Coxsackievirus A6 and Hand, Foot, and Mouth Disease, Finland

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During fall 2008, an outbreak of hand, foot, and mouth disease (HFMD) with onychomadesis (nail shedding) as a common feature occurred in Finland. We identified an unusual enterovirus type, coxsackievirus A6 (CVA6), as the causative agent. CVA6 infections may be emerging as a new and major cause of epidemic HFMD.

Hand, foot, and mouth disease (HFMD) is a common childhood illness characterized by fever and vesicular eruptions on hands and feet and in the mouth (Figure 1). It is caused by members of the family Picornaviridae in the genus Enterovirus. Complications are rare, but pneumonia, meningitis, or encephalitis may occur. Outbreaks of HFMD have been mainly caused by 2 types of enterovirus A species, coxsackievirus (CV) A16 (CVA16) or enterovirus 71 (EV71). Some outbreaks have been associated with CVA10, but only sporadic cases involving other members of the enterovirus A species have been reported (2,3).

During fall 2008, a nationwide outbreak of HFMD occurred in daycare centers and schools in Finland, starting in August and continuing at least until the end of the year and possibly into the following year. From vesicle fluid specimens of hospitalized children, we identified the etiologic agent as coxsackievirus A6.

The Study

In August 2008, vesicle fluid specimens were collected from 2 children and 1 parent with HFMD at the Central Hospital of Seinäjoki, Southern Ostrobothnia. Specimens were sent to the Department of Virology, University of Turku, for identification of the causative agent. After detection of CVA6 in these index cases, the virus was also found in specimens obtained from the Pirkanmaa Hospital District (Tampere), Turku University Hospital (Turku), Pori Central Hospital (Pori), and Central-Ostrobothnia Central Hospital (Kokkola) (Table).

Nucleic acids were extracted from specimens by using the NucliSens EasyMag automated extractor (bioMérieux, Boxtel, the Netherlands). When the extracts were analyzed for enteroviruses by using real-time reverse transcriptase–PCR (RT-PCR) specific for the 5′ noncoding region (NCR) of picornaviruses (4), amplicons with melting points indistinguishable from each other and typical to enteroviruses were obtained.

To identify the enterovirus type in the specimens, RT-PCR, specific for a partial sequence of the viral protein 1 (VP1) region, was performed by using the COnsensus-DEgenerate Hybrid Oligonucleotide (CODEHOP) Primers (bioinformatics.weizmann.ac.il/blocks/codehop.html) (5). The amplicons were separated by agarose gel electrophoresis, purified with the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany), and sequenced in the DNA Sequencing Service Laboratory of the Turku Centre for Biotechnology. The virus type in the 3 index specimens, 3 samples of vesicular fluids, and 1 throat swab was successfully identified with sequencing and BLAST (www.ncbi.nlm.gov/BLAST) analysis as CVA6. Phylogenetic relationships of the sequences were examined by using CVA6 (Gdula strain), CVA16 (G10), and enterovirus 71 (BrCr) prototype strains as well as selected clinical CVA6 isolates obtained from GenBank. Sequence alignments were generated with the ClustalW program (www.ebi.ac.uk/clustalw), and the phylogenetic tree was computed by using the Jukes-Cantor algorithm and the neighbor-joining method. Phylogenetic analyses were conducted by using MEGA4 software (www.megasoftware.net) and the bootstrap consensus tree inferred from 1,000 replicates (6) (Figure 2).

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Phylogenetic analysis placed all CVA6 strains from the HFMD outbreak in 1 cluster (97%–100% identity), whereas the nucleotide identities between those isolates and CVA6 prototype strains Gdula, CAV16, G-10, and enterovirus 71 BrCr were 82.5%–83.2%, 55.6%–56.6%, and 55.6%–57.3%, respectively. The closest preceding CVA6 strain was isolated from cerebrospinal fluid in the United Kingdom in 2007 and had 92%–94% nucleotide identity with the strains described here (7).

To improve the detection of the novel CVA6 strains in clinical specimens, we designed specific VP1 primers from the aligned sequences. CVA6vp1 reverse primer (5′-ACTCGCTGTGTGATGAATCG-3′) and CVA6vp1 forward primer (5′-CGTCAAAGCGCATGTATGTT-3′) generated a 199-bp amplicon. First, cDNA was synthesized in a 20-μL reaction mixture containing 1 μmol/L CVA6vp1 reverse primer, 2.5 mmol/L of each dNTP, 20 U ReverTase H Minus M-MuLV reverse transcriptase (Fermentas, St. Leon-Rot, Germany), reaction buffer (Fermentas), 4 U RiboLock RNase inhibitor (Fermentas), and 5 μL RNA incubated at 42ºC for 1 h. Then, 5 μL of cDNA was added to 20 μL of master mixture containing 0.4 μmol/L of each CVA6vp1 primers and Maxima SYBR Green qPCR Master Mix (Fermentas). PCR with melting curve analysis was performed in a Rotor-Gene 6000 real-time instrument (Corbett Research, Mortlake, Victoria, Australia) by...
Onychomadesis was 1 characteristic feature in patients during this HFMD outbreak; parents and clinicians reported that their children shed fingernails and/or toenails within 1–2 months after HFMD (Figure 1). Only a few published reports of nail matrix arrest in children with a clinical history of HFMD exist in the medical literature (9–11). We obtained shed nails from 2 siblings who had HFMD 8 weeks before the nail shedding. The nail fragments were stored at −70°C for a few weeks and treated with proteinase K before nucleic acid extraction. The extracts were enterovirus positive in 5’ NCR RT-PCR. The virus in one of them was identified as CVA6 by the specific RT-PCR and yielded a 5’ NCR sequence that was similar to the novel CVA6 strains.

Conclusions
Enterovirus CVA6 was a primary pathogen associated with HFMD during a nationwide outbreak in Finland in autumn of 2008. HFMD epidemics have primarily been associated with CVA16 or enterovirus 71 infections; those caused by enterovirus 71 have occurred more frequently in Southeast Asia and Australia in recent years (12). Reportedly, CVA10 has been found in minor outbreaks; other coxsackievirus A types have been found in only sporadic cases of HFMD (2,3). In general, CVA6 infections have been seldom detected and mostly in association with herpangina (13,14). In Finland, CVA6 has been identified only on 4 occasions over 8 years during enterovirus surveillance from 2000 to 2007 (15).

Although the CODEHOP primers were elementary for rapid genotyping of the novel CVA6 strains, we identified more viruses with the designated CVA6-VP1 specific primers. Onychomadesis was a hallmark of this HFMD outbreak. To our surprise, we detected CVA6 also in a fragment of shed nail. The same virus could have given rise to the outbreak in Spain in 2008 (10). Supposedly, virus replication damages nail matrix and results in temporary nail dystrophy. Whether nail matrix arrest is specific to CVA6 infections remains to be shown. This study demonstrates that CVA6, in addition to CVA16 and enterovirus 71, may be emerging as a primary cause of HFMD.

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Ms Österback is a doctoral candidate at the University of Turku in Finland. Her research interests are laboratory diagnostics and molecular epidemiology of viruses.

References

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