	Sense	448–466	This study
NRZ 340	VP4	TGT GAT ATA GGR ATc CCA GCA TCR AG	Antisense	1471–1446	This study
NRZ 341	VP3	AGY AAR TTC CAY CAA GGR GCR C	Sense	1293–1314	This study
NRZ 342	VP3	TCR ATC ATR CTC TCR TCA CTA G	Antisense	2635–2611	This study
NRZ 343	VP3	GCY CCA ATY TCA GCR GCT TG	Antisense	2599–2580	This study
NRZ 360	VP3 seq	GCT GGA GCT GTG TCA GGT GG	Sense	1841–1860	This study
NRZ 361	VP3 seq	TGC GTG CCC AGC ATA GCG	Antisense	2173–2156	This study
NRZ 131	VP1	CCN TGG ATH AGY AAC ACN CAY T	Sense	2220–2241	(4)
NRZ 209	VP1	CTR ACY GGR TAR TGY TTY CT	Antisense	3553–3534	This study
NRZ 210	VP1	THT GGT AYC ARA CAA AYT WYG TNG THC	Sense	2299–2326	This study
NRZ 211	VP1	CCM ACR TAD ATD GCN CCN GAY TGY TGN CC	Antisense	3367–3339	This study
NRZ 337	VP1 seq	ACG TTC GGT GAG CAC AAG C	Sense	3066–3084	This study
NRZ 344	2ABC	ACR TTC GGT GAR CAC AAG CAG	Sense	3066–3086	This study
NRZ 345	2ABC	TGG TGA TYA GGA TTT ACA TGA GGA TG	Sense	3178–3203	This study
NRZ 346	2ABC	GGT GTT TGC TCT TGA ACT GCA T	Antisense	4431–4410	This study
NRZ 353	2ABC seq	GGC CAG TGA GTA TTA CCC GG	Sense	3584–3603	This study
NRZ 347	3ABC	TCC AAC CTT GAG CAG TCY GC	Sense	4257–4276	This study
NRZ 348	3ABC	ATG GAA TGT TTC ATA GAG GTG CC	Antisense	6533–6511	This study
NRZ 349	3ABC	GCC ATT CTA AGG TAA ACT GAG TC	Antisense	6505–6483	This study
NRZ 362	3ABC seq	CGG ATC TTG GCC GAT TGG A	Sense	4843–4861	This study
NRZ 363	3ABC seq	TGC CAC CAA TGT GAA TGC C	Antisense	5880–5862	This study
NRZ 350	3D	TGA GCA TRG ADG ADG CYT G	Sense	6220–6237	This study
NRZ 351	3D	ATG AAR TTY TAY ATG GAY AAG TAT GG	Sense	6357–6382	This study
NRZ 352	3D	AGA TTH CTG GTG GGG TTS AGB T	Antisense	7360–7339	This study
NRZ 370	3D seq	ATG AYT CAG TGT ACY TSA GRA TGR C	Sense	6481–6505	This study
NRZ 371	3D seq	GCT GAW CCR GTK AYY GTM CCW GG	Antisense	6562–6540	This study

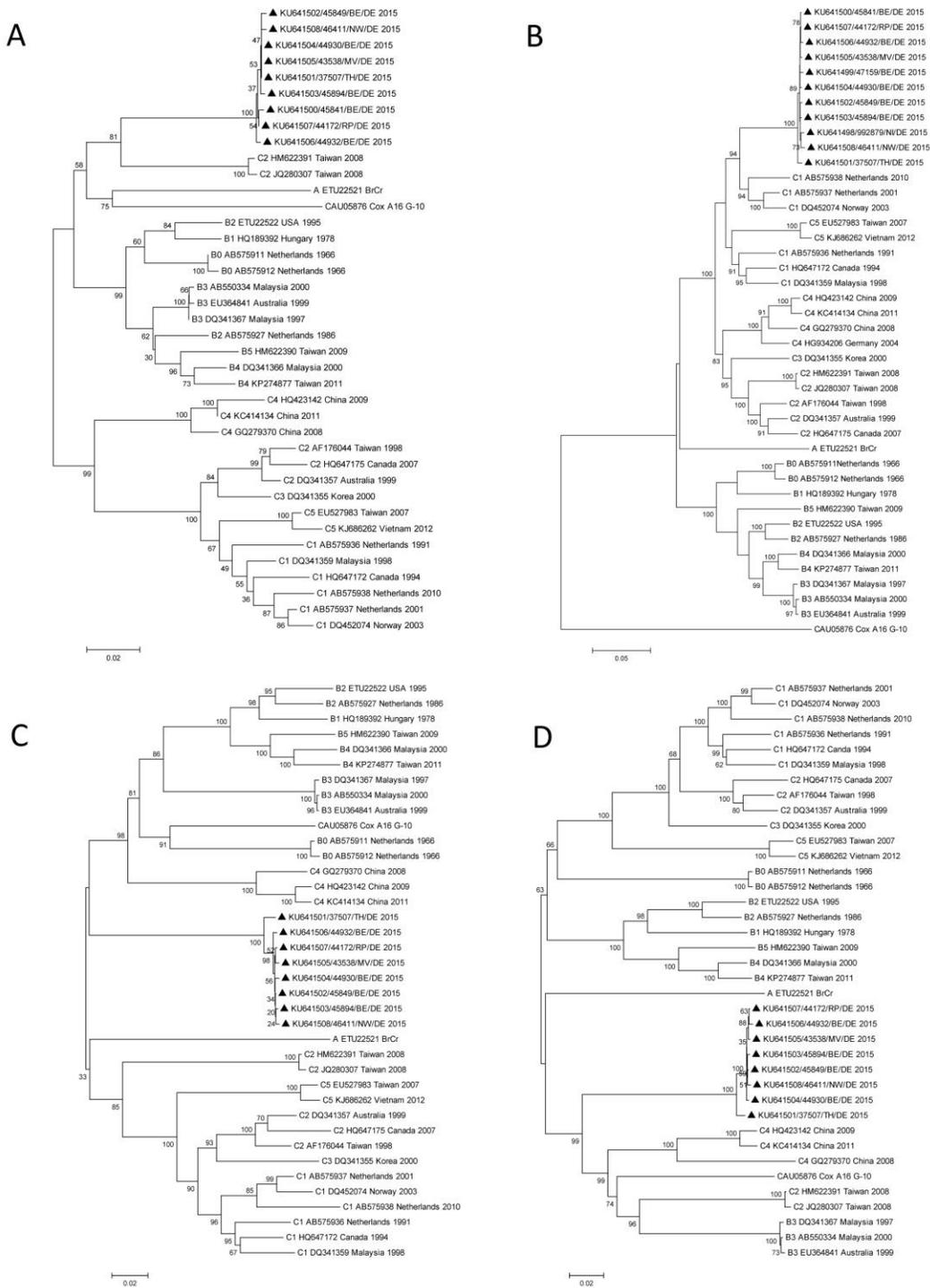
\*UTR, untranslated region; VP, viral protein; seq, used for sequencing only.

†Location according to EV-A71 prototype strain BrCr (ETU25521).

**Technical Appendix Table 3.** Number of samples analyzed in the German enterovirus surveillance and enterovirus A71 genotyping results, 2006–2015\*

Samples	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Total no. samples tested	1,856	2,135	3,207	2,526	2,804	2,992	2,859	3,413	2,461	2,158
No. EV positive	645	657	1,139	501	772	740	670	1,239	506	419
No. EV typed	358	370	928	383	575	649	581	1,118	482	392
EV-A71 positive	3	25	8	13	73	15	16	96	13	44
EV-A71/EV typed, %	0.8	6.8	0.9	3.4	12.7	2.3	2.8	8.6	2.7	11.2
Subgenogroup										
B5			2					1		
C1					1	1	1	4	1	19
C2	2	13	1	3	52	7	8	58	7	17
C4						5	3	12		
ND	1	12	5	10	20	2	4	21	5	8

\*EV, enterovirus; ND, not determined.



**Technical Appendix Figure.** Phylogenetic tree based on complete 5'untranslated region (A, 562 ntd), P1 (B, 2,586 nt), P2 (C, 1,734 nt), and P3 (D, 2,262 nt) region of the strains identified within the German enterovirus surveillance and a representative set of enterovirus A71 available from GenBank. Trees were

constructed by using the neighbor-joining method (Kimura 2-parameter model) with 1,000 replicates through MEGA 6.06 (<http://www.megasoftware.net/>). Coxsackievirus A16 prototype (CAU05876) was used as the outgroup. Only bootstrap values >70 are shown. Scale bar represents nucleotide substitutions per site. Genogroup and subgenogroup assignment, GenBank accession number, country, and year of isolation are provided in the virus names. Virus names of the German strains contain strain number, abbreviation of federal state (BB, Brandenburg; BE, Berlin; MV, Mecklenburg Western Pomerania; NI, Lower Saxony; NW, Northrhine-Westphalia; RP, Rhineland Palatinate; TH, Thuringia), country (DE, Germany), and year of isolation.