Emerging Hand Foot Mouth Disease in Bangladeshi Children- First Report of Rapid Appraisal on Pocket Outbreak: Clinico-epidemiological Perspective Implicating Public Health Emergency [version 3; peer review: 1 approved, 1 approved with reservations]

Md. Azraf Hossain Khan¹, Kazi Selim Anwar², A. K. M. Muraduzzaman³, Md. Abid Hossain Mollah⁴, S. M. Akhter-ul-Alam⁵, Kazi Munisul Islam¹, Sheikh Ariful Hoque⁶, Md. Nazrul Islam¹, Md. Ahasan Ali⁷

¹Department of Dermatology and Venereology, Pabna Medical College and General Hospital, Pabna, 6600, Bangladesh
²US-CDC’s GHSA Project, Institute of Epidemiology, Disease Control and Research (IEDCR), Dhaka, 1212, Bangladesh
³Department of Virology, Institute of Epidemiology, Disease Control and Research (IEDCR), Dhaka, 1212, Bangladesh
⁴Department of Pediatrics, Ibrahim Medical College & Hospital, Institute of Research & Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Dhaka, 1200, Bangladesh
⁵Infectious Disease Division, International Center for Diarrheal Diseases Research, Bangladesh (icddr,b), Dhaka, 1212, Bangladesh
⁶Tissue Culture Laboratory, Centre for Advanced Research in Sciences (CARS), University of Dhaka, Dhaka, 1000, Bangladesh
⁷Microbiology Section, Institute of Public Health (IPH), Mohakhali, Dhaka, 1212, Bangladesh

Abstract

Background: Hand, foot and mouth disease (HFMD) is a common contagious disease among children under 5 years, particularly in the Asia-Pacific-region. We report a localized outbreak of childhood HFMD for the first time from Bangladesh, diagnosed only based on clinical features due to lack in laboratory-diagnostic facilities.

Methods: Following the World Health Organization’s case-definition, we conducted a rapid-appraisal of HFMD among all of the 143 children attending Pabna Medical College and General Hospital with fever, mouth ulcers and extremity rash. Data were collected between September and November 2017 using a preset syndromic approach and stringent differential diagnostic-protocols.

Results: The mean age of children was 2.9±2.3 years. There was a significant difference among the age and sex of children (P=0.98), first sibling being more belonging to middle-income families (62%). Younger children (<5 years) were more likely to suffer with moderate-to-high (38.5°C) fever (P<0.04), painful oral ulcers (P<0.03) and painful/itchy rash (P<0.01). Sex did not differ with other symptoms, but boys had less painful oral ulcers than girls (P<0.04). Fever (63%) and chicken-pox-like-rash
(62%) was observed more in mid-October to mid-November than September to mid-October (P<0.01 and P<0.03, respectively). No differences in symptoms (fever, oral ulcers and extremity rash) were observed with precipitation, nor with ambient temperature. Children <5 years (85%) had quicker recovery (within 5 days) than those ≥5 years (69%), (P<0.04), with marginal differences in sex (P<0.05).

Conclusions: Our findings highlight potential usefulness in diagnosing HFMD based on clinical parameters, although stringent differential diagnosis remains indispensable, which is particularly applicable for resource-constrained countries lacking appropriate virology/essential laboratories. Since no specific treatment or effective vaccination is available for HFMD, supportive therapy and preventive measures remain the primary methods to circumvent disease-transmission augmented by climate-related factors. Standardized virology laboratory warrants appropriate diagnosis and globally representative multivalent-vaccine deem essential towards preventing HFMD.

Keywords
Emerging Childhood-HFMD, Bangladesh, Rapid-Appraisal, Pocket-Outbreak

Corresponding author: Kazi Selim Anwar (kselim2256@gmail.com)

Author roles: Hossain Khan MA: Conceptualization, Data Curation, Funding Acquisition, Methodology, Project Administration, Resources, Supervision, Visualization, Writing – Review & Editing; Anwar KS: Conceptualization, Data Curation, Formal Analysis, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation; Muraduzzaman AKM: Conceptualization, Data Curation, Formal Analysis, Methodology, Supervision, Validation, Writing – Original Draft Preparation; Hossain Mollah MA: Formal Analysis, Resources, Supervision, Visualization, Writing – Review & Editing; Akhter-ul-Alam SM: Data Curation, Project Administration, Resources, Supervision, Visualization; Munisul Islam K: Data Curation, Formal Analysis, Methodology, Resources, Software, Writing – Review & Editing; Hoque SA: Formal Analysis, Methodology, Resources, Software, Supervision, Writing – Review & Editing; Nazrul Islam M: Data Curation, Project Administration, Resources, Supervision, Visualization; Ali MA: Data Curation, Formal Analysis, Project Administration, Resources, Software, Validation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

Copyright: © 2019 Hossain Khan MA et al. This is an open access article distributed under the terms of the Creative Commons Attribution Licence, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Data associated with the article are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC0 1.0 Public domain dedication).

How to cite this article: Hossain Khan MA, Anwar KS, Muraduzzaman AKM et al. Emerging Hand Foot Mouth Disease in Bangladeshi Children- First Report of Rapid Appraisal on Pocket Outbreak: Clinico-epidemiological Perspective Implicating Public Health Emergency [version 3; peer review: 1 approved, 1 approved with reservations] F1000Research 2019, 7:1156 (https://doi.org/10.12688/f1000research.15170.3)

First published: 30 Jul 2018, 7:1156 (https://doi.org/10.12688/f1000research.15170.1)
Introduction

Of all commonly occurring febrile illness and rash syndromes, hand, foot and mouth disease (HFMD) remains the most among young children. Although this viral infection remains largely contagious, it is self-limiting and benign. Severe cases reportedly occur in lower incidences (3.2% to 8.5%) and fatalities are rare. Starting in the West during the mid-1970s, HFMD emerged in the Asia-Pacific region in mid-1990s heralding as a major public health hazard. Epidemiologically, it follows a 2–3 years cyclical pattern but may break out anytime as has occurred in India (Orissa and Calcutta), bordering with Bangladesh.

With the complaints of mild-to-moderate fever (≥38.5°C; 101.3°F) childhood HFMD, characteristically manifest with body rashes, mostly of the knees and buttocks, augmented by painful oral/buccal ulcers and blisters. Papulo-vesicular rash in the extremities consequently forms pustules. Most children recover/heal within 7–10 days. Of the few complications, neuro-respiratory syndromes (encephalitis, aseptic meningitis and acute flaccid paralysis) occur mainly in younger children; these are rare but seldom fatal. HFMD is caused by several serotypes of enterovirus A, the most common being enterovirus A71 (EV A71) and coxsackievirus A16 (CV-A16) and more recently, also (CV A-6, and CV A-10). EV-A71 is associated with a higher proportion of severe illnesses. Reportedly, these viruses are transmitted through direct contact/blister-fluid, droplets, oro-fecally and also spread out through contaminated environment, water and food.

Reportedly, clinical diagnosis of HFMD is usually established depending on physicians’ suspicions as the sole diagnostic modality. The diagnosis is primarily based on history of illness, disease-onset, presenting clinical-features and, socio-demographic profile. Small erythematous maculopapular lesion (1–5 mm) enlarge (3–15 mm) and progress to vesicular eruptions with a prominent erythematous halo. It is essential to perform stringent differential diagnosis (DD) to distinguish HFMD from a group of diseases. DD includes chickenpox, scabies, measles, erythema multiforme, herpangina, herpetic gingivitis, drug eruption and others. Laboratory diagnosis is usually not essential, and has been described by the World Health Organization (WHO) as optional. Conversely, the sophisticated laboratory tests used for definitive diagnosis (virus isolation, molecular analysis, PCR, genotyping) are not available in most resource-constrained countries like Bangladesh.

Since there is no specific treatment, for HFMD, care largely remains palliative with antipyretics/analgesics and antihistamines. Topical anesthetics are rarely used for oral ulcers for soothing and comfort. Povidone-iodine used as a mouth wash/topical application that can relieve pain. Since no effective vaccine against HFMD-viruses is available, preventive measures remain the primary method of circumventing HFMD transmission to break infection-chains (droplets, oral-fecal route, and direct contact). Effective prevention requires personal hygiene, hand washing and a pollution-free environment including food and water. Meteorological variations in precipitation and ambient temperature often impact on HFMD occurrences in the Asia-Pacific region, along with atmospheric pressure and the relatively higher humidity in summer and early autumn.

Extraction from extensive reviews, when compared with our intensive observations on upsurge of unusual febrile, rash-associated childhood illnesses in the two periods, were indicated as a short-term standardized-surveillance. Following a pre-set case-definition and syndromic approach (according to the WHO HFMD guidelines), a strategic plan was adopted to conduct this comprehensive study from September to November 2017.

Methods

Set up, patients and research design

Utilizing a pre-set syndromic approach based on case-definition following the WHO’s HFMD guidelines, this rapid appraisal was conducted among all the 143 children attending Pabna Medical...
College and General Hospital (PMC-GH) between September and November, 2017. PMC-GH is a 250-bed secondary care hospital serving a targeted population of nearly 2.81 million from its 2,371.5 km² catchment area situated in a small poverty-stricken north-western flood-prone plain land on the Ganges Delta basin in Bangladesh.

Research instruments used
Clinical diagnostic tool. Prepared based on syndromic case-definition following the WHO’s HFMD guidelines¹, similar to a prior study conducted in Thailand⁷. Most of the contents of this tool have been shown in Figure 4 (4 A), showing the algorithm of Clinical diagnosis of HFMD¹.

Clinical case management protocol. This was prepared incorporating a history of disease, onset, chief complaints and duration of illness, clinical diagnosis and therapeutic intervention. We ascertained clinical outcome by through post-treatment follow-up in the outpatient department of PMC-GH or through cellphone-based enquiry. We performed the clinical diagnosis following WHO guidelines¹, predominantly based on three main signs and/or symptoms: fever, oral ulcers and rash in extremities. Fever was graded into moderate-to high (38.5°C) and none-to-low (37-38.4°C), oral ulcers were grouped into three stages: more painful, less painful & painless; and, rashes in extremities into three types: painful and itchy, painless and itchy; and painful but not itchy.

Pain assessment/scoring tool
Since pain remains subjective in younger children in expressing pain intensity properly, we arbitrarily categorized the pain intensity based on following clinical grounds:

i. Nullifying any history of similar disease/disorders in near past
ii. Facial expression of a child with body rash and/or oral ulcer on touch
iii. Impression and/or opinion of child’s parent/guardian in respective cases
iv. Finally, clinician’s judgements based on history and presented signs/symptoms

Therapeutic management guideline
A therapeutic guideline was prepared to treat childhood HFMD cases following standard therapeutic plan consisting of: antipyretic/analgesics, antihistamines, anesthetic-cream for topical applications.

Epidemiological tool
This tool consisted of socio-demographic variables and household (HH) income. We categorized the monthly (mon) income (in Bangladeshi taka: BDT) of child’s family according to World Bank (WB) Data Help Desk 2016¹¹ as follows:

- Low-income group: HH income of ≤ 6,946/mon
- Lower-mid income group: HH income: 6,947–27,336/mon
- Upper-mid-income group: HH income:27,337–84,564/mon
- High-income group: HH income of ≥ 84,564 BD/mon

(Calculated using USD rate: 1US $=84.31 BDT dated 11.06.18)

Seasonal data collection
Seasonal data on local weather/climate for average temperature and rain precipitation were collected from Pabna Meteorology Department, Bangladesh over the period of September through November 2017. In Bangladesh, early autumn runs from September to mid-October, followed by late autumn/fall from mid-October to mid-November.

All these tools (developed or followed) were duly pre-tested for this rapid-appraisal (small-scale disease surveillance)¹².

Data analysis
Crosschecked data were subjected to Pearson’s chi-squared test, Fisher’s exact test and Spearman correlation analysis using SPSS for Windows v.21, taking P<0.05 as indicating statistical significance (at 95% CI).

Inclusion criteria/patient enrolment
Any child, irrespective of age and sex, attending PMC-GH between September and November 2017 with suspected HFMD (meeting WHOs’ recommended criteria) were included in this study. Suspected cases having other serious disease/co-morbidities were excluded, although patients were referred to concerned department for proper clinical management.

Ethical considerations
Following standard procedure of ethical issues³⁵, written informed consent was obtained from the parents of children with suspected HFMD prior to enrolment. We detailed the parents/guardian of all children on the purpose and procedures of this study. We also informed the parents on the lack of risk of harm/damage involved in procedures and did not collect body fluids or other biological samples. We informed the parents that they could remove their child at any stage of the study. Complete privacy and anonymity of clinical data was ensured, including its protected use research purposes only. This study had prior approval through the Ethical Committee of Pabna Medical College and General Hospital, Government of the Peoples’ Republic of Bangladesh (Memo No. 1577, dated: 26/08/2017).

Results
Demographic information
The mean (±SD) age of the 143 children was 2.9±2.3 years; 80 (56%) were boys and 63 (44%) were girls. Of the total, 70% were under 5 years old. Age did not differ with sex (P=0.98). Data on HH structure yielded an average size of children’s family as 5.5±6.9 persons/per HH. Of them, 62% having only one (no siblings) and 38% two (first sibling) children, (Table 1).

Following Word Bank, (2016) standard³¹ family/HH income-group evidenced that majority families (85%) belonged to middle-income HH/families (34% belonged to upper-middle
Table 1. Socio-demographic characteristics and household income of child’s family attending the Pabna Medical College and General Hospital with the complaints of hand, foot and mouth disease (n=143 cases).

| Variable                | Groups               | N (%)   |
|-------------------------|----------------------|---------|
| Age                     | 2 months–3 years     | 78 (54.5) |
|                         | 3.1–5 Years          | 32 (22.4) |
|                         | >5.1 Years           | 33 (23.1) |
| Sex                     | Male                 | 80 (55.9) |
|                         | Female               | 63 (44.1) |
| Age vs. sex             | χ²                   | p=0.98  |
|                         | Likelihood ratio     | p=0.98  |
|                         | Spearman’s correlation| p >0.87 |
| Siblings                | Child 1              | 89 (62.2) |
|                         | Child 2+             | 54 (37.8) |
| Household income         | Low income           | 21 (14.7) |
|                         | Low-mid-income       | 73 (51.0) |
|                         | Upper-mid-income     | 49 (34.3) |
|                         | High income          | 0       |
| Sibling number vs. income| χ²                   | p <0.01 |
|                         | Likelihood ratio     | p =0.01 |
|                         | Spearman’s correlation| p <0.01 |

*Following World Bank Data Help Desk, 2016\(^3\)

and 51% to lower-middle income-groups living with a modest HH budget). The rest (14.7%) belonged to low-income groups lived with a tight HH-budget. Notably, HFMD cases were significantly more common among children from mid-income-HHs and among first siblings (P<0.01), (Table 1).

Assessment of symptoms

Child’s age was significantly associated with three major clinical signs/symptoms. Younger children (under 5 years old) suffered more (74/91, 81%) with moderate-to-high fever than older children (17/91, 19%; p<.04). Similarly, painful oral ulcers (82/111, 74%) and painful itchy rash in extremities (92/116, 79%) were more common in younger than older children (p<0.03 and p<0.01, respectively). Notably, skin rash in extremities of younger children’s were predominantly more like papulo-vesicular (59/68, 87%) than chicken pox-like (43/75, 57%) lesions (p<0.001). However, sex did not differ with other signs/symptoms except oral ulcers: boys had less painful ulcers (23/32, 72%) than girls (9/32, 28%), (P<0.04), (Table 2).

None of the three major signs/symptoms of HFMD (fever, oral-ulcers/blisters and extremity rash) was associated with seasonal variations except fever and characteristics of rash. Moderate-to high fever (57/91, 63%) was observed more in fall/late-autumn (mid-October through mid-November) than in early autumn (September through mid-October), yielding 37% of cases (34/91), (p<0.01). Similarly, papulovesicular rashes were more common in fall (42/68, 62%) than in early autumn (26/68, 38%) (P<0.03) (Table 3).

Findings of post-treatment clinical outcome was associated with age. Time to recover from HFMD varied with child’s age. More young children (<5 years) recovered in <5 days (63/74, 85%) than older peers (≥5 years) (47/69, 69%) who were more likely to recover in >5 days (P<0.05). However, clinical disease/outcome was not associated with children’s sex, although boys were more likely to suffer with the illness for 6–7 days, whereas girls tended to recover within 5 days. However, this was only marginally significant (P<0.05) (Table 4).

Discussion

Basis of this rapid appraisal on HFMD outbreak

Clinico-epidemiological insights from an extensive review on latest literature on HFMD augmented by our careful clinical observations on unusual events of febrile-rash (following
Table 2. Composite table showing association of HFMD clinical features with age and sex.

| Variables      | Clinical manifestation |
|----------------|------------------------|
|                | Body temperature       | Oral ulcers | Rash in extremities | Rash characteristics |
|                | ≥38.5°C (n=91)         | ≥37–38.4°C (n=52) | Painful (n=111) | Painless/less-painful (n=32) | Painful (n=116) | Painless/less painful (n=27) | Chicken pox-like (n=75) | Papulovesicular (n=68) |
| Child’s age    |                        |             |                   |                     |                 |                             |                            |                         |
| <3 years (n=78)| 57                     | 21          | 54                | 24                  | 70               | 08              | 32                           | 46                        |
| ≥3 but <5 years (n=32) | 17                 | 15          | 28                | 4                   | 22               | 10              | 19                           | 13                        |
| ≥5 years (n=33) | 17                     | 16          | 29                | 4                   | 24               | 09              | 24                           | 09                        |

χ²: P<0.04 (2-sided); P<0.03 (1-sided)  
Spearman’s correlation: P<0.01 (2-sided); P<0.01 (1-sided)  
Fisher’s exact test: P>0.73 (2-sided); P>0.42 (1-sided)  
Spearman’s correlation: P>0.71 (2-sided); P<0.04 (1-sided)  
Fisher’s exact test: P<0.18 (2-sided); P<0.49 (1-sided)  
Spearman’s correlation: P>0.15 (2-sided); P>0.11 (1-sided)  
Fisher’s exact test: P<0.28 (2-sided); P<0.26 (1-sided)  

*Mean ± SD = 2.9±2.3.

Table 3. Composite table showing association of HFMD clinical features with season/local climate.

| Variables                  | Clinical manifestation |
|---------------------------|------------------------|
|                           | Body temperature       | Oral ulcers | Rash in extremities | Rash characteristics |
|                           | ≥38.5°C (n=91)         | ≥37–38.4°C (n=52) | Painful (n=111) | Painless/less-painful (n=32) | Painful (n=116) | Painless/less painful (n=27) | Chicken pox-like (n=75) | Papulovesicular (n=68) |
| Seasons                   |                        |             |                   |                     |                 |                             |                            |                         |
| September-mid-October     | 34                     | 8           | 33                | 9                   | 36               | 6               | 16                           | 26                        |
| (n=42)                    |                        |             |                   |                     |                 |                 |                             |                            |
| Mid-October-mid-November  | 57                     | 44          | 78                | 23                  | 80               | 21              | 59                           | 42                        |
| (n=101)                   |                        |             |                   |                     |                 |                 |                             |                            |
| Fisher’s exact test       | P<0.01 (2-sided); P<0.01 (1-sided) | p>1.0 (2-sided) & 0.53 (1-sided) | p>0.48 (2-sided) & 0.26 (1-sided) | p>0.03 (2-sided) & 0.02 (1-sided) |
| Spearman’s correlation test | p<0.01                  | p>0.86            | p>0.37             | p<0.03             |
| Average rainfall on admittance |                        |             |                   |                     |                 |                             |                            |                         |
| 0.0 mm (n= 107)           | 67                     | 40          | 85                | 22                  | 86               | 21              | 56                           | 51                        |
| 0.1-7 mm (n= 22)          | 15                     | 7           | 17                | 5                   | 19               | 3               | 9                            | 13                        |
| >20.1 mm (n= 14)          | 9                      | 5           | 9                 | 5                   | 11               | 3               | 10                           | 4                         |
| χ²- Chi-square test       | p =0.88                | p>0.44      | p <0.78           | p <0.20            |
| Spearman’s correlation test | p >0.70                  | p =0.32            | p <0.76           | p <0.77            |
| Ambient temperature on admittance |                        |             |                   |                     |                 |                             |                            |                         |
| 24.4–29.9°C (n= 22)       | 11                     | 11          | 20                | 2                   | 16               | 6               | 14                           | 8                         |
| ≥30°C (n=121)             | 80                     | 41          | 91                | 30                  | 100              | 21              | 61                           | 60                        |
| Fisher’s exact test       | p>0.16 (2-sided) and 0.12 (1-sided) | p>0.16 (2-sided) & 0.08 (1-sided) | p>0.37 (2-sided) & 0.21 (1-sided) | p>0.35 (2-sided) & 0.18 (1-sided) |
| Spearman’s correlation test | p<0.15                  | p>0.11            | p>0.28           | p>0.26            |

*a* Comparatively lower temperature: Arbitrarily set cut-off values of lower temperature (on average).  
*b* Comparatively higher temperature: Arbitrarily set cut-off values of higher temperature (on average). The three major sign/symptoms of HFMD among these children were more prevalent on those days when the rain precipitation was recorded 0.0 mm, in our outbreak areas. Rain had no significant impact on any of the three major sign/symptoms, unlike on dry days with no rainfall (0.0 mm). Similarly, all major sign/symptoms prevailed more in hot and humid days when the ambient temperature was recorded at ≥30°C (up to a maximum of 36.2°C), with no significant difference among three major sign/symptoms (Table 3).
WHO’s “Clinical management and public health response for HFMD” made us enabled to establish the primary clinical diagnosis of childhood HFMD (M Azraf H Khan: Personal Observations, June–July 2017). Further, concurrent agreement from similar reports attested our diagnosis of HFMD in children, as correct.

Gauging the potential of a sudden upsurge in HFMD cases in children (during July 2017) attending PMC-GH from its catchment area made us aware on an upcoming localized outbreak. A strategic plan was thus urgently adopted to conduct this rapid appraisal (short-term standardized surveillance) on childhood HFMD utilizing a pre-set case-definition/syndromic approach based on WHO’s HFMD guidelines as depicted in Figure 4 (having fever or a history of fever, papulo-vesicular rash in extremities with or without oral ulcers), similar to a study conducted in Thailand.

The principal objective of this study (rapid appraisal) was to combat the impending HFMD outbreak with a secondary aim of disseminating the existence of newly emerged disease, thus to create a mass awareness. We also aimed to stir-up the local public health emergency squad to cumulate further strength towards combating such upcoming outbreaks in future. Finally, we desired to gauge strength of administrative drive, technical knowhow and clinical skill of PMC-GH team in combating that HFMD cases based on strong yet rational suspicions, as reported by others.

Keys to success of combating that on-going pocket outbreak, were: i) Sincerity and devotion of PMC-GH team despite huge limitation in manpower and resources, ii) strong clinical eye suspecting HFMD as appropriate diagnosis, iii) instituting supportive therapy instantly, and iv) diagnosing HFMD despite gross lack in diagnostic facilities, though it often remains not essential in such emergencies.

Table 4. Composite table showing association of HFMD clinical features with season/local climate.

| Variables | Post-treatment clinical outcome of childhood HFMD like-disease |
|-----------|---------------------------------------------------------------|
|           | Cured in >5 days (n=69) | Cured in <5 days (n=74) |
| Age of children (Mean= 2.9 ± 2.3 years) | | |
| <1–3 years (n=78) | 32 | 46 |
| 3.1–5 yrs (n=32) | 15 | 17 |
| 5.1–10 years (n=33) | 22 | 11 |
| Chi-square (χ²) test: | p <.04 | |
| Correlations | p <.02 | |
| Sex | | |
| Male (n= 80) | 44 | 36 |
| Female (n=63) | 25 | 38 |
| Fisher’s exact test | p <0.09 (2-sided), p<0.05 (1-sided) | |
| Pearson’s correlation | p <.07 | |

Potentials and dynamics of HFMD outbreaks

HFMD, emerged as a major public health problem in recent years was first recognized in the Western world during mid-1970s. It was then, spread out in Asia-Pacific region since mid-1990s, mostly in Malaysia, Taiwan, China and Singapore. Though a longer time series is required to ascertain EV71 outbreaks of HFMD, it generally occurs in 2-to 3-year cyclical pattern in West Pacific Region (WHO/WPRO, 2010) as reported from Singapore, UK, Malaysia and Japan. However, HFMD CA16 outbreak in Singapore also occurred periodically: in 2002, 2005 and 2007 but in 2006 but it was caused by EV71. HFMD outbreaks were also reported from Orissa and Calcutta in India that borders with Bangladesh but strange is no published data or report exist in Bangladesh, yet (until June 2018). In China, incidence and mortality were reported to be the highest among 12–23 months-old children.

All these facts and figures, including epidemiological hunches and variabilities support our strong speculation of this localized outbreak of HFMD in Pabna that we could combat successfully. We also postulate that HFMD might have emerged in Bangladesh earlier, but, swept on unnoticed being ‘underestimated’ due to its benign nature and self-limiting features, or such latent HFMD cases or small localized outbreaks might remained under-reported or un-reported (Kazi Selim Anwar and Md. Abid Hossain Mollah, personal observation, June 2017). Our postulations partly remain similar to that of Xing et al. from China.
In addition to history, onset and presenting clinical features we considered child’s socio-demographic characteristics and a positive history of similar sign/symptoms in child’s family, nursery/schools.

Our data yielded a significant association between age groups and three major clinical signs/symptoms. Moderate-to-high fever, painful oral ulcer and itchy-painful rash were more common in younger children- which remain consistent with other findings. Moderate-to-high fever remains an important, but not mandatory or principal sign of HFMD, as the WHO’s guidelines for clinical and public health response indicate, in agreement with our findings. Oral and/or axillary temperature in 64% of cases revealed a moderate fever (38.5°C), ranging mostly between 37.5°C and 38.2°C; the rest (36%) had no or a low-grade fever (ranging between 37.0 to 38.4°C). These observations resembles with Van Pham et al. though others reported high fever in HFMD-cases.

Literature reveals papulo-vesicular rash as the most important characteristic symptoms for HFMD often manifesting as painful chicken-pox-like rashes in 60% cases (Figure 3) though the rest 40% had it less painful or painless. Our findings on itchy rashes remain consistent with others, particularly in its distributions (knees and/or buttock). Itchy rash in child’s extremities that formed small pustules were filled with turbid fluid (Figure 1) and in some cases it crusted off consequently after 3–4 days- as other reported, as well

Secondly, most of the children (78%) had characteristic oral ulcers and/or painful blisters in tongue/mouth (Figure 2), that remain similar to several reports. However, the exact reason of less pain or painless oral ulcers/blisters in 22% cases in our study remain unclear. We guess it could be due to a varied perception and/or different tolerance, unwilling to mention, feeling shy or even being scared. Some of them may have taken analgesics at home prior to attending the hospital which they did not disclose despite repeated probing. Notably, sex of HFMD cases did not significantly differ with any sign/symptom except oral ulcers. More boys had it less painful than girls. Although a study in India reported an overall male-female ratio of 21:17.

**Differential Diagnosis of HFMD**

Stringently examined thorough DD was performed to differentiate HFMD from closely similar diseases, like chicken-pox/varicella, scabies, measles, erythema multiforme, herpangina, herpetic gingivitis, drug eruptions, as several reports mentioned. Mosquito bite was also included as report from India, underlined it as a simple yet valuable DD-point. Particular attention in the DD was paid on examining the characteristics of skin lesions (macules and papules quickly evolve into small vesicles manifesting on their palms, soles, and buttocks). We observed small vesicles in majority of these children that ruptured with the formation of erosions and crusts as ascribed by Sharma et al. Alike his finding, we also observed those vesicles as 1–5 mm in size as erythematos maculopapular lesions that rapidly enlarged by 3–15 mm progressing to vesicular eruption with prominent erythematos halo being comparable to that of a report by Bhumesh et al. from India.

![Figure 1](image1.png)

**Figure 1.** Multiple vesicular lesions containing turbid fluid seen in right knee of 4-year old girl.

![Figure 2](image2.png)

**Figure 2.** An oral ulcer on tongue with surrounding erythema of a 5-year old boy.
Laboratory diagnosis for HFMD

Laboratory diagnosis often remain unnecessary\textsuperscript{19} to establish a diagnosis of HFMD. Use of WHO clinical case definition and exclusion of other similar syndromes through a stringent differential diagnosis usually remains adequate in most cases\textsuperscript{12,23}. Moreover laboratory tests, such as serotyping, molecular, PCR and genotyping\textsuperscript{3} and virus culture\textsuperscript{1,13,24}, may not be feasible, available and more importantly not affordable in resource-constrained countries\textsuperscript{12,13}, like Bangladesh, particularly in hard-to-reach/remote areas. Although few studies report high WBC count or blood glucose, as associated with HFMD severity\textsuperscript{12,13,23,24}, it remains scarcely seen in recent literature.

Virological assays remains the main diagnostic tool. Of the four species in the family of Picornaviridae (groups EV-A, B, C and D) that cause HFMD in children, EV 71 remain the most, followed by coxsackie-virus A6, A10, A16\textsuperscript{3,7,8,10,24}. All these viruses are transmitted rapidly\textsuperscript{15,17} through direct contact, respiratory droplets, via feces/blister-fluid and through contaminated environment\textsuperscript{19}.

Specific treatment for HFMD viruses

There is no specific treatment\textsuperscript{22,25} or pharmacological intervention\textsuperscript{4} available for HFMD yet. Since it largely remain supportive\textsuperscript{19,25} we prepared a standardized therapeutic guide that was followed to take all therapeutic measures against HFMD cases. It consisted

---

**Figure 3.** Papulo-vesicular lesions surrounded by erythematous zones on the left palm of a 1.5-year-old boy.

**Figure 4.** Decision tree for the clinical diagnosis and management of hand, foot and mouth disease.

Adopted from WHO: A guide to Clinical management and public health response for Hand, Foot & Mouth Disease.\textsuperscript{4}(WHO, 2011)
of: i) antipyretic/analgesics, ii) antihistamines, and iii) anesthetic gel or ointment. Since, skin lesion in these cases usually got healed within 3–4 days; we did not prescribe any acyclovir due to its reported adverse effects (nephropathy & neurotoxicity). Since oral acyclovir is poorly absorbed, we had to prescribe it for 5 days only in 8 severe cases (mean age, 2.4 years), exceptionality, in recommended dosage of oral syrup (20 mg/kg body-weight). But we found them (with profuse skin-lesions with severe pain) to respond to it dramatically, with early recovery. Reasons or basis of pathogenicity or pharmaco-dynamics is not fully understood demanding further investigations.

Vaccination of HFMD

Though no effective vaccine available yet against HFMD viruses, scientists have been attempting to develop it in Malaysia (since 2010)6, in China (since 2012)7, and in Taiwan (since 2014)8,9. Cai et al. demonstrated how active immunization with an experimental inactivated CA16 vaccine can confer full protection by developing inactivated whole-virus vaccines against CA16 infection in human. Similarly, Chih-Wei Lin et al. dissected ‘prospect & challenges’ with critical bottlenecks of developing multivalent HFMD vaccines. They demonstrated that combined vaccine will reduce number of shots that will simplify WHOs ongoing child immunization schedule, along with protecting kids from several viruses, viz., H5N1, EV71 & JEV at the same time. Yican Cui et al. attempted to develop a combined bivalent-vaccine comprising EV71 and A16 for receiving a balanced protective immunity10 along with other developments in developing multivalent vaccines for broader protection for HFMD.11

Clinical outcome

Our clinico-epidemiological data, in agreement with other reports, revealed that younger children (<5 years old) recovered quickly (in <5 days) than their elder peers (>5 years old) who recovered in 6–7 days (>5 days). This was similar to a report from China, too. There was a marginal significant difference in sexes: boys had seemingly quicker recovery than girls (P<0.05). However, among Chinese children: boys had HFMD in 1.6 times more than the girls. Nonetheless, latest literature attest most HFMD-cases recover within 7–10 days.12,13 These findings remain consistent with that of others from Asia-pacific countries, including India14,15,16.

Complications

Though complications of childhood HFMD remain few, younger children may develop it more often.17,20. In our study, three cases (2.09%) developed complications of mild to moderate severity requiring special care. The first case (a 4-year-old girl) developed pneumonia requiring I/V antibiotics & was discharged following recovery after 2 days. The second one was an admitted case of pyoderma (a 5-year-old boy), who received appropriate antibiotics and was discharged after 2 days. The 3rd case, a 1.5 year old girl having post-HFMD Onychomadesis18 who were clinically diagnosed HFMD 25 days before. She had shedding of skin (right little finger) since few days. On repeated observations (weekly) her nail resumed in original position with 3 weeks without any medication. This scenario remains comparative to that of a report from South Korea.18 However, mechanisms of Onychomadesis and its association with HFMD is not yet fully understood as literature reveals that some viruses are responsible for onychomadesis as a temporal variation.

Although CA16 and EV71 are mostly associated with neuro-respiratory syndromes, we did not observe any of such severe diseases or serious complications, alike Vietnam study reporting 8.5% of severe cases4, nor we encountered any death in our HFMD cases - finding that remain consistent with several reports5,6,9,15.

HFMD cases and local weather/climate

Several studies carried out in the Asia-Pacific region reporting an association of HFMD cases with a wide range of meteorological findings (weather, climate, ambient temperature, humidity, rain, etc.).5,10,15,17,18. Reports on meteorological factors showing an association with HFMD outbreaks are: Singapore1, China19 and Hong Kong20. Mostly, rainy season and short-term temperature variations10,19,20 had an impact on HFMD occurrence in this region. This includes atmospheric pressure, relative humidity and rain precipitation21 that peaks in summer and in autumn partly remain similar to that of ours. We observed this pocket outbreak of HFMD among children in early autumn (September to mid-October) and in late autumn/fall (mid-October to November), 2018. Interestingly, while in North China HFMD peaks in June, it is more in May and September-October southern China22, which nearly corroborated with our findings22.

One limitation is we could not conduct a proper meteorological study as reported from some Asian countries5,10,15,20. Contrarily, we only tried to find out briefly if local weather has any impact on HFMD just to acquire a preliminary idea in this aspect. A report from China also remain alike ours that seasonal patterns were weakly associated with climate and demographic factors15.

However, the literature did not reveal any such study/report detailing the symptom-specific association of HFMD with seasons that we did, though some of our findings remain comparable with that of others.5,6,9,10 Thus, data from this rapid appraisal (short-term surveillance) demonstrated certain seasonal characteristics of local weather were associated with fever and rash characteristics. Moderate-to-high fever was observed more often in fall/late-autumn (mid-October to November) than in early autumn (September to mid-October). We found similar result for rashes that predominantly occurred in fall than in early autumn. We did not observe an impact of rainfall/precipitation or ambient temperature on any of the 3 major signs/symptoms that we evaluated. We observed that childhood HFMD cases occurred mostly in dry weather with no rainfall (0.0 mm) almost equally in all three major signs/symptoms of HFMD including disease severity. These findings on local climatic factors did not corroborate with others5,9,11.

Socio-demographic characteristics and household economy of child’s family

Another unique strength of our study was to associate socio-demographic &/or HH-economy with child’s family with HFMD. Child’s age (mean ± SD, 2.9± 2.3 years) group remained
similar to other reports[1,2,7,8,14–21]. Child’s age did not differ, significantly with sex. The HH structure revealed an average size of children’s family as 5.5±0.7 persons/HH, 62% of whom were the first kids and 38% the second ones. Following World Bank categorized family/HH income/grades[13] majority of children’s family (85%) belonged to middle-income HHs living on a modest budget; 34.3% being in upper and 51% in lower, mid-income HHs. The rest 14.7% belonging to low-income HH are compelled to live with a very tight HH budget. Notably, a logical but unique finding, based on ecology, environment and health care utilizations, we observed HFMD cases more among first siblings than their siblings and who used to live in tight/low HH-budget. Of multifaceted reasons for this, we postulate that gross limitation in health care expenditure, distance of PMC-GH from HHs and low level of HH-income remains the major reasons. While it demands further explorations, few reports associating HFMD cases with personal hygiene and surrounding environment[13,17,27] remains important to stop transmitting HFMD-virus among adjacent communities.

Prevention and control measures for HFMD
Due to a lack of available vaccines against HFMD-viruses[15,17,18], preventive measures remain the primary tool to circumvent HFMD-virus. Preventive methods include good personal hygiene, proper hand washing[19] particularly the post-defecation hand wash, pollution free environment[17,18] and hygienic sewage management[17], ensuring germ-free drinking water and food[19]. Although avoid person-to-person contact[2] through isolation remain justified, it often may not be practical in unprivileged communities of low-income countries having resource-constrained healthcare budget like Bangladesh. But it remains imperative that mass awareness be increased both among the communities and physicians.

Insights on principal findings
- Our clinico-epidemiological observation indicates that childhood-HFMD has emerged in Bangladesh. Earlier outbreaks in Calcutta, India (bordering with Bangladesh) remains indicative of its introduction in this country since few years but remained unreported and thus, unnoticed.
- The physicians’ strong yet rationales judgment clinical suspicion (based on signs/symptoms) could establish a correct diagnosis, of HFMD cases.
- Stringent differential diagnosis remain indispensable to exclude similar fever- or rash-causing illness.
- Laboratory diagnosis seems unessential, particularly during HFMD outbreak situations when proper laboratory-diagnosis (virus culture, serology, molecular analysis) is not readily available.
- We experienced that early forecasting may aid in combating HFMD outbreaks in catchment areas to curb complications more successfully.

- Small-scale/localized outbreaks can be combated utilizing existing health-care/hospital set up/facilities.
- No specific treatment for HFMD exists, although supportive therapy can treat cases of HFMD in a week.
- Healthcare workers must remain aware on the prevention and treatment of HFMD, and, particularly on the warning signs of its severe illness.
- It is imperative to increase mass awareness to stop transmission of HFMD viruses (air/droplet, environment).
- Personal hygiene, hand washing and a pollution-free environment are mainstays of HFMD prevention.

Conclusion
We could diagnose cases of childhood HFMD successfully based on clinical signs/symptoms only and all cases recovered well within a week. Stringent differential diagnosis on similar rash and/or fever diseases/syndromes were deemed indispensable. The local climate may influence HFMD. Time consuming and costly laboratory diagnosis (virological/molecular) is not essential in resource-constrained settings, particularly during outbreak situations. No specific treatments or effective vaccinations exist for this often-underestimated disease yet. Supportive therapy and strict preventive measures is able to circumvent/destroy EV or CA viruses to combat ongoing HFMD-outbreaks/threats.

Recommendations
Development of a globally representative multivalent HFMD vaccine remains necessary, particularly in countries where HFMD widespread, before it becomes pandemic. Both the government health services and meteorology departments should work together since climate is shown to be an early indicator of potential HFMD outbreaks. Our findings warrant that the countrywide public health emergency operations teams be more alert towards the effective prevention and control of HFMD in resource-constrained countries like Bangladesh. The governments of such countries should come up with a well-designed, sustainable strategic plan to combat upcoming HFMD outbreaks, in close-cooperation with national and global NGOs and UN organs to prevent its pandemic threat in the near future.

Data availability
Dataset 1. Complete raw data from each child assessed as part of this study. DOI: 10.5256/f1000research.15170.d21103

Consent
Written informed consent was obtained from the parents/guardians of each child for the publication of this report and the images contained within it.

Grant information
The author(s) declared that no grants were involved in supporting this work.
Acknowledgments
We sincerely thank Prof. Tetsuya Matsumoto, MD, PhD, Head, Dept. of Infectious Disease, School of Medicine, International University of Health and Welfare (IUHW), Narita Campus, Japan for editing the manuscript and incorporating valuable suggestions. We thank Dr. Asadur Rahman, Dept. of Pharmacology, IUHW, for assisting in figure designing/artwork and editing some part of the manuscript. We also thank Januka Khatiwada, Dept. of Public Health, IUHW, for sorting out few technical issues with the SPSS-software. Special thank goes to the PMC-GH authority for allowing us to conduct study successfully. We remain indebted and thankful to all those parents/guardians who allowed their children to take part in the study without which this endeavor would have been in futile.

Author information
Kazi Selim Anwar is currently a Faculty at Department of Infectious Diseases, School of Medicine, International University of Health & Welfare (IUHW), Chiba, Japan.

Supplementary material
Supplementary File 1
STROBE checklist
Click here to access the data

References
1. WHO: A guide to Clinical management & public health response for Hand, Foot & Mouth Disease. Sec. 5: Clinical Features & case management. 2011; 19. Reference Source
2. Chang PC, Chen SC, Chen KT: The Current Status of the Disease Caused by Enterovirus 71 Infections: Epidemiology, Pathogenesis, Molecular Epidemiology, and Vaccine Development. Int J Environ Res Public Health. 2016; 13(9): pii: E890. Published Abstract | Publisher Full Text | Free Full Text
3. Ooi MH, Wong SC, Leithwaite P, et al.: Clinical features, diagnosis, and management of enterovirus 71. Lancet Neurol. 2010; 9(11): 1097–105. Published Abstract | Publisher Full Text
4. Aswathy NJ, Anurukumar G, Alidjnoou EK, et al.: Hand, foot and mouth disease (HFMD): emerging epidemiology and the need for a vaccine strategy. Med Microbiol Immunol. 2016; 205(5): 397–407. Published Abstract | Publisher Full Text
5. Wang P, Goggin WB, Chan EY: Hand, Foot and Mouth Disease in Hong Kong: A Time-Series Analysis on Its Relationship with Weather. PLoS One. 2016; 11(8): e0161006. Published Abstract | Publisher Full Text | Free Full Text
6. Andreoni AR, Colton AS: Coxsackievirus B5 associated with hand-foot-mouth disease in a healthy adult. JAAD Case Rep. 2017; 3(2): 165–68. Published Abstract | Publisher Full Text | Free Full Text
7. Stock I: [Hand, foot and mouth disease—more than a harmless “childhood disease”]. Med Monatschr Pharm. 2014; 37(1): 4–10; quiz 11–2. Published Abstract
8. Van Pham H, Hoang TNA, Duong HT, et al.: Clinical characteristics of hand, foot and mouth disease in Daklak Province, Vietnam and associated factors of severe cases. Virusdisease. 2017; 28(4): 430–433. Published Abstract | Publisher Full Text | Free Full Text
9. Hi YL, Rookij J, Ng N: Short term effects of weather on hand, foot and mouth disease. PLoS One. 2011; 6(5): e16796. Published Abstract | Publisher Full Text | Free Full Text
10. Liu Y, Wang X, Liu Y, et al.: Detecting spatial-temporal clusters of HFMD from 2007 to 2011 in Shandong Province, China. PLoS One. 2013; 8(5): e63447. Published Abstract | Publisher Full Text | Free Full Text
11. Ang LW, Koh BK, Chan KP, et al.: Epidemiology and control of hand, foot and mouth disease in Singapore. 2001-2007. Ann Acad Med Singapore. 2008; 37(2): 106–12. Published Abstract
12. Kar BR, Dibedi B, Kar SK: An outbreak of hand, foot and mouth disease in Bhubaneswar, Odisha, Indian Pediatr. 2013; 50(1): 139–42. Published Abstract | Publisher Full Text
13. Sharma N, Sarkar A, Mukherjee A, et al.: Epidemic of hand, foot and mouth disease in West Bengal, India in August, 2007: a multicentric study. Indian J Dermatol. 2009; 54(1): 26–30. PubMed Abstract | Publisher Full Text | Free Full Text
14. Sarma N, Chakraborty S, Dutta A, et al.: Hand, Foot and Mouth Disease in West Bengal, India: A Preliminary Report on Clinicoepidemiological Trend over 3 Successive Years (2013-2015), Indian J Dermatol. 2017; 62(5): 485–490. PubMed Abstract | Free Full Text
15. Li T, Yang Z, Liu X, et al.: Hand-foot-and-mouth disease epidemiological status and relationship with meteorological variables in Guangzhou, southern China, 2008-2012. Rev Rev Inst Med Trop Sao Paulo. 2014; 56(6): 533–539. PubMed Abstract | Publisher Full Text | Free Full Text
16. Venturina O, Bordon L, Silverberg N: Update on hand-foot-and-mouth disease. Clin Dermatol. 2015; 33(3): 340–46. PubMed Abstract | Publisher Full Text | Free Full Text
17. Chan JH, Law CK, Hamblin E, et al.: Best practices to prevent transmission and control outbreaks of hand, foot, and mouth disease in childcare facilities: a systemic review. Hong Kong Med J. 2017; 23(2): 177–90. PubMed Abstract | Publisher Full Text
18. Rajtar B, Majek M, Polataiski L, et al.: Enteroviruses in water environment—a potential threat to public health. Ann Agric Environ Med. 2008; 19(2): 199–203. PubMed Abstract
19. Nervi SJ, Bronze MS: Hand-Foot-and-Mouth Disease Workup. Medscape Drugs & Di. 2013; Updated: Jun 16, 2017. (Accessed on 09/02/2018). Reference Source
20. Cheng Q, Bai L, Zhang Y, et al.: Ambient temperature, humidity and hand, foot, and mouth disease: A systematic review and meta-analysis. Sci Total Environ. 2018; 625: 828–36. PubMed Abstract | Publisher Full Text
21. Kumar KB, Kiran AG, Kumar BU: Hand, foot and mouth disease in children: A clinico epidemiological study. Indian J Paediatr Dermatol. 2016; 17(1): 7–12. Publisher Full Text
22. Wolff K, Johnson RA, Saavedra AP, et al.: Fitzpatrick’s color Atlas and Synopsis of Clin Dermatol. 2nd ed. Mc Graw - Hil Medical. 2013: 653–655. Reference Source
23. Sarkar PK, Sarkar NK, Tayeb MA: Hand, Foot and Mouth Disease (HFMD): An Update. Bangladesh J Child Health. 2016; 40(2): 115–119. Publisher Full Text
24. Bian L, Wang Y, Yao X, et al.: Coxsackievirus A6: a new emerging pathogen causing hand, foot and mouth disease outbreaks worldwide. Expert Rev Anti Infect Ther. 2015; 13(9): 1061–71. PubMed Abstract | Publisher Full Text | Free Full Text
25. Hand-foot-and-mouth disease: Diagnosis and treatment. Mayo Clinic. (Accessed
26. Ruan F, Yang T, Ma H, et al.: Risk factors for hand, foot, and mouth disease and herpangina and the preventive effect of hand-washing. Pediatrics. 2011; 127(4): e808–904. PubMed Abstract | Publisher Full Text

27. Connell C, Tong HI, Wang Z, et al.: New approaches for enhanced detection of enteroviruses from Hawaiian environmental waters. PLoS One. 2012; 7(5): e32442. PubMed Abstract | Publisher Full Text | Free Full Text

28. Liao J, Yu S, Yang F, et al.: Short-Term Effects of Climatic Variables on Hand, Foot, and Mouth Disease in Mainland China, 2008–2013: A Multilevel Spatial Poisson Regression Model Accounting for Overdispersion. PLoS One. 2016; 11(1): e0147054. PubMed Abstract | Publisher Full Text | Free Full Text

29. Puenpa J, Chieochansin T, Linsuwanon P, et al.: Hand, foot, and mouth disease caused by coxsackievirus A6, Thailand, 2012. Emerg Infect Dis. 2013; 19(4): 641–643. PubMed Abstract | Publisher Full Text | Free Full Text

30. Bangladesh Population Census 2001: Bangladesh Bureau of Statistics: Cultural survey report of Pabna District 2007; Cultural survey report of upazilas of Pabna District. 2007. Reference Source

31. How are the income group thresholds determined? World Bank Data Help Desk. 2016. Reference Source

32. WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. Adopted by 64th WMA General Assembly, Fortaleza, Brazil. 2013. Reference Source

33. NATION: Firm working on vaccine to treat HFMD. 2010; (Accessed 25 April, 2018). Reference Source

34. Xing W, Liao Q, Viboud C, et al.: Hand, foot, and mouth disease in China, 2008-12: an epidemiological study. Lancet Infect Dis. 2014; 14(4): 308–18. PubMed Abstract | Publisher Full Text | Free Full Text

35. Cai Y, Liu Q, Huang X, et al.: Active immunization with a Coxsackievirus A16 experimental inactivated vaccine induces neutralizing antibodies and protects mice against lethal infection. Vaccine. 2013; 31(18): 2215–2221. PubMed Abstract | Publisher Full Text

36. Lin CW, Chang CY, Chen WL, et al.: Formulation and immunological evaluation of a trivalent vaccine comprising emulsified submicron particles and inactivated virions of H5N1/EV71/JEV. Hum Vaccin Immunother. 2013; 9(11): 2378–85. PubMed Abstract | Publisher Full Text | Free Full Text

37. Cai Y, Ku Z, Liu Q, et al.: A combination vaccine comprising of inactivated enterovirus 71 and coxsackievirus A16 elicits balanced protective immunity against both viruses. Vaccine. 2014; 32(21): 2406–2412. PubMed Abstract | Publisher Full Text

38. Kim EJ, Park HS, Yoon HS, et al.: Four cases of onychomadesis after hand-foot-mouth disease. Ann Dermatol. 2014; 26(6): 777–778. PubMed Abstract | Publisher Full Text | Free Full Text

39. Hossain Khan MA, Anwar KS, Muraduzzaman AKM, et al.: Dataset 1 in: Emerging Hand Foot Mouth Disease in Bangladeshi Children- First Report of Rapid Appraisal on Pocket Outbreak: Clinico-epidemiological Perspective Implicating Public Health Emergency. F1000Research. 2018. http://www.doi.org/10.5281/zenodo.10170.2211038
Open Peer Review

Current Peer Review Status: ? ✓

Version 3

Reviewer Report 08 July 2019

https://doi.org/10.5256/f1000research.21485.r50587

© 2019 Haque M. This is an open access peer review report distributed under the terms of the Creative Commons Attribution Licence, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Md. Azizul Haque
Department of Medicine, Rajshahi Medical College (RMC), Rajshahi, Bangladesh

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Infectious disease, toxicology and rheumatology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 11 Jul 2019

Kazi Selim Anwar, Institute of Epidemiology, Disease Control and Research, Dhaka, Bangladesh

I, on behalf of all authors cordially thank reviewer-2 for reviewing our on-line published paper so brilliantly.

We do extend all our best wishes for reviewing our paper with so much patience and upmost care with largely valuable comments and section/topic specific valuable suggestions being seriously careful towards adding values to improve the quality of our paper.

We cordially wish that any reviewer should possess such qualities, which we believe most reviewers do have those (except few).

We also recommend that the F1000Research should take up this reviewer (the 2nd one) in their reviewing panel if can not be taken in the editorial board right now.

We wish all the bests for our 2nd reviewer, per se.

Competing Interests: No competing interest declared!

---

Page 14 of 25
© 2019 Haque M. This is an open access peer review report distributed under the terms of the Creative Commons Attribution Licence, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Md. Azizul Haque
Department of Medicine, Rajshahi Medical College (RMC), Rajshahi, Bangladesh

This is the first reported outbreak of HFMD from Bangladesh. This article will add some valuable information about distribution and burden of HFMD. Following observations has been made regarding this manuscript:

1. In abstract (background): “We report a localized outbreak of childhood HFMD for the first time from Bangladesh, diagnosed only based on clinical features due to gross lack in laboratory-diagnostic facilities.” The word “gross” should be removed from this sentence.
2. In abstract (results): please clarify and rewrite this sentence “Age did not differ with sex (P=0.98)
3. In Introduction “Severe cases occur red with a low incidence (3.2% to 8.5%) and fatalities are rare”, the word “red” should be replaced by “rarely”
4. In results (demographic information), the sentence “Notably, children from mid-income-HHs contracted significantly more HFMD which was more among the first siblings (P<0.01)” should be rewritten as “HFMD cases were significantly more common among children from mid-income households and in the first siblings (p <0.001)
5. In results (assessment of symptoms) in the sentence “Similarly, papulo-vascular rashes were more common in fall…” papulovascular should be replaced with papulo-vesicular.
6. The sentence should be replaced by “The three major sign/symptoms of HFMD were more prevalent on days where 0.0 mm precipitation was recorded.”
7. In the same section “Time to recover from HFMD varies with age of the patient”. In the sentence “More young children (<5 years) recovered in <5 days (63/74, 85%) than older peers (≥5 years) (47/69, 69%) who were more likely to recover in >5 days) (P<0.05).”
8. In results (Clinico-epidemiological perspectives) section, the sentence “However, we neither observed such high incidences of sever disease alike from Vietnam (8.5%) nor recorded any fatal case in like others reporting as ‘none’ or ‘rare’ looks out of context and should be removed altogether or moved to complications section. If moved to the complications section, this sentence should also be rewritten as
   “In our series, we neither observed high incidence of severe disease, nor recorded any fatality.” Then comparison with data from other countries may be done.

1. While discussing severe disease and death, citation from Xing et al should be included, as his group published the largest epidemiologic study to date and showed the rate of severe disease and death in patients affected by HFMD (Xing W., Liao Q., Viboud C. Hand, foot, and mouth disease in China, 2008-12: an epidemiological study)
2. In results (Clinico-epidemiological perspectives) section, the sentence “Moderate-to-high fever, painful oral ulcer and itchy-painful rash were directly proportional to younger children which remain
consistent with other findings” should be rewritten as “Moderate-to-high fever, painful oral ulcer and itchy, painful rash were more common in younger children; this finding is consistent with other studies”.

3. In results (Clinico-epidemiological perspectives) section, the sentence “This observation led us to postulate variation in body temperature led us to postulate that fever itself should not be considered as the sole symptom to confirm HFMD, rather this remain consistent with Van Pham et al.8 though others reported high fever in HFMD-cases5,9,” is redundant and should be removed altogether. This is unnecessary repetition of a statement made in the same para (Moderate-to-high fever remains an important, but not mandatory or principal sign of HFMD).

4. In results (Clinico-epidemiological perspectives) section, citation is needed for the sentence “Literature reveals papulo-vesicular rash as the most important characteristic symptoms for HFMD often manifesting as painful chicken-pox-like rashes in 60% cases (Figure 3) though the rest 40% had it less painful or painless.”

5. In results (Clinico-epidemiological perspectives) section, the sentence “Since pain remains subjective in younger children in expressing pain intensity, we categorized HFMD cases based on having no history of recent pain issues, facial expression of child on slight touch on rash/oral ulcer including mother’s impression plus clinician’s rational judgement.” is redundant and should be removed altogether. Pain assessment/scoring tool has been described in detail in the Methods section.

6. In results (Clinico-epidemiological perspectives) section, the sentence “However, the exact reason in 22% less pain or painless oral ulcers/blisters remain unclear.” should be rewritten as “However, the exact reason of less pain/painless oral ulcers/blisters in 22% cases in our study remain unclear.”

7. In Laboratory Diagnosis for HFMD, the sentences “Laboratory diagnosis is usually not essential12,23 to confirm a readily diagnosed HFMD case based on rational judgement of existing clinical features. Even, lab diagnosis often remains unnecessary19” should be rewritten as “Laboratory diagnosis often remain unnecessary to make a diagnosis of HFMD. Use of WHO clinical case definition and exclusion of differential diagnosis is adequate in most cases.”

8. Reference number 12 may be omitted as authors of that article used RT PCR for virus isolation in 7 cases.

9. The para “Furthermore, raised blood glucose level may be due to other viral diseases rather than HFMD, and may confound by other infections and/or inflammatory processes. Moreover, drawing intravenous blood from younger children possessing thin veins may be extremely difficult if not possible particularly in primary care health centers in grass-root level. Children often exhibits agitation when attempts are made to draw blood showing grossly non-compliance and non-cooperative” is redundant and should be removed.

10. In complications, the sentence “Our finding yielded three cases (2.09%) complications of mild-to-moderate severity who we had to pay particular attention to” should be rewritten as “In our study, three cases (2.09%) developed complications of mild to moderate severity requiring special care”.

References
1. Xing W, Liao Q, Viboud C, Zhang J, Sun J, Wu J, Chang Z, Liu F, Fang V, Zheng Y, Cowling B, Varma J, Farrar J, Leung G, Yu H: Hand, foot, and mouth disease in China, 2008–12: an epidemiological study. The Lancet Infectious Diseases. 2014; 14 (4): 308-318 Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Yes
Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Infectious disease, toxicology and rheumatology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

---

Author Response 23 May 2019

Kazi Selim Anwar, Institute of Epidemiology, Disease Control and Research, Dhaka, Bangladesh

I, on behalf of all authors whole heartedly thank the reviewer for reviewing our online published paper with so much care and attention, yet so productively and meaningfully. We, the authors, remain totally satisfied on the way the reviewer looked into every bits of our paper & in so in-depth, yet so positively.

**Comment:** Yes we agree to all of the the well-chalked & thoughtful queries, along with all his suggested points to bring some minor changes in our paper, soon.

**Final comment for the Reviewer (only if the F1000Research allow me to do so, please):**
This is one of the best review I have had encountered ever so far (I am engaged in reviewing at least 6 globally reputed Scopus indexed journals having good impact factors).

May I, thus, recommend the F100Research group/authority to take this reviewer as one of its regular reviewers, like me, who would definitely add more significant values of International Board of Reviewers or Editorial Panel of F100Research. It would facilitate more wider scopes towards more standardized publishing, I believe, that the journal has been doing that always, of course!

Dr. Kazi Selim Anwar, in favour of all authors of this online published paper.

**Competing Interests:** No competing interests were disclosed.
I thank the authors for addressing a number of my comments.

I still think the discussion is too long and deals with a large of number of topics that are irrelevant to the data presented, being a clinically diagnosed first-time outbreak of HFMD in Bangladesh in a single hospital.

There is already a large body of available literature on HFMD. This manuscript adds the description of an outbreak or upsurge in Bangladesh. That is what needs to be described and interpreted / compared with other outbreaks. There is no need in this publication for the discussion section to provide a general review on HFMD or to provide extensive general recommendations on HFMD diagnosis, treatment and prevention.

The fourth paragraph of the discussion is an interpretation for which the authors present no data, this shouldn't be in a scientific publication. Sections on laboratory diagnosis, specific treatment and vaccination are not needed in this report. The list of principal findings should also be limited to findings from THIS study.

In the introduction, rephrase the sentence on "pollution free environment". HFMD prevention is through avoiding contact with infected persons and their secretions and excretions through hygiene/hand-washing and distancing (e.g. school closure or sending sick kids home).

Is the work clearly and accurately presented and does it cite the current literature? Yes

Is the study design appropriate and is the work technically sound? Yes

Are sufficient details of methods and analysis provided to allow replication by others? Yes

If applicable, is the statistical analysis and its interpretation appropriate? Yes

Are all the source data underlying the results available to ensure full reproducibility? Yes

Are the conclusions drawn adequately supported by the results? Yes
**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Clinical Microbiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

**Version 1**

Reviewer Report 02 October 2018

https://doi.org/10.5256/f1000research.16525.r38927

© 2018 van Doorn H. This is an open access peer review report distributed under the terms of the Creative Commons Attribution Licence, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

H Rogier van Doorn

Oxford University Clinical Research Unit, University of Oxford, Oxford, UK

The authors describe the results from a prospective observational study of children attending a single hospital in Bangladesh using WHO diagnostic criteria. If this is the first time HFMD has been described from Bangladesh, this is of major relevance locally and regionally. I recommend the authors to use the STROBE guidelines/checkbox to verify whether all required data are included.

The main findings are that a number of cases of HFMD among young children was described from one hospital in Bangladesh, the symptom and age distribution are relatively similar to what is known from the region. Healthcare workers should be aware of this illness, its prevention and treatment and warning signs of severe illness.

I have made some comments and suggestions below, the most important being to shorten and bring more focus on the current data in the discussion.

Specific comments:

- Add this sentence on the aetiology, to replace the sentence in the second paragraph of the introduction: "HFMD is caused by several serotypes of Enterovirus A, the most common being Enterovirus A71 (EV-A71) and Coxsackievirus A16 (CV-A16) and more recently also CV-A6 and CV-A10. EV-A71 is associated with a higher proportion of severe illness."
- Please add the exact case definition that was used to enrol children.
- 143 children were included, how many children were eligible during the period of enrolment? How many were not enrolled because of exclusion criteria or otherwise, how many didn't consent?
- Can an epidemiological curve be added?
- Any further information on cases in the region, nearby hospitals?
- Were any warning signs detected during the study?
- Because of the epidemiology of HFMD, the preferred age stratification would be 0-6, 6-12, 12-24, 24-60 and >60 months or similar (e.g. Xing et al)
● It is common to study the effects of precipitation allowing for a lag period of few days (incubation period)
● Reviews on the epidemiology, mortality and long-term outcome of HFMD have been published recently. These can be referenced in the discussion for clarity.
● The discussion deals with a broad spectrum of general topics. This is appropriate for a report to be circulated among local healthcare workers, but not for the current scientific publication. I would suggest to focus on the data from the current study for the discussion here, to broadly describe the findings and if there were any striking differences with what has been described from the region. The authors should not overinterpret the data from this relatively small sample size to look for potential associations.
● In the third paragraph of the discussion the authors state that outbreaks occurred in 1997 and 1998, despite forecasts. The referenced forecasts were derived from timeseries from Malaysia from 1998-2006 and could not have predicted the 1997-8 outbreaks. There are syndromic and serotype specific timeseries from Japan dating back to the 1980s that may have forecasted these, but to my knowledge no major HFMD outbreaks had occurred in Malaysia and Taiwan prior to these.
● In the laboratory diagnosis section, it is important to realise that diagnosis of EV-A71 as the main pathogen is important as it is associated with a higher proportion of severe illness.

References
1. Xing W, Liao Q, Viboud C, Zhang J, Sun J, Wu J, Chang Z, Liu F, Fang V, Zheng Y, Cowling B, Varma J, Farrar J, Leung G, Yu H: Hand, foot, and mouth disease in China, 2008–12: an epidemiological study. *The Lancet Infectious Diseases*. 2014; 14 (4): 308-318 [Publisher Full Text]

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Clinical Microbiology
I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 19 Oct 2018

Kazi Selim Anwar, Institute of Epidemiology, Disease Control and Research, Dhaka, Bangladesh

Comment specific response from the authors:
The authors thankfully appreciate the reviewer-1 for approving the paper with reservations and thank for the kind review and comments.

The followings remains the comment specific response from the authors:

Comment-1: The authors describe results from a prospective observational study of children attending a single hospital in Bangladesh using WHO diagnostic criteria. If this is the first time HFMD has been described from Bangladesh, this is of major relevance locally and regionally.

Reply-1: Yes, this observational study in our hospital remains the first report on HFMD from Bangladesh. We, therefore, thank you sincerely, for such an important comment…. “it remains of major relevance locally and regionally”.

Comment-2: I recommend the authors to use STROBE guideline/checkbox to verify if all required data included.

Reply-2: Yes, agreed. We have uploaded STROBE guideline to verify our data in revised version.

Comment-3: The main findings that a number of cases of HFMD in young children was described from one hospital in Bangladesh, symptom & age distribution are relatively similar to what is known from the region.

Reply-3: Yes, we are glad to see your valuable comment that HFMD symptoms in younger children remain relatively similar with that of other reports from South/SE- Asian region.

Comment-4: Healthcare workers should be aware of this illness, its prevention and treatment and warning signs of severe illness.

Reply-4: Yes, we appreciate your comment that healthcare workers must be aware of HFMD its prevention & treatment, particularly on warning signs of severe illness. Mentioned in 8th bullet point of “Insights on principal findings”.

Comment-5: I have made some comments and suggestions below, the most important being to shorten and bring more focus on the current data in the discussion.

Reply-5: Yes, agreeing to your most suggestion, shortened the discussion part focusing on current data—that really makes sense. Shortened discussion part as suggested.

Comment-6: Add this sentence on the aetiology, to replace the sentence in 2nd para-graph of introduction ….“HFMD is caused by several serotypes…. with a higher proportion of severe illness.”

Reply-6: We thankfully agreed to replace it in the 2nd paragraph of introduction.. HFMD is caused by several serotypes of enterovirus A, the most common being enterovirus A71 (EV A71) and coxsackievirus A16 (CV-A16) and more recently, also (CV A-6, and CV A-10). EV-A71 is associated with a higher proportion of severe illnesses *
**Comment-7:** Please add the exact case definition that was used to enroll children.

**Reply-7:** Though it is mentioned in Fig. 4, we have re-emphasized the WHO recommended exact case definition of HFMD: having i) fever or history of fever, ii) papulovesicular rash on hand & foot iii) with or without oral ulcers.

Added this case definition in 2nd line of method and in clinical diagnostic tool, as well.

**Comment 8:** … 143 children were included how many were eligible during enrolment period? How many were not enrolled because of exclusion criteria or otherwise, how many didn’t consent?

**Reply-8:** Since it was an outbreak situation, we had to enroll all 143 children attending our hospital from Sept. to Nov., 2017 with suspected HFMD cases (who met WHO criteria). Guardians of all children provided written consent to enroll.

**Comment 9:** Can an epidemiological curve be added?

**Reply-9:** Well yes, but we have described almost all epidemiological features in tables.

**Comment 10:** Any further information on cases in the region, nearby hospitals?

**Reply-10:** No. We explored to determine that among surrounding families, nurseries or kindergarten/primary schools, but none revealed any positive information.

**Comment 11:** Were any warning signs detected during the study?

**Reply-11:** No, not as such. Of the 3 complications that we observed, only girl had onychomadesis, 1 child had pneumonia and the other had pyoderma. These may well be regarded as ‘cautionary’, if not ‘warning’ signs.

**Comment 12:** Because of the epidemiology of HFMD, the preferred age stratification would be 0-6, 6-12, 12-24, 24-60 and >60 months or similar (Xing et al.).

**Reply-12:** Yes. But during that pocket outbreak our hospital team categorized the HFMD victimized children into two groups of <5 and >5 years only. Since 77% of them fell under <5 years it was further categorized into <3 years & 3.1 to <5 years. This age-stratification was done to fit aged-matched cases facilitating analysis.

**Reply 13:** It is common to study the effects of precipitation allowing for a lag period of few days (incubation period)

**Reply 13:** Yes. But we could not do that due to paying more attention in tackling/combating the on-going emergency of that pocket outbreak.

**Comment 14:** Reviews on the epidemiology, mortality and long-term outcome of HFMD have been published recently. These can be referenced in the discussion for clarity.

**Reply 14:** Well, yes. But we have described some of those in our discussion already.

**Comment 15:** The discussion deals with a broad spectrum of general topics. This is appropriate for a report to be circulated among local healthcare workers, but not for the current scientific publication.

I would suggest to focus on the data from the current study for the discussion here, to broadly describe the findings and if there were any striking differences with what has been described from the region. The authors should not overinterpret the data from this relatively small sample size to look for potential associations.

**Reply 15:** Thanks for the good suggestions. We have shortened the discussion part, focused on
our data from our current study and tried to describe the striking findings only that yielded some regional differences. And we also tried to avoid over-interpreting our data (relatively small sample size).

**Comment 16:** In the third paragraph of the discussion the authors state that outbreaks occurred in 1997 and 1998, despite forecasts. The referenced forecasts were derived from time series from Malaysia from 1998-2006 and could not have predicted the 1997-8 outbreaks. There are syndromic and serotype specific time series from Japan dating back to the 1980s that may have forecasted these, but to my knowledge no major HFMD outbreaks had occurred in Malaysia & Taiwan prior to these.

**Reply 16:** Thanks for pointing it out rightly. After cross checking on the contents of this sentence we have removed the following sentences 'Despite epidemiological forecasts that HFMD outbreaks occur in a 2–3-year cyclical pattern two large epidemics broke out in 2 consecutively years: one in Malaysia during 1997 and the other in Taiwan, the following year." Corrected this part as edited in the 2nd paragraph of ‘Potentials & dynamics of HFMD outbreak’.

**Comment 17:** In the laboratory diagnosis section, it is important to realise that diagnosis of EV-A71 as the main pathogen is important as it is associated with a higher proportion of severe illness.

**Reply 17:** Yes. Good point. We have added this point in lab diagnosis sect giving importance to diagnose EV-A71 as the main pathogen causing proportionately more severe cases of HFMD. It was reflected in 3rd paragraph of laboratory diagnosis, properly.

Finally the authors thank the reviewer-1 for his kind comments and suggestions once again.

**Competing Interests:** No competing interests were disclosed.

---

**Comments on this article**

**Version 2**

Author Response 05 Jun 2019

Kazi Selim Anwar, Institute of Epidemiology, Disease Control and Research, Dhaka, Bangladesh

REVISED Amendments from Version 2/Reviewer 2:

To comply with both reviewer’s suggestion a paper was added (Ref.#39: Xing W, Liao Q, Viboud C, Zhang J, Sun J, T Wu J, et al. Hand, foot and mouth disease in China, 2008-12: an epidemiological study. Lancet Infect Dis 2014; 14:308-18) and, some information/data was included to discuss.

The following minor errors were corrected in abstract, including few in introduction but mostly in results section:

- The word ‘gross’ was removed from the 1st paragraph in abstract, page-4.
- The 1st sentence on Page-9 (Notably, HFMD cases…. first siblings (p <0.001) was amended.
• The word ‘papulovascular’ is replaced with ‘papulovesicular’ (last sentence of 2nd para, page-10).
• Rephrased the 1st sentence of 2nd para (The three major sign… recorded at 0.0 mm), page-12.
• A sentence ‘Time to recover from HFMD varies with age of patient’ was added on page-12.
• The last sentence on page-14 (‘However, we neither observed… or, rare’) was removed.
• To comply with reviewer’s opinion changed the 2nd sentence of 1st paragraph, Page-15).
• Removed the last phrase of 1st para on page-5. “This observation led us to postulate…..others. reported high fever…” with a little modification keeping ref. 5, 8-9.
• Complying with reviewer’s comment, cited 4 references in 1st sentence of 2nd para, page-15.
• Removed the 2nd sentence of 2nd para on page-15 (“Since pain remains…. rational judgement)
• The 2nd sentence of 3rd par on p15 was altered a bit (However, the exact reason ….. remain unclear).
• The 1st sentence of 2nd para was replaced as suggested page-16 (discussion: Lab diagnosis).
• The 2nd paragraph of the manuscript on page-17 was taken out as commented.
• The 2nd sentence of 1st paragraph on page-18 was a bit changed following reviewer’s advice.

REVISED Amendments from Version 2/Reviewer 2: To comply with both reviewer’s suggestion a paper was added (Ref.#39: Xing W, Liao Q, Viboud C, Zhang J, Sun J, T Wu J, et al. Hand, foot and mouth disease in China, 2008-12: an epidemiological study. Lancet Infect Dis 2014; 14:308-18) and, some information/data was included to discuss. The following minor errors were corrected in abstract, including few in introduction but mostly in results section:
• The word ‘gross’ was removed from the 1st paragraph in abstract, page-4.
• The 1st sentence on Page-9 (Notably, HFMD cases…. first siblings (p <0.001) was amended.
• The word ‘papulovascular’ is replaced with ‘papulovesicular’ (last sentence of 2nd para, page-10).
• Rephrased the 1st sentence of 2nd para (The three major sign… recorded at 0.0 mm), page-12.
• A sentence ‘Time to recover from HFMD varies with age of patient’ was added on page-12.
• The last sentence on page-14 (‘However, we neither observed… or, rare’) was removed.
• To comply with reviewer’s opinion changed the 2nd sentence of 1st paragraph, Page-15).
• Removed the last phrase of 1st para on page-5. “This observation led us to postulate…..others. reported high fever…” with a little modification keeping ref. 5, 8-9.
• Complying with reviewer’s comment, cited 4 references in 1st sentence of 2nd para, page-15.
• Removed the 2nd sentence of 2nd para on page-15 (“Since pain remains…. rational judgement)
• The 2nd sentence of 3rd par on p15 was altered a bit (However, the exact reason ….. remain unclear).
• The 1st sentence of 2nd para was replaced as suggested page-16 (discussion: Lab diagnosis).
• The 2nd paragraph of the manuscript on page-17 was taken out as commented.
• The 2nd sentence of 1st paragraph on page-18 was a bit changed following reviewer’s advice.

Competing Interests: No competing interests were disclosed.
• You can publish traditional articles, null/negative results, case reports, data notes and more
• The peer review process is transparent and collaborative
• Your article is indexed in PubMed after passing peer review
• Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com