Int.j.pathol.2022;20(4): 182-188

Original Article

Measles Vaccine Failure OR Emergence of New Virus strain: 2018 Measles Outbreak Peshawar, Pakistan

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ABSTRACT

Introduction: Measles vaccines are included in routine immunization programs started in 1978[4] but still, a large number of cases have been reported in the recent past across the country

Objective: The frequency and genotypes of measles Virus (MeV) in children vaccinated against Measles Virus (MeV) were investigated through PCR using gene-specific primers during the 2018 measles outbreak in Peshawar, Pakistan.

Methodology: Throat swabs and urine samples, as well as clinical and demographic data, were collected from 156 children with measles-like symptoms, admitted to different tertiary care hospitals in Peshawar.

Results: Most prevalent genotypes found were D3, G2, and B3.1. The distribution of MeV genotypes was statistically significant in unvaccinated children ($p<0.005$). Mixed genotypes (D3 and D7) were identified in 2.45% of children administered with single or double dose and 14.7% of the samples were not typed *viz* in Clade-D (7.4%), Clade- B (4.1%), and Clade- G (3.3%). The predominant genotype found in vaccinated and unvaccinated children was D3 followed by B3.1 and G2. Most of the children were found positive for MeV of age 1-5 years and the notable complications were severe pneumonia (5.6%) and diarrhea (26.8%). Furthermore, MeV and Rubella Virus (RV) coinfection was found in 6.4% of the total children, especially in unvaccinated children of the study.

Conclusion: It is concluded that MeV genotype D3 was prevalent in the study population, importantly in the vaccinated children. Moreover, a substantial number of un-typed samples seem the emergence of new variants.

Keywords: Measles, Rubella, Children, Vaccines, Genotypes, Co-infection.

Introduction

Measles outbreaks are reported in various countries and the death toll is about one million each year across the globe[1, 2]. Despite the measles and rubella strategic plan 2012-2020, Pakistan is still experiencing large measles outbreaks[3]. Although measles vaccines are included in routine immunization programs started in 1978[4] but still, a large number of cases have been reported in the recent past across the country [2, 5-7].

The generally administered vaccines include MCV (measles-containing vaccine), MR (measles rubella), and MMR (measles mumps rubella). MMR vaccines are in use in many countries to boost immunity against measles, mumps, and rubella[8] but still, cases are reported from those countries.

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In Pakistan, live attenuated (Edmonston strain) measles vaccine has been used since 1986 [9] and MMR vaccines are available in private sectors in Pakistan[10] but are not a routine practice due to their high cost.

Measles outbreaks are reported from New York, USA[11], Iran[12], India[13], China[14], and Afghanistan[15] and are linked to a lack of vaccination. It is shown that an average of 380 deaths per day was recorded in some of the previous measles epidemics in Asia, Africa, and Europe[16]. Recently, in the first quarter of 2018, measles cases were reported from the entire Khyber Pakhtunkhwa (KP) province of Pakistan but in the capital city Peshawar, the main epidemics were observed where measles-infected children were admitted in hospitals[17].

According to WHO recommendations, the genotypes from every outbreak should be determined[18]. The virus is classified into eight clades from A to H and about 24 different genotypes.[5, 13] In the present study, a non-sequencing method was used targeting the hyper variable region *i.e.* 450 C-terminal nucleotides of the Nucleoprotein gene to know the various clades

and genotypes in Peshawar the capital city of KP, Pakistan.

Material and Methods

The study was approved from Ethical Committee University of Peshawar.This study was conducted in Peshawar, KP Pakistan. A total of 156 suspected children included in this study were admitted to major hospitals of Peshawar, Khyber Teaching Hospital and Lady Reading Hospital. All the cases fulfilled the WHO clinical definition criteria for measles infection. As the core medical facilities are available in the capital city as compared to the rest of the areas in the province, the number of patients admitted to various hospitals in Peshawar is high.

Throat swab/urine samples were collected from the suspected measles patients and transported to the Laboratory of Virology and Immunology, Department of Zoology, University of Peshawar and stored at low temperature till further analysis. Data about the patient was also collected using an already-designed questionnaire after the patient/attendant's consent.

RNA was extracted from the throat swab and urine samples using a Trizol RNA isolation kit (ThermoFisher USA) according to the supplier's instructions. cDNA was synthesized in a total volume of 20µL reaction using 200U/uL of reverse transcriptase enzyme (RevertAid RTase-ThermoFisher USA) and Random hexamer primer at 42°C for 60 minutes. MeV was amplified through nested PCR using the protocol of Kremer *et al.*[18]. While RV was detected using the protocol of Bosma *et al.*[19]. The amplified product of MeV and RV was electrophoresed in 2% agarose gel, visualized under UV light using trans-illuminator, and compared with 100bp DNA ladder marker (ThermoFisher USA).

The amplified product was processed for clade-typing and genotyping through multiplex PCR using the protocol as described in Kremer *et al.*[20]. Specific primers were used to amplify Clade-B (genotype B3.1 and B3.2), Clade-D (genotype D2-D9), Clade-G (genotype G2 and G3), Clade-H (genotype H1 and H2) and detected according to their amplified product sizes.

The chi-square test was applied using SPSS V25 and a *P*-value less than 0.05 was considered statistically significant.

Results

The demographic and clinical characteristics of the children included in the study are given in Table-1. All the children were divided into three age groups *viz*. Group-I (<1 year), Group II (1-5 years), and Group III (> 5 years).

Table-1: General characteristics of the study population

Characteristics		N/Total (%)
Gender	Male	86/156 (55.1)
	Female	70/156 (44.9)
Complications	Pneumonia	68/156 (43.6)
	Diarrhea	32/156 (20.5)
	Others	56/156 (35.9)
Vaccination	2 doses	22 /156 (14.1)
	Single dose	38//156 (24.4)
	None	96/156 (61.5)
Sign & symptoms	Fever	156/156 (100.0)
	Rash	148/156 (94.9)
	Conjunctivitis	142/156 (91.0
	Coryza	148/156 (94.9)
	Cough	142/156 (91.0)

Complications of MeV positive, Rubella positive and Co infected (MeV+RV) positive children shown in Table-4.

Table-2: Gender-wise Prevalence

Infection	Total (N=156)		Male (N=86)		Female (N=70)		P- value
	N (%)	95% CI	N (%)	95% CI	N (%)	95% CI	
MeV+	112 (71.8)	0.6842-.4955	62 (72)	0.5527-0.9242	50 (71.4)	0.5302-0.9417	0.94
RV+	04 (2.6)	0.05900-11.229	02(2.3)	0.6842-1.4955	02 (2.9)	0.00346-0.10321	0.88
Co-infection+	10 (6.4)	0.02105-1.0197	02 (2.3)	0.00282-0.08401	08 (11.4)	0.0493-0.2252	0.10

Table-3: Age-wise distribution of vaccinated and unvaccinated children

Age group	MeV +				RV +		Co-infection +		
	Double dose	Single dose	Unvaccinated	Total	Unvaccinated	Total	Double dose	Unvaccinated	Total
I (54)	-	2	28	30	4	4	-	6	6
II (94)	8	14	52	74	-	-	1	3	4
III (8)	2	-	6	8	-	-	-	-	-
Total (156)	**10**	**16**	**86**	**112**	**4**	**4**	**1**	**9**	**10**

Table- 4: Complications in MeV, RV, and co-infected children N= Number of samples

Complications	MeV + N (%)	RV + N (%)	Co-infection + N (%)	Negative N (%)
Pneumonia	60 (53.6)	2 (50.0)	6 (60.0)	8
Diarrhea	30 (26.8)	2 (50.0)	-	12
Pneumonia +diarrhea	10 (8.9)	-	4 (40.0)	-
Chest infection	3 (2.7)	-	-	2
Vomiting, dysentery, and miscellaneous of above	9 (8.0)	-	-	8
Total	**112 (71.8)**	**4 (2.6)**	**10 (6.4)**	**30 (19.2)**

Among the 96 unvaccinated children for measles, 86 (76.8%) were positive for MeV. RV-infected children were also un-vaccinated and negative for MeV .None of the measles-vaccinated children were found positive for RV only. The result was significant in unvaccinated children ($P<0.05$)

Table-5: Prevalence of MeV and RV in vaccinated and non-vaccinated children

Vaccination status (N)	MeV + N (%)	RV + N (%)	MeV + RV N (%)
Two doses (22)	10 (45.5)	-	1 (4.5)
Single dose (38)	16 (42.1)	-	-
Unvaccinated(96)	86 (89.6)	4 (4.2)	9 (9.4)
Total (156)	**112 (71.8)**	**4 (2.6)**	**10 (6.4)**

All the positive samples were processed for genotyping. The most prevalent Clade was Clade-D (52.5%) followed by Clade-G (25.4%) and Clade-B (22.1%). All three Clades detected, were found prevalent in un-vaccinated children *i.e.* Clade D (N=48), Clade-G (N=26), and Clade-B (N=21). Among the Clade-D, Genotype D3 was more predominant (N=43), followed by D7 (N=5) and D8 (N=4). The mixed genotype of D3 +D7 was found only in three samples, while a few samples of Clade-D were not typed by the primers used (Figure-1). Among Clade-G, the prevalent genotype was G-2 (N= 27) while the remaining 4 were found un-typed. Likewise, in Clade-B the widespread genotype was B3.1 (N=22) and 5 remained un-typed (Table-6). The genotype distribution was significant in unvaccinated children ($P<0.05$).

Table-6. Distribution of measles clade-types and genotypes in vaccinated and unvaccinated children

Vaccination status	Clade-type N (%)			Genotype N (%)								
	B	D	G	B		D					G	
				B 3.1	NT	D3	D7	D8	Mixed (D3+D7)	NT	G2	NT
Two Doses (n=11/22)	2 (18.1)	6 (54.5)	3 (27.3)	2 (100.0)	-	3 (50.0)	-	1 (16.6)	1 (16.6)	1 (16.6)	3 (100.0)	-
Single dose (n=16/38)	4 (25.0)	10 (62.5)	2 (12.5)	2 (50.0)	2 (50.0)	5 (50.0)	1 (10.0)	-	2 (20.0)	2 (20.0)	2 (100.0)	-
Unvaccinated (n=95/96)	21 (22.1)	48 (50.5)	26 (27.4)	18 (86.0)	3 (14.3)	35 (73.0)	4 (8.3)	3 (6.2)	-	6 (12.5)	22 (85.0)	4 (15.4)

* NT= Not Typed

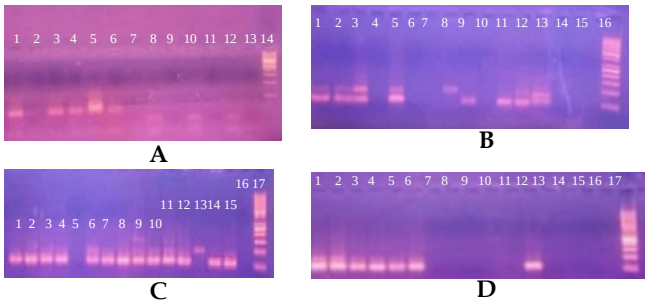

Figure 1. Agarose gel electrophoresis, the pattern of PCR products

A) L1,3-6 Positive for genotype B3.1 (84bp), L2, 7-13 negative, L-12 100bp ladder marker.

B) L1-2 positive for D7 (98bp), L3 coinfection of D3+D7 (151bp, 98bp), L5 coinfection of D3+D7 (151bp, 98bp), L8 positive for D3 (151bp), L9 positive for D7 (98bp), L11-12 positive for D7, L13 positive for D3+D7 (151bp, 98bp), L4,6-7,10,14,15 negative, L16 100bp ladder.

C) L1-4 positive for D3, L6-12 d3, lane 13 positive for D8, L14-15 positive for D3, L5, 16 negative, L17 100bp ladder.

D) Lane 1-6, 7 positives for G2, Lane 7-11, 13-16 negative, L17 100bp ladder.

Discussion

In this study males were more in number as compared to females. Different studies reported either male[21] or female[22] with high infection but MeV can infect both irrespective of gender[23]. Among the measles-positive children, rubella-infected children were also found. The immune system becomes weak because of MeV infection, providing room to other pathogens like RV which enhance the severity of the disease[24]. Co-infection of MeV and RV was also reported from India with similar complications and situations[1]. Measles can affect any age as the current study reports the highest prevalence (60.1%) in age group II (01 – 05 years) with no vaccine history except a few. Children of the same age range with MeV infection were previously reported from other parts of the country.[2,]

Infection in infants is reported all over the world and also found in the current study could be dreadful that is normally associated with weak immunity, insufficient maternal antibodies[25], or declined herd immunity[26] that makes the child vulnerable to infection. The gap between the disappearance of maternal antibodies and protection provided by immunization should be as small as possible in the case of all vaccine-preventable infections due to the chance of infection in the early stages of an infant's life[27]. Administering the first dose of the measles vaccine at the 6th month is found safe and effective in reducing infection in infants[28]. Due to the high incidence rate of measles in infants in China, it has been recommended there to revaccinate the women before pregnancy against MeV, this could increase the placental antibodies transfer and can help in reducing the infection rate in infants[29]. In highly endemic and epidemic conditions, the vaccine should be administered earlier than the recommended age[29].

The current study found pneumonia and diarrhea as major complications. Studies in other areas of Pakistan also illustrate the major complications in children infected with MeV as diarrhea and pneumonia[5, 24] and it is thought that it might be due to vitamin A deficiency or secondary bacterial/viral infection in response to immunosuppression[30,] .

Various factors are thought that contributed to measles outbreaks in Pakistan, noteworthy are lack of awareness, parent's education, their carelessness towards immunization, inaccessibility to the vaccination center, fear of side effects of the vaccine, low socioeconomic status, and poor interest of the government in vaccination program[5, 27, 31]. If a measles eradication campaign is started along with, polio immunization programs it will be of great worth[32]. Single-dose vaccine provides little immunity which declines with time and in persistent protection, booster dose is essential[33]. Inadequate protection after the first dose could be due to vaccine interference with maternal antibodies and reduced nutritional status of a child[1]. In remote areas, mishandling and interruption of vaccine cold chains may also provide an opportunity for infection. This little or no vaccine coverage could be the reason for the current measles outbreak. Various studies in Pakistan concluded that ignorance of the vaccine booster dose could be due to a lack of parent assurance on vaccination program[30].

Infection in vaccinated children contributes to the pool of current break and deems questions about vaccine storage and their transportation in the study area. Vaccines need proper temperature for transportation and storage, failure in either of these may decline the efficiency[34]. Some reports are available from other countries showing MeV RNA in vaccinated children where it was attributed to child poor health and vaccine failure due to improper storage or its poor quality[1]. Others linked it with vaccine failure due to the presence of maternal antibodies[35]. It should be explored whether any new serotype may emerge in the study area which declined the available vaccine. It might be investigated if a new type of MeV emerged in the study area.

Similar to our study other researchers[36] reported measles in both vaccinated and unvaccinated children

due to improper vaccination programs or may be due to failure of vaccination. A study showed that outbreaks in vaccinated populations were observed due to loss of antibodies titer from immunized people with time[37]. Some researchers revealed that the replacement and prevalence of MV strains depend on different factors *i.e.* the importation rate of various strains per year and vaccine coverage or it might occur by chance[38]. One of the studies from Pakistan reported B3, the prevalent genotype in the 2013-15 measles outbreak in Islamabad, Pakistan[37] but in the current study genotype, D3 is prevalent. It is demonstrated that most of the measles cases resurgence occurred due to the vaccination gap in different age groups[39]. Various countries faced MV outbreaks because of endemic measles genotypes like India and Nepal (D4, D8) and Indonesia (G2, G3)[40]. Different genotypes detected in our study which were not previously observed in any study in Pakistan might be imported from these neighboring countries where these strains were endemic or circulating in communities.

Some of the cases in the current study were found to have a combination of two genotypes; it seems that dual infection may not be covered through available vaccines. Moreover, a few samples were not typed although the clades were identified, indicating that variants are circulated in the study area which should be confirmed through sequencing as these un-typed genotypes may be linked with vaccine failure.

Patients were reported with signs/symptoms of measles but the negativity could be either due to a low viral load, longer duration between the rash onset and the collection of a specimen or due to the presence of pathogens other than MeV and RV causing the complications like pneumonia and diarrhea[43]. Negative children may have other infections as other viruses like parvovirus B19, human herpesvirus type6 (HHV6), enteroviruses, varicella, chikungunya, and dengue can also cause fever and maculopapular rash[27,29] they should further be investigated.

Conclusion

In conclusion, MeV was found prevalent in vaccinated children. D3 was found as the major genotype in all study populations.

Recommendations

Some of the genotypes were found un-typed therefore, it is recommended that sequence analysis of un-typed genotypes should be done to know if there is any other new variant emerged.

Moreover, the infection in vaccinated children raises many questions about the vaccine whether the failure was due to a cold chain factor or the emergence of a new strain.

Conflict of Interest: Authors declare no conflict of interest.

Funding: No funding was received for this project

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HISTORY	
Date received:	18-11-2022
Date sent for review:	10-12-2022
Date received reviewers comments:	15-12-2022
Date received revised manuscript:	20-12-2022
Date accepted:	20-12-2022

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