(30%), and religious taboos (27.5%). The EBM was received in a clean, sterile and covered container in only 8 (20%) of the cases.

The limitations of our study were that though the fathers and caretakers were sensitized regarding the importance of early administration of EBM to their neonate and method of safe transportation of EBM, their understanding was not assessed. Also, we could not ensure whether the mothers admitted at some other hospital were counselled and offered assistance in expression of breast milk.

This study highlights that ensuring the availability of EBM in optimal condition to the neonates admitted in extramural centres remains a challenge in our country. Certain interventions like education and motivation of families regarding the importance of EBM in preterm survival soon after delivery, assisting mothers in early expression of breast milk and sensitization of fathers and caretakers regarding appropriate transport conditions of EBM may result in better and early availability of EBM for preterm infants admitted in extramural NICUs.

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Clinical and Molecular Investigations of Hand, Foot and Mouth Disease Outbreak in Navi Mumbai, India

An outbreak of Hand, Foot and Mouth Disease (HFMD) was reported in Navi Mumbai in July-October 2018. Of 15 HFMD cases, two had recurrences within a month while three had lesions extending to trunk. Coxsackie virus A6 and A16 were detected from 13 cases (CV-A6 from 10 cases and CV-A16 from 3 cases) indicating co-circulation of these viruses.. The present study highlights an urgent need of HFMD surveillance.

Keywords: *Coxsackie virus, Epidemiology, Epidemic.*

Navi Mumbai city witnessed an unprecedented rise in cases of Hand, Foot and Mouth Disease (HFMD) in July-October 2018 with two children reporting recurrences in the same year and lesions extending beyond the characteristic distribution over the body. Clinical characteristics were studied and the virological and molecular laboratory diagnosis was carried out to identify the etiological agents.

All children with clinical suspicion of HFMD upto age 18 years during the study period September- October 2018 were included. Stool specimens along with swabs from the throat and vesicles were collected from these children. The specimens (stool, vesicle fluid and throat swabs) were transported in cold chain to the ICMR-National Institute of Virology, Mumbai Unit, Mumbai for molecular diagnosis and virus isolation.Written consent for specimen collection was obtained from the parents of the children by the hospital authorities. Enterovirus (EV) isolation was performed as per the WHO Laboratory Manual protocol (2004) by inoculating human rhabdomyosarcoma (RD) cells. The cultures briefly were incubated at 36ºC and cytopathic effect (CPE) was

observed for five days. Once CPE was observed, tissue culture material was harvested for RNA extraction using QIAGEN Mini RNA Extraction Kit according to the manufacturer's instructions. Clinical specimens were used for RNA extraction for direct detection of enteroviruses. Partial VP1 (RT-snPCR) was performed using primers as shown in ***Table* I**.

A total of 17 (four stool, nine vesicle fluids and four throat swabs) specimens were collected from 15 HFMD cases. Stool and vesicle fluid specimens were collected simultaneously from only 2 cases whereas only one specimen was collected from the remaining cases. Of the 15 HFMD cases aged between nine months and 12 years, 13 (13/15) were confirmed to be caused by EV. EV was isolated by culture from different specimens (4/4 stool, 4/9 vesicle fluids and 1/4 throat swabs). Stool specimens yielded highest virus isolations as compared to swabs from vesicles. In 4 children, EV was detected from clinical samples by RT-PCR alone.

Coxsackievirus A6 (CV-A6) was detected in ten cases while Coxsackievirus A16 (CV-A16) was reported in three cases. No mixed infection due to either CVA6 or CVA16 was observed from any case.

Interestingly, three children identified with CV-A6 had extensive rashes, extending to the trunk. Out of these three cases, two had previous history of HFMD. A 2-year-old female child from China excreted CV-A16 in the stool. She was vaccinated twice against enterovirus-A71 (EV-A71) vaccine in China in 2017. No complications were reported in any case.

HFMD presents as sudden appearance in crops of erythematous papulovesicular rashes with a characteristic distribution over the hands, feet, knees, buttocks and intraoral areas. It is commonly seen in children less than 10 years. It is mostly caused by CV-A16 and EV-A71, but can also be caused by serotypes CV-A 2-8, 10, 12 and 14 [1]. The diagnosis of HFMD in India is usually clinical due to limited availability of molecular testing. The disease is by and large self-limiting and resolves within 7-10 days. However, neurological and/or cardiopulmonary complications such as encephalitis, aseptic meningitis, pulmonary oedema and cardio respiratory failure can occur in about 1% of HFMD patients. EV-A71 infections are more severe than CV-A16 across all ages [2].

Recurrences occurred in two cases which could be due to absence of immunity or due to infection with another enterovirus serotype. Extensive lesions over trunks in HFMD with CV-A6 have been reported in Japan [3]. CV-A16 and CV-A6 have also been identified as major causes of HFMD in Southern and Eastern parts of India [4,5].

EV-A71 vaccines are available in mainland China with >90% protective efficacy against EV-A71-associated HFMD [6]. Cross-protection of EV-A71 vaccine to CV-A16 infections does not occur [7].

A similar HFMD outbreak was reported during May-June 2018 in South Mumbai, which is about 25 km away from Navi Mumbai [8]. The phylogenetic analysis of CV-A6 and CV-A16 viruses isolated from these cases from both outbreaks showed that they are genetically closely related (Data not shown).

Co-circulation of CV-A6 and CV-A16 was observed in the reported HFMD outbreak in Navi Mumbai, highlighting an urgent need of virological surveillance to study recurrences and the changing clinical pattern of the disease.

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TABLE I Primers used for EV PCR Amplification and Sequencing

Primers	*Sequences (5'-3')*	*Region*	*Nucleotide positions*
AN89	CAGCACTGACAGCAGYNGARAYNGG	VP1	2602-2627
AN88	TACTGGACCACCTGGNGGNAYRWACAT	VP1	2977-2951
224	GCIATGYTIGGIACICAYRT	VP3	1977-1996
222	CICCIGGIGGIAYRWACAT	VP1	2969-2951
011	GCICCIGAYTGITGICCRAA	2A	3408–3389
240	ATICCICCRCARTCICCIGG	2A	3690-3671

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