Circulation of Multiple Serotypes of Enterovirus Causing Hand, Foot and Mouth Disease (HFMD): An Emerging Infection in Andaman Islands, India

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Summary
Present communication is intended to document the outbreak of the disease caused by enterovirus serotypes in these remote islands and children health care.

Abstract
Hand foot mouth disease is a viral infection seen in worldwide. It has never been reported in Andaman and Nicobar archipelago till 2010. Present communication notifies the outbreak occurred in 2010 and the cases reported during 2011 in these islands demonstrates that, CVA16 and CVA6 may be emerging as a primary cause of HFMD. Though HFMD is a self-limiting disease in children, usually more severe cases with HFMD requiring hospitalization and less severe which can subside within 6 days without any treatment. However, the circulation of different virus strains and gives an alarm to the health authorities/public needs special attention to control the disease.

Keywords
Hand Foot Mouth Disease; Vesicular Lesions; Human; Coxsackievirus a 16; Coxsackievirus A6; Andaman & Nicobar Islands; India

Introduction
Hand, Foot, And Mouth Disease (HFMD) is a common febrile illness of early childhood, characterized by 3-4 days of fever and the development of vesicular enanthem on the buccal mucosa, gums, palate and papulovesicular exanthem on the hands, feet, and buttocks [1]. Coxsackievirus A16 (CA16) and enterovirus 71 are the two major causative agents and Coxsackievirus A4, A5, A6A8, A10, B3, B7 are usually associated with minor etiologies [2,3]. It was first reported in Toronto in 1957 [4]. As per the data from Infectious Agents Surveillance Report, CVA6 was detected in 709 HFMD cases and 156 herpangina cases throughout Japan [5].

In 2008, HFMD was declared a type C legally notifiable communicable disease in China [6]. Previous studies showed that the HFMD was predominantly occurred in children under 5 years old, especially those less than 3 years old. Most adults presented with subclinical infection when exposed to EV71 or CAV16, and then developed protective antibodies, which can transplacentally pass to newborns [7].

In India there was a largest outbreak of HFMD occurred in 2007 in the eastern part of the country in West Bengal,38 cases of HFMD noticed in and around Kolkata [8]. There is no past record of HFMD cases in Andaman & Nicobar Islands till the year 2010.

Methods
This study was hospital-based, observational study on pediatric patients who were clinically diagnosed with HFMD at outpatient department in INHS Dhanwantri Hospital and GB Pant Hospital, Port Blair. We reported here an outbreak and sporadic cases of HFMD among primary school going children in a Naval Hospital (INHS Dhanwantri) in Andaman Islands. During 27th August and 7th October 2010 several patients presenting with fever;
Figure 1: Genetic relatedness of the etiological agent CV A16 sequences obtained from the Andaman Islands and other sequences from NCBI.
papulovesicular rash on the buttocks, knees, hands, feet and lesions on the oral mucosa were seen at Naval hospital in Paediatric OPD in Port Blair. During 25th September and 21st November 2011, with the same clinical picture few patients were observed in South and Middle Andaman. Clinically it was suspected of HFMD.

All the patients with suspected signs & symptoms were included in the study. The patients were interviewed using a structured questionnaire that elicited information about symptoms and signs and clinical course. Throat & lesion swabs were collected in viral transport medium from all suspected patients and stored at -80°C freezer until processed. Stools samples were also collected for virus isolation.

Genomic viral RNA was extracted from 140 μl of clinical sample using the QIAamp Viral RNA Mini Kit Qiagen, USA), following the manufacturer’s instructions. RT-PCR was performed for enterovirus by using primers for 5`Non Coding Region and VP1as has been described [9,10]. PCR products of Enterovirus positive were purified by using a Gel Extraction Kit (QIAquick, Qiagen). Both strands were sequenced by using Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Bio systems, Carlsbad, CA, USA) in ABI PRISM.

![Figure 2: Genetic relatedness of the etiological agent CV A6 sequences obtained from the Andaman Islands and other sequences from NCBI](image-url)
The complementary DNA sequences of enterovirus were assembled using SeqMan II version 5.03 (DNASTAR). The sequences of Enterovirus reported in these Islands were aligned with worldwide diverse Enterovirus sequences using ClustalW multiple alignment and pairwise alignment for neighbour-joining phylogenetic tree analysis with bootstrap resampling analysis (1000 repetitions) using MEGA version 4.0. [11].

Results and Discussion

Forty two children have reported to the clinician with classical sign of HFMD with an age group of 3months to 15years with clinical sign of hemorrhagic lesions in mouth and hands. Out of these 19 children with the age group of 12-71 months were studying in primary schools. The attack rate in the school was 6.5%.

Vesicular eruption/Lesions (100%), fever 47.61%, rashes 28.57%, are the common symptoms observed. Papulovesicular lesion were observed in different parts of the body includes hand 54.76%, Palm 30.95%, Leg 42.85%, sole 28.57%. Mouth/Pharynx/Tonsil 11.90%, face 4.76%, Gluteal region 21.42%. Hands were found to be more common site of involvement. Whereas majority of the maculopapular lesions were observed in hands (8, 27.58%).

Thirty Eight children’s samples were available and been processed by RT PCR analysis. Eleven samples (28.94%) were found positive for enterovirus5’ non coding region. Among the 4 samples which were reported during 2011 as sporadic cases. These were processed for VP1 region of Enterovirus by RT-PCR.

The assembled nucleotide sequences were blasted in NCBI nucleotide BLAST search site,5’NCR nucleotide sequences obtained from 2010 outbreak showed 100% query coverage, 0.00% Error value and 97% maximum identity with enterovirus A16 and nucleotide sequences obtained from sporadic cases in 2011 showed 98% identity with the CV-A6 isolate (HM190277) from India.

Analysis was done along with reference sequences of different genus, species and serotype of Picornaviridae family using ClustalW multiple alignment and pair wise alignment for phylogenetic and molecular evolutionary analyses using MEGA version 4.1. The phylogenetic analysis (Figure 1) of the 5’NCR gene sequences obtained from HFMD cases in Port Blair in the year 2010 was closely related to Coxsackievirus A16. And the phylogenetic analysis of the VP1 gene sequences obtained from the sporadic cases reported in the year 2011 were closely related to Coxsackievirus A6. The pair wise distance along with different genus, species and serotypes were analyzed. The virus 5’NCR gene sequences (2010) of Port Blair showed very small distance with Human Enterovirus Coxsackievirus A16 (0.024) followed by A4 (0.032), A6 (0.063), A14 (0.063), B3 (0.066), A3 (0.071). Echovirus (0.071), A5 (0.074), A7 (0.077), A7 (0.088) and A7, A10 (0.088).

The VP1 virus gene sequences (2011) of Port Blair showed very small pair wise genetic distance with Human Enterovirus Coxsackievirus A6 reported from India (HM190277, HM190268) and China (JN316023) than Japan, Atlanta and other Enterovirus A4 (0.060), A16 (0.593), B3 (1.256), Echovirus 19 (1.179), A5 (0.572), A71 (0.663), A8 (0.536) and A10 (0.535), poliovirus (0.998) (Figure 2).

The molecular analysis reveals that presence of CV-A16 and CV-A6 in these islands. Though HFMD is a self-limiting disease in children, usually more severe cases with HFMD requiring hospitalization and less severe which can subside within 6 days without any treatment [12]. HFMD outbreaks caused by CVA6 were reported in Finland and few part of India [8-10]. This study demonstrates that, CVA16 and CVA6 may be emerging as a primary cause of HFMD.

The significant increases in HFMD morbidity and mortality have caused an enormous burden on public health. There are no effective drugs or clinical treatments so far. In these Remote Islands existing of these emerging viruses is giving an alarm to health authorities to initiate the control measures which can lower the risk of infection and to carryout extensive surveillance to prevent the epidemics.

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