Journal of Infection and Public Health xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: http://www.elsevier.com/locate/jiph

Identification of measles virus genotype B3 associated with outbreaks in Islamabad, Pakistan, 2013–2015

Syed Sohail Zahoor Zaidi[a,b,*], Abdul Hameed[a,c], Muhammad Suleman Rana[b], Muhammad Masroor Alam[b], Massab Umair[b], Uzma Bashir Aamir[b], Maqbool Hussain[d], Salmaan Sharif[b], Shahzad Shaukat[b], Mehar Angez[b], Adnan Khurshid[b]

[a] Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan
[b] Department of Virology, National Institute of Health, Park Road, Chak Shahzad, Islamabad, Pakistan
[c] International Islamic University, Islamabad, Pakistan
[d] Pakistan Institute of Medical Sciences, Islamabad, Pakistan

ARTICLE INFO

Article history:
Received 28 March 2017
Received in revised form 2 October 2017
Accepted 12 October 2017

Keywords:
Measles virus
Epidemiology
Outbreak

ABSTRACT

Background: Measles virus infection remains a significant cause of childhood mortality and morbidity despite continued global efforts and the availability of a safe and effective vaccine. Molecular analysis of indigenous measles viruses could provide critical information on outbreak linkages and transmission pathways that can aid the implementation of appropriate control programs in Pakistan.
Methods: Blood samples and throat swabs were collected from subjects suspected with measles in Islamabad, Pakistan from 2013 to 2015. Serum samples were tested for the presence of measles immunoglobulin M (IgM) antibodies using enzyme-linked immunosorbent assay (ELISA) while throat swabs were used for the isolation (Vero/SLAM cell line) and subsequent characterization and phylogenetic analysis of measles strains.
Results: Of 373 blood samples, 66% tested positive for measles IgM. Male subjects were more often infected (58%) than female (42%) with the highest frequency of positive cases (63%) in the 0–5-years age group. Among the positive cases, only 13% had received one or two doses of the measles vaccine, while 87% were unvaccinated. Of 80 throat swabs, 29 (36%) showed a measles virus-specific cytopathic effect (CPE) and were characterized as genotype B3 through partial sequencing of the nucleoprotein (N) gene. Phylogenetic analysis revealed the Pakistani B3 strains to be closely related to strains from neighboring countries (Iran and Afghanistan) as well as with B3 viruses from the USA, Germany, and the UK.
Conclusions: The study results showed that despite the availability of an effective vaccine, the burden of measles infections is very high in Pakistan due to poor routine immunization coverage even in major cities, including the capital city of Islamabad. It is imperative that national health authorities take urgent strategic steps to improve routine immunization and implement adequate molecular identification methods to tackle future measles outbreaks.

Introduction

Measles virus (MV) is a member of the genus *Morbillivirus* and belongs to family Paramyxoviridae. It is an enveloped, non-segmented, and negative-sense single-stranded RNA virus with a genome size of 15,894 nucleotides (nt) that encodes six structural proteins [1–3] Although MV is serologically monotypic, antigenic and genetic analysis of the virus has defined eight clades (A-H) that are further segregated into 24 genotypes (A, B1-3, D1-11, E, F, G1-3, and H1-2) [4,5]. Molecular epidemiological studies of MV have established links to allow the detection of imported and indigenous cases and classification of suspected cases as caused by vaccine or wild-type strains.

Despite the World Health Organization (WHO)'s strategic plan for 2012–2020 and effective vaccination strategies that have resulted in up to 71% reduction in measles-associated mortality between 2000 and 2011 worldwide, MV remains the major cause of mortality and morbidity among children in developing countries, with an estimated 15,000 or more deaths globally each year [6]. The WHO Eastern Mediterranean Region has set 2020 as the target date for the elimination of measles from the region [7]. Despite success-

* Corresponding author at: Department of Virology, National Institute of Health, Park Road, Chak Shahzad, Islamabad, Pakistan.
E-mail address: sohailz@live.com (S.S.Z. Zaidi).

https://doi.org/10.1016/j.jiph.2017.10.011

Table 1
Characteristics of measles cases in Islamabad, Pakistan, 2013–2015.

Year	Total samples tested	Type of specimens		Male	Female	Vaccination status		
		Blood	Throat swabs			1 dose	Two doses	Unvaccinated
2013	286	286	62	169	117	22	15	249
2014	65	65	11	34	31	15	5	45
2015	22	22	7	13	9	4	2	16
Total	373	373	80	216	157	41	22	310

ful nationwide campaigns since 2013 that have covered over 63 million children and markedly improved the administration of a second vaccine dose at 15 months of age, Pakistan is still struggling to control measles transmission [8]. Furthermore, few comprehensive studies have described the molecular epidemiology of MV in Pakistan.

However, significant efforts have been made to strengthen these activities, including the implementation of case-based surveillance and laboratory analysis of suspected measles cases. The National Measles Laboratory (NML) was established with WHO support in 2005 at the Department of Virology of the National Institute of Health in Islamabad, which receives samples collected from suspected measles and rubella cases across Pakistan for diagnostic testing using serological and molecular methods [9].

Islamabad is the capital city of Pakistan and is believed to have high vaccination coverage compared with many other districts in the country; despite this, measles remains highly endemic [10,11]. Limited baseline molecular data in Pakistan are available on the circulating MV genotypes, which are crucial for the monitoring and elimination of MV. The NML of the Department of Virology of the National Institute of Health in Islamabad provides diagnostic services for serological and molecular testing (genotyping) of MV to the entire country.

This study described the epidemiological and molecular analysis of MV genotypes circulating is Islamabad during 2013–2015.

Materials and methods

Sample population

Serum (n = 373) and respiratory; i.e., throat swab (n = 80) samples were collected from subjects suspected with measles (fever, rash, cough, coryza, and conjunctivitis) admitted to the Pakistan Institute of Medical Sciences (PIMS) in Islamabad. All subjects between 2 months and 28 years of age were included and samples were collected after obtaining informed consent from the parents or patients as applicable. Patient history including epidemiological, clinical, and vaccination status data were noted on the measles case investigation forms. All collected sera and respiratory samples were transported to the NML while maintaining a cold chain.

Detection of anti-measles immunoglobulin M (IgM) antibodies

Anti-measles IgM antibodies from patient serum samples were detected using an IgM enzyme-linked immunosorbent assay (ELISA kit, SIEMENS Enzygnost Test, OWL115, Siemens health care diagnostics products GmbH Germany) according to the manufacturer instructions.

MV isolation

A total of 80 throat swab samples collected in viral transport medium was used for the isolation of MV in cell culture. Of these throat swabs, 62, 11, and seven were from 2013, 2014, and 2015, respectively. A sample volume of 300–500 μL was inoculated into 25 cm^2 tissue culture flasks containing Vero/SLAM cell monolayers with 70–80% confluence. The flasks were observed daily for cytopathic effect (CPE) for up to five days post-inoculation.

CPE-positive cell suspensions were also confirmed by one-step reverse transcription polymerase chain reaction (RT-PCR) and sequencing [12].

MV genotyping

The MV isolates (n = 29) were genotyped according to WHO standard protocols for sequencing and phylogenetic analysis. Briefly, 140 μL of sample volume was used to extract viral RNA using an RNA extraction kit (Qiagen GmBH, Hilden, Germany) according to the manufacturer's protocol, eluted in 60 μL buffer, and stored at −70° C until further analysis. For the amplification of the MV nucleoprotein (N) gene, primers MeV-216, 5′-TGGAGCTATGCCATGGGAGT-3′ and MeV-214 5′-TAACAATGATGGAGGGTAGG-3′ were used [11]. One-step RT-PCR was performed using a QIAgen one-step RT-PCR kit (QIAgen GmBH, Hilden, Germany) according to manufacturer instructions [12,13].

The amplified genomic fragments were sequenced on an ABI PRISM-3100 genetic analyzer (Applied Biosystems USA), using a Big dye terminator kit v3.1. The sequence reads were analyzed for closest matches using an online Basic Local Alignment Search Tool (BLAST) tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The nucleotide alignments were performed using CLUSTALW incorporated into MEGA v5.0. Phylogenetic analysis and trees were constructed by neighbor-joining methods and the relatedness of the strains was assessed by using 1000 bootstrapping replicates [14].

Results

Demographic and epidemiological findings

During the study period from January 2013 to December 2015, serum samples (n = 373) and throat swabs (n = 80) were collected from subjects with suspected measles at NML of the National Institute of Health in Islamabad, Pakistan. Of the 373 serum samples, 58% (216/373) and 42% (157/373) were from male and female subjects, respectively. The number of tested samples was highest in 2013 (77%; 286/373), followed by 2014 (17%; 65/373) and 2015 (6%; 22/373). Review of measles vaccination status showed that 11% (41/373) of subjects had received at least one dose of measles vaccine, 6% (22/373) had a history of two vaccine doses, and 83% (310/373) were unvaccinated (Table 1).

The majority (79%; 191/246) of suspected measles cases were received between March and July each year (Fig. 2).

From 2013-2015, 66% (246/373) of cases were confirmed by the detection of measles IgM antibodies. Of 246 positive cases, 58% (143/246) were from male and 42% (103/246) from female patients (Table 2). Based on the medical record available on the National immunization cards, 54% (22/41) of measles-positive cases had a history of having received at least one dose of measles vaccine, 41%(9/22) had received two vaccine doses, and 69% (215/310) IgM-positive cases were unvaccinated (Fig. 1).

Please cite this article in press as: Zaidi SSZ, et al. Identification of measles virus genotype B3 associated with outbreaks in Islamabad, Pakistan, 2013–2015. J Infect Public Health (2017), https://doi.org/10.1016/j.jiph.2017.10.011