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Onychomadesis after an hand, foot and mouth disease outbreak in Spain, 2009

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Summary

To date few reports exist regarding the association between onychomadesis and an enterovirus infection presenting clinically as hand, foot and mouth disease. In February 2009, an outbreak of hand, foot and mouth disease occurred in a Spanish nursery school followed by onychomadesis two months later. During nail shedding, enterovirus was detected in stool samples from 8 (47%) of the 17 children who suffered hand, foot and mouth disease, but only in three of them an unusual enterovirus serotype (coxsackievirus B1) could be identified. The epidemiological results of this study confirm onychomadesis as a newly recognized complication in the course of hand, foot and mouth disease, but in future outbreaks, enterovirus molecular characterization of appropriate clinical samples should be studied.

Hand, foot, and mouth disease (HFMD) is a common childhood illness characterized by fever and vesicular lesions on hands, feet and in the mouth. It is caused by members of the genus *Enterovirus,* belonging to the *Picornaviridae* family. Two species A enterovirus serotypes, coxsackievirus A16 (CVA16) and enterovirus 71 (EV71), are mainly associated with HFMD [1, 2], but cases involving other types have been reported [3-5]. Infections are usually sporadic, but epidemics can also occur. Indeed, this disease is endemic in Asia where large outbreaks of EV71 associated HFMD with severe neurological complications have been found in recent years [6, 7]. Onychomadesis describes complete nail shedding from the proximal portion; it is consecutive to a nail matrix arrest and is a rare disorder in children. It has been related to a variety of drug exposures and systemic illnesses, including infections, and is a newly recognised complication in the course of a HFMD, although up to now, there are few studies in literature associating them [8, 9]. In Spain, however, two onychomadesis outbreaks with a clinical history of HFMD were reported in 2008 [10, 11].

In April 2009, onychomadesis cases occurred in a nursery school in Arzúa, a small town in La Coruña region (Spain), two months after clinical HFMD. The aim of this study was to relate both illnesses and to attempt to identify the causative agent of the outbreak.

Clinical samples (stools) were collected from 34 children of the nursery school and 8 adults (staff). Specimens were sent to the Enterovirus Laboratory of the National Centre for Microbiology in Madrid, for analysis. Twelve children presented nail shedding, and 11 (92%) of them referred having suffered HFMD symptoms two months before (from 36 to 69 days). In addition, 6 other children had clinical HFMD but did not show signs of onychomadesis. Sample collection was from 5 to 20 days after nail shedding. The

rest of the individuals (16 children and 8 adults) had no clinical symptoms. The symptomatic patients' mean age was 1.8 years old (range, 8 months to 3 years old).

Viral RNA was extracted from specimens using a QIAamp Viral RNA kit following the manufacturer's instructions (Qiagen, Hilden, Germany) and 5 µl was analysed for enterovirus detection by a RT-nested PCR in the 5'-non-coding region (5'-NCR) of the viral genome [12]. To enterovirus typing, a species HEV-A, B and C specific RT-nested PCR in the 3'-VP1 region previously described [13] was carried out.

Enterovirus was detected in 14 clinical samples, from 8 (47%) of the 17 HFMD cases (four with onychomadesis) and 6 (25%) of the 24 asymtomatic individuals (two children and 4 adults). None of them, however, were positive by the typing assay, probably because the sensitivity of the RT-PCR in VP1 region is from 10 to 100-fold less than that the 5'-NCR-RT-PCR [13]. To increase the amount of virus, isolation from cell culture was carried out with the 14 positive samples. For this, an aliquot of each stool was suspended in 4 ml of Eagle minimum essential medium with 2% fetal bovine serum, and centrifuged at 15000rpm/4ºC/25 minutes. Then, 0.2ml of each clarified sample was inoculated in two different cell lines, rhabdomyosarcoma (RD) and human embryo fibroblast (HEF). The culture supernatants were collected at tenth day, although no cytopathic effect was observed. However, positive results with the RT-PCR assay in VP1 region were obtained in 6 of the isolates (two in RD and 4 in HEF), corresponding to 3 cases (two with HFMD and onychomadesis symptoms and one with HFMD only) and 3 asymtomatic individuals (two children and one adult). The virus type in all of them was successfully identified by sequencing and BLAST (www.ncbi.nlm.gov/BLAST) analysis as coxsackievirus B1 (CVB1).

To determine the relationships between the six CVB1 sequences obtained, a phylogenetic analysis was carried out, including reference strain, sequences deposited in

the GenBank from different countries and, also, several Spanish CVB1 characterized by our laboratory in 2008 and 2009. These latter strains were from patients with aseptic meningitis or fever, and were isolated from May to July in different Spanish regions to La Coruña. Multiple sequence alignments were performed by the ClustalW program. Genetic distances were calculated using the Kimura 2-parameter model of nucleotide substitution, and statistical significance of phylogenies estimated by bootstrap analysis with 1000 pseudoreplicate datasets. A phylogenetic tree was constructed using the neighbor-joining method in the MEGA 3.0 software.

The reconstructed tree (Figure) showed the six CVB1 strains from outbreak to be closely related (98.3-99.5% identity), and formed a subgroup (nucleotide distance <0.01) within a major cluster represented by all Spanish CVB1 from 2008 and 2009 that placed together (bootstrap value of 99%), and separated from the sequences detected in other countries and years.

Onychomadesis, as a complication in the course of enteroviral HFMD, has rarely been reported [5, 8, 9]. In 2008, however, two large Spanish outbreaks were described [10, 11] one of them seemed to be produced by a CA10 (Salazar et al., communication in Spanish National Congress of Epidemiology, Gac sanit. 23, 2009: 13-203), but no typing analysis was carried out in the other.

The results of this study suggested that the onychomadesis cases which occurred in a nursery school from a town in the Northwest of Spain in April 2009 were a consequence of a previous clinical HFMD, as 92% of the cases presenting nail shedding referred had suffered this viral infection two months before. The clinical and epidemiological characteristics of the patients from this study were similar to those described in the other Spanish outbreaks which occurred in 2008 [10, 11].

Enterovirus was detected in 47% of patients with HFMD. Stool specimens collected during onychomadesis illness are not the most appropriate clinical samples for viral diagnosis of HFMD but, unfortunately, no vesicular samples at the acute phase of the primary illness were available. Also, only 6 of the 14 detected enteroviruses (three of them from patients with HFMD and onychomadesis or with HFMD only) could be further typed as CVB1. So, there was insufficient information to confirm the tentative hypothesis regarding this serotype as the causative agent of the HFMD outbreak, followed by onychomadesis described in this study. However some data might help to support this. First, enterovirus detection two months after the initial infection are in accord with a recent report where the authors detected an enterovirus in a fragment of shed nail two months after a primary HFMD [5]. Second, HFMD cases associated with different echovirus or coxsackievirus B serotypes, including CVB1, have been previously reported, although infrequently [3]. Finally, phylogenetic results showed that the 6 sequences detected had very high identity and formed a cluster separate from other Spanish CVB1 strains (Figure).

In conclusion, this report confirm that onychomadesis can be a complication during the course of viral infections presenting clinically as HFMD, but further case-control studies with suitable samples (mainly exanthematic specimens as vesicular fluids or shed nail fragments) during the onychomadesis symptoms and/or primary HFMD are needed to know what enterovirus serotypes are implicated in this type of infections.

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Declaration of interest

None

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Figure Legend

Phylogenetic tree of CVB1 3'-VP1 sequences (390 nucleotides) showing the relationship between the Spanish strains (6 detected in this study (black spots) and 15 from other cases), several CVB1 sequences from other countries available in Genbank, and the prototype Conn strain. The tree is rooted with E30 Bastianni strain.

Dendogram were constructed by the neighbor-joining method, with 1000 bootstrap pseudoreplicates. Only bootstrap values >64% are shown at nodes. Genetic distances were calculated with Kimura 2-parameter model of evolution (values<0.01 are not shown), and horizontal branch lengths are drawn to scale.

The Spanish sequences have been deposited in the GenBank database, under accession numbers HM584455-HM584475.