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Molecular Detection of Measles Virus from Febrile Rash Illness Cases in Lagos State, Nigeria

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ABSTRACT

Measles virus, an RNA virus of the genus *Morbillivirus* in the family *Paramyxoviridae,* is the etiological agent of measles disease, the fifth worldwide cause of death in children < 5 years of age. Despite the ongoing immunization progress in Nigeria, several sporadic cases and outbreaks of measles are still being reported annually, even among the immunized in the country. Continuous surveillance and early detection by laboratory diagnosis is of importance for early management of cases and prompt containment of community spread. Reverse Transcription Polymerase Chain Reaction (RT-PCR) testing was used for prompt diagnosis of all febrile rash illness (FRI) cases captured during routine disease surveillance activities in selected Health Facilities in Lagos State from 2016 to 2018. Whole blood or throat swab samples were collected and screened by RT-PCR from 140 consenting FRI patients accessing selected health facilities in Lagos State, Nigeria. Nine (6.4%) out of the 140 samples screened by RT-PCR were positive for Measles RNA. All the 9 measles positive cases were from children ages 1 – 5 years with females being more infected than males in ratio 3:1, although without any statistical significance (p= 0.7735). Out of eight Local Government Areas (LGAs) where FRI cases were sampled, only two of them (Eti-Osa and Lagos Mainland LGAs) account for the nine measles positive cases detected in this work. It is however a possibility that the number of LGAs with positive measles cases could have been more than two if all health facilities in the sampled LGAs were selected for the work, but within the limit of available resources, all health facilities could not be sampled.

Keywords: Measles virus; Reverse Transcription Polymerase Chain Reaction (RT-PCR); Febrile Rash Illness.

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INTRODUCTION

Molecular study is useful in the identification of measles virus (MV) [1], which is a single stranded, negative sense, non-segmented RNA virus belonging to the genus *Morbillivirus* in the family *Paramyxoviridae*. The detection of measles virus by reverse transcription polymerase chain reaction (RT-PCR) is prompt and diagnostic in clinical specimens. Laboratory confirmation is essential for all outbreaks and all sporadic measles cases. Clinical specimens are collected from suspected cases to aid in case confirmation as well as for genetic characterization of measles virus [1,2,3]. It is important to pay critical attention to the samples to ensure that the integrity of the sample is maintained, because the integrity of the viral RNA can be compromised at any point during sample collection, processing, storage, and transport [1]. Oral fluid (OF), throat swabs (TS), or nasopharyngeal (NP) aspirates or swabs are good sources of RNA to detect MV [4,5,6,7]. Urine samples are acceptable but may be more difficult to transport and may contain substances that are inhibitory to RT-PCR [7]. The likelihood of a successful RNA detection and genotype identification is enhanced when multiple clinical specimens are collected for a suspected case in a setting where measles elimination strategies are pursued [8]. Serum specimens (including eluate from dried blood spots) [7] have been utilized as a source of virus-specific RNA, particularly from sporadic cases or when routine virologic samples could not be obtained. However, successful detection of viral RNA in serum specimens requires that the serum is collected very close to rash onset (<3 days) [8]. Even when collected early, the low copy number of measles-specific viral RNA in serum makes successful RNA detection very unlikely and this approach is rarely attempted [7,8]. The most important factor for successful amplification and identification of measles RNA by any detection method is the timing of the sample collection [8]. The specimens should be collected as soon as possible after onset of rash as the sensitivity of detection decreases with time after onset of rash. The sensitivity of RNA detection from most types of specimens declines considerably after 5 days, although the possibility for successful RNA detection exists beyond this window, albeit at a much-reduced likelihood. Measles RNA detection has been reported from specimens collected >10 days after rash onset [8,9]. Measles virus detection rate has been enhanced with highly sensitive and specific multiplex RT-PCR measles detection kits, which also reduces or eliminates the tendency for cross reactivity with other viruses and demonstrated high reproducibility [9]. This will help to improve measles detection rate in developing nations like Nigeria and at the same time reduce or eliminate under reporting of cases due to missed detection in the laboratory.

The conventional RT-PCR for measles that amplifies a fragment to generate the N-450 sequencing window is used by some laboratories for case confirmation. Several sensitive conventional RT-PCR assays targeting the nucleoprotein (NP) or Haemagglutinins (H) genes for either detection and/or genotyping have been described with lower limits of detection in the range of 1,000-10,000 copies of measles viral RNA per reaction [10]. Real-time RT-PCR assays that target regions of the measles NP or H genes can typically detect as few as 10-100 copies of viral RNA per reaction and can provide quantitative results [9,11,12,13].

In real-time RT-PCR assays, result of the virus-specific RT-PCR determines the final outcome of the result of cellular-gene specific RT-PCR. For example, a clinical sample that is positive by both virus- and cellular- gene specific RT-PCR (or RT-qPCR) is considered positive, but samples that are negative by the virus-specific RT-PCR but positive by the cellular-gene specific RT-PCR, are considered negative. On the other hand, in multiplex assays, if the virus- specific RT-PCR is positive, it is acceptable for the cellular gene to have a negative result- this does not invalidate the positive virus-specific result when expected results are obtained for the positive and negative controls. However, if both the cellular-gene specific RT-PCR and the virus-specific RT-PCR have negative results, the quality of the RNA in the sample may have been compromised and the result is reported as undetermined (inconclusive). It is critical to carefully evaluate the data for each sample. The amplification curves must cross the set threshold for the virus-specific RT-PCR to be considered positive and visual inspection of the amplification with a sigmoid curve is suggested to verify positive result [9].

If MV is detected by RT-PCR, the viral isolate can be used for molecular epidemiology to distinguish between measles disease caused by a wild-type MV or by a measles vaccination derived strain [1,7,11,14]. RT-PCR is important for molecular epidemiologic surveillance to help determine: Source of the outbreak (viral strain), which viral strains are circulating, and whether these viral strains have become endemic in the country. Molecular identification of MV is prompt and of high significance, as it confirms measles disease. Molecular diagnosis is therefore critical to the early detection and management of measles.

In the last decade, cases of measles have been on the increase globally. In the years 2014 - 2018, the

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average number of measles cases reported by case-based surveillance was 68,299 cases in the African Region [15]. However, an increase in the number of cases was observed in 2019, when a total of 869,770 cases were reported with 207,500 deaths. Similarly, the United States of America that had declared elimination of measles in year 2000 reported 1,300 cases in 2019 [16]. The upsurge in the number of cases detected by measles case-based system were as a result of outbreaks that took place in three main countries, namely: Madagascar, Democratic Republic of Congo and Nigeria, who accounted for 84% of the entire reported cases for the African Region [16].

Therefore, this study was aimed to confirm the diagnosis of Measles from Febrile Rash Illness (FRI) cases in Lagos State, Nigeria using Polymerase Chain Reaction Technique. This became imperative because the number of measles cases worldwide, and particularly in Africa has been rising, thus posing a serious public health problem globally. There is also a need to differentiate FRI caused by MV from other viral etiological agents**.**

MATERIALS AND METHODS

Study Design and Location

A cross sectional study design was utilized, targeting individuals with febrile rash illnesses who reported in selected health facilities (table 1) spread across the three senatorial districts in Lagos State, Nigeria. These individuals were captured during routine disease surveillance in health facilities between 2016 and 2018.

Sample Collection, Transportation and Processing

Blood or throat swab samples were collected from 2016 to 2018. During each time, samples were transported using triple level packaging and stored according to the procedure described by CDC [17,18]. Samples were processed immediately after collection, but in case they were not processed immediately, they were stored at -20°C. Each stored sample was tested for MV [19] within one week from time of collection.

Measles virus RNA extraction

Ribonucleic Acid (RNA) extraction was performed using method previously described by McMahon *et al.* [19], with the process conducted inside a biosafety cabinet class II.

RT-PCR for Measles virus

Reverse Transcription Polymerase Chain Reaction (RT-PCR) was performed using the extracted RNA with the cycling conditions for the 1st round PCR as described by McMahon *et al.* [19]**.** Nested PCR was performed on the 1st PCR product [19]. The nested fragment was then subjected to agarose electrophoresis as described by Lee *et al.* [20].

Measles virus primer sequence

REVERSE TRANSCRIPTION: **MN5** (5'-GCCATGGGAGTAGGAGTGGAAC-3')

1st ROUND PCR: **MN5** and **MN6** (5'-CTGGCGGCTGTGTGGACCTG-3'

NESTED PCR: **Nf1alt5** (5'-CGGGCAAGAGATGGTAAGGAGGTCAG-3') and **Nr7alt1g** (5'-AGGGTAGGCGGATGTTGTTCTGG-3')

FRAGMENT LENGTHS: 1st round PCR: **660bp 2nd round PCR: 526bp**

RESULTS

Summary of result

Table 1 shows the list of randomly selected health facilities where blood or throat swabs were collected from febrile rash illness patients. In Table 2, a total number of 140 samples (blood from 84 patients and throat swabs from 56 patients) were analyzed by RT-PCR. Of this total, 74 (52.9%) were from males while 66 (47.1%) were from females. Table 3 shows that the 140 samples were obtained from individuals aged < 1 year to > 50 years, of which only 9 (6.4%) of them were positive for MV. Table 4 reveals that, out of the 9 positive cases, a total of 2 (22.2%) and 7 (77.8) were from male and female respectively.

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Table 1: Distribution of PCR positive and negative measles among febrile rash illness cases by health facility/community across LGAs in Lagos State

S/No	Health Facility/Community	LGA	Total Sample	Total Positive for Measles by PCR	Total Negative for Measles by PCR
1	Ikate PHC/Gbame (PRY H/F)	Eti Osa	15	7	8
2	Ajeabo PHC (PRY H/F)	Mushin	5	0	5
3	Kajola PHC (PRY H/F)	Mushin	4	0	4
4	Palm Avenue PHC (PRY H/F)	Mushin	4	0	4
5	Regina Mundi (SEC H/F)	Mushin	2	0	2
6	Gen Hospital Lagos (SEC H/F)	Lagos Island	2	0	2
7	Massey Children Hospital (SEC H/F)	Lagos Island	9	0	9
8	Odumbaku PHC (PRY H/F)	Agege	10	0	10
9	Sango PHC (PRY H/F)	Agege	38	0	38
10	Orile Agege Gen. Hospital (SEC H/F)	Agege	3	0	3
11	Betta Hospital (SEC H/F)	Agege	4	0	4
12	Kwaka Uku PHC (PRY H/F)	Agege	5	0	5
13	Powerline PHC (PRY H/F)	Agege	4	0	4
14	Kajola PHC (PRY H/F)	Agege	4	0	4
15	Ajegunle Health Post (PRY H/F)	Agege	1	0	1
16	Ajao PHC (PRY H/F)	Oshodi-Isolo	14	0	14
17	Isaga PHC (PRY H/F)	Oshodi-Isolo	5	0	5
18	Ipaja PHC (PRY H/F)	Alimoso	2	0	2
19	Blessed El Rapha MCC (PRY H/F)	Alimoso	1	0	1
20	Kosofe (PRY)	Kosofe	1	0	1
21	Okobaba PHC/Okobaba (PRY H/F)	Lagos Mainland	7	2	5
	Total		**140**	**9**	**131**

Table 2: Gender distribution across blood and throat swab samples

Gender	Sample type (%)		Total sample (%)	Statistics
	Blood	Throat swab	Total	X^2=1.72
Male	42(56.76)	32(43.24)	74 (52.9)	df=4
Female	42(63.64)	24(36.36)	66 (47.1)	p=0.7735
Total	84	56	140	

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Table 3: Percentage of febrile rash illness samples and RT-PCR Positive Measles Cases across age groups

Age Group	Number of febrile rash illness Samples	%Febrile illness sample distribution among age groups	Number RT-PCR Positive Samples (%)	%Measles cases
<= 5	78	55.7	9 (6.4)	100.0
6-15	33	23.6	0 (0.0)	0.0
16-24	10	7.1	0 (0.0)	0.0
25-39	4	2.9	0 (0.0)	0.0
40-49	4	2.9	0 (0.0)	0.0
>=50	1	0.7	0 (0.0)	0.0
Unknown	10	7.1	0 (0.0)	0.0
Total	**140**	**100.0**	**9 (6.4)**	**100.0**

Table 4: Distribution of RT-PCR positive and negative measles samples across gender

Gender	Blood Sample	Throat Swabs	Total Samples	% Sample Distribution across Gender	Number of Measles Positive Cases across Gender	% Measles Cases	Number of Measles Negative Cases across Gender	% Negative Measles Cases
Male	42	32	74	52.9	2	22.2	72	54.9
Female	42	24	66	47.1	7	77.8	59	45.0
Total	**84**	**56**	**140**	**100.0**	**9**	**100.0**	**131**	**100.0**

LGAs recording Positive RT-PCR Measles Result

Table 5 shows the names of the LGAs where samples were collected, with only two (25.0%) out of the eight LGAs sampled accounting for all the nine RT-PCR positive cases. Additionally, Eti-Osa and Lagos Mainland LGAs only account for 7 (77.8%) and 2 (22.2%) of the RT-PCR positive cases respectively. On the other hand, Figure 1 shows the distribution of RT-PCR positive cases among Febrile Rash illness patients across the sampled LGAs in Lagos State.

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Table 5: Distribution of RT-PCR Positive and Negative Measles among Febrile Rash Illness (FRI) patients across LGAs in Lagos

LGA	Number of Samples	% Samples Distribution Across LGAs	Number Measles Positive Cases by RT-PCR	% Measles Positive among total FRI Cases	Number Measles negative Cases by RT-PCR	% Measles negative Cases
Eti Osa	15	10.7	7	77.8	8	6.1
Mushin	15	10.7	0	0.0	15	11.5
Oshodi-Isolo	19	13.6	0	0.0	19	14.5
Lagos Island	11	7.9	0	0.0	0	0.0
Agege	69	49.3	0	0.0	69	52.7
Alimoso	3	2.1	0	0.0	3	2.3
Lagos Mainland	7	5.0	2	22.2	5	3.8
Kosofe	1	0.7	0	0.0	1	0.7
Total	**140**	**100.0**	**9**	**100.0**	**131**	**100.0**

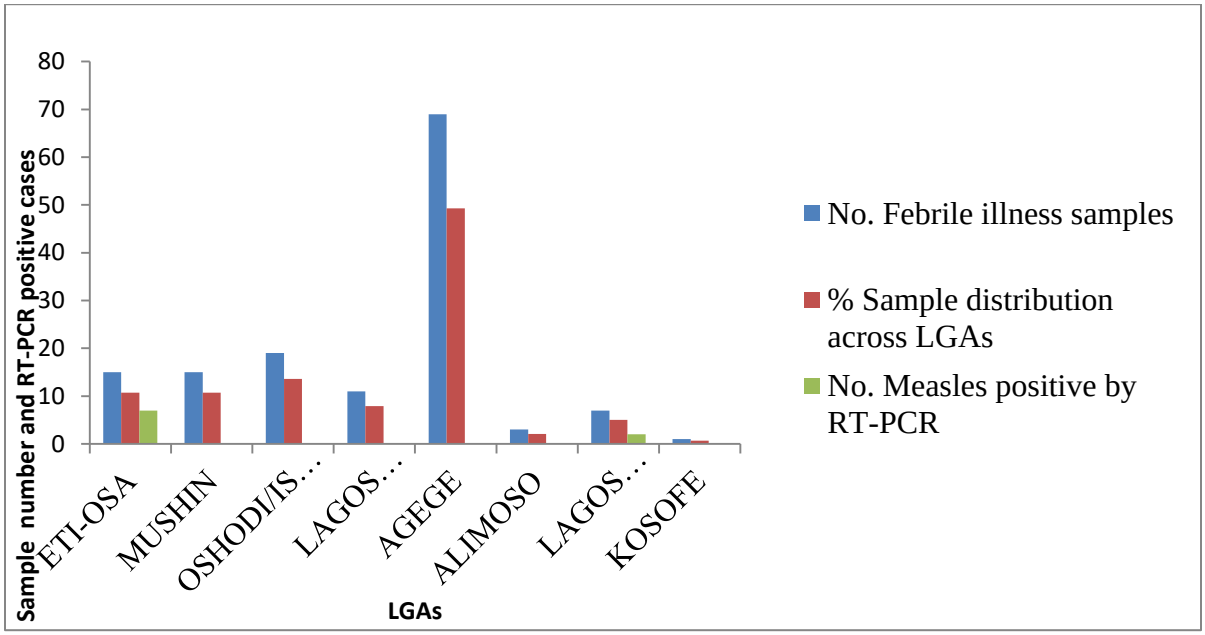

Figure 1: Distribution of RT-PCR Measles positive cases among Febrile Rash illness patients across LGAs in Lagos State

RT-PCR amplification result by electrophoresis
Figures 2 and 3 depict the RT-PCR amplification products separated by electrophoresis on 1.5% agarose gel containing batch 1 (7 Nos.) and batch 2 (2 Nos.) positive MV cases respectively with their characteristic bands at 526bp representing NP gene.

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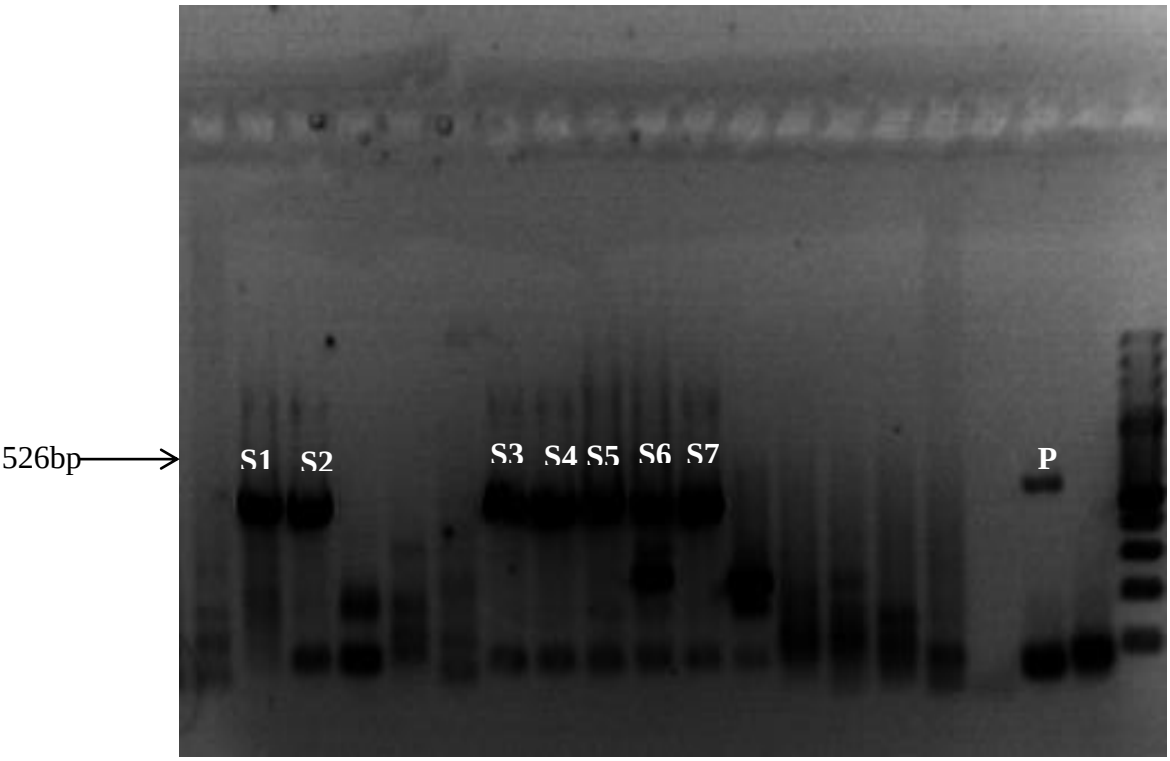
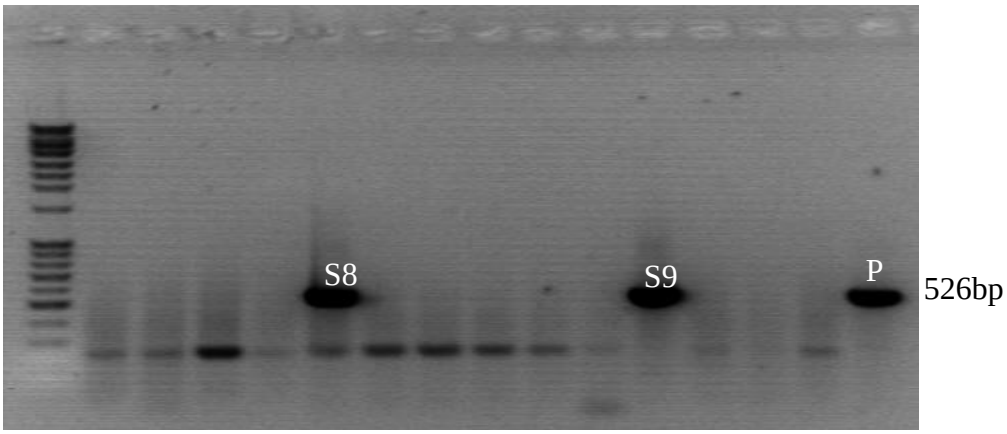


Figure 2: BATCH 1- RT-PCR amplification products separated by electrophoresis on 1.5% agarose gel with seven positive measles strains and the characteristic bands at 526bp representing NP gene from febrile rash illness cases in Lagos, Nigeria. P - Positive Control, S1 to S7 - PCR Positives (NP gene).



P -Positive Control, S8 to S9 - PCR Positives (NP gene)

Figure 3: BATCH 2- RT-PCR amplification products separated by electrophoresis on 1.5% agarose gel with two positive measles strains and the characteristic bands at 526bp representing NP gene, from Febrile rash illness cases in Lagos, Nigeria.

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RT-PCR results by age group

Tables 6a and 6b show the age group distribution of RT-PCR positive measles cases across eight sampled LGAs, with majority (55.7%) of the sampled population within the age group 0 – 5 years old, followed by 6 – 15 years, 16 – 24 years and those with unknown ages. It also shows that all the nine RT-PCR positive samples (100.0%) were found within the age group 0-5 years.

Table 6a: Age Distribution of RT-PCR Positive Measles among Febrile Rash Illness Cases across LGAs in Lagos

Age Group	Eti Osa		Mushin		Oshodi-Isolo		Lagos Island	
	PCR +	PCR -	PCR +	PCR -	PCR +	PCR -	PCR +	PCR -
<= 5	7	8	0	4	0	7	0	2
6-15	0	0	0	2	0	7	0	6
16-24	0	0	0	5	0	2	0	2
25-39	0	0	0	0	0	0	0	0
40-49	0	0	0	0	0	2	0	0
>=50	0	0	0	0	0	1	0	0
Unknown	0	0	0	4	0	0	0	1
Total	**7**	**8**	**0**	**15**	**0**	**19**	**0**	**11**

Table 6b: Age distribution of RT-PCR positive measles among febrile rash illness cases across LGAs in Lagos

Age Group	Agege		Alimosho		Lagos Mainland		Kosofe	
	PCR +	PCR -	PCR +	PCR -	PCR +	PCR -	PCR +	PCR -
<= 5	0	43	0	1	2	4	0	0
6-15	0	14	0	2	0	1	0	1
16-24	0	1	0	0	0	0	0	0
25-39	0	4	0	0	0	0	0	0
40-49	0	2	0	0	0	0	0	0
>=50	0	0	0	0	0	0	0	0
Unknown	0	5	0	0	0	0	0	0
Total	**0**	**69**	**0**	**3**	**2**	**5**	**0**	**1**

RT-PCR positive rate by sample type

Table 7 depicts the fact that a higher number of 6 out of 56 (10.7%) throat swab samples were RT-PCR positive for MV while 3 out of 84 (3.6%) blood samples were RT-PCR positive for MV. Table 7 also shows that, overall positive rate of 6.4% was obtained from the total number of 140 samples. In addition, the table also explains that out of the nine RT-PCR positive cases, 6 (66.7%) and 3 (33.3%) were obtained from throat swab and blood respectively.

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Table 7: Distribution of RT-PCR Positive and Negative samples

Type of Sample	Number of Febrile Illness Samples	% Distribution of Febrile Rash Illness Samples	Number and percentage RT-PCR Positive Febrile Rash Illness Samples (%)	Number of RT-PCR confirmed Measles Cases (%)
Blood	84	60.0	3 (3.6)	3 (33.3)
Throat Swabs	56	40.0	6 (10.7)	6 (66.7)
Total	**140**	**100.0**	**9 (6.4)**	**9 (100.0)**

DISCUSSION

Measles remains a major cause of childhood mortality and morbidity in Nigeria, although measles case fatality has been on the decline over the years and laboratory confirmed cases have been considerably low. However, the detection rate of 6.4% of measles virus RNA in febrile rash illnesses, particularly among children aged 0 – 5 years, is still a major public health concern in Lagos State and Nigeria at large. The possible reasons for this could be as a result of immigration into Lagos of very many children from the neighbouring Countries and from the insecure Northern part of Nigeria who are likely not to have been vaccinated or a lot of unvaccinated children who are below five years but had missed their vaccination schedule at nine months to one year, which the national measles vaccination campaigns cover. The positive cases detected in this study is in concomitance with the standard set by World Health Organization (WHO), regarding the occurrence of minimum of three cases of measles virus in a location as an indication of an outbreak of the infection.

The detection rate of 6.4 % from this study was a little above the range of prevalent data of between 1.3 – 5.1 % available in the country by various authors [30,31,32,33,34,35]. The current rate is an indication of the growing trend in the incidence of measles in Lagos State, Nigeria, which calls for urgent attention.

The nine positive measles cases detected in this study is a confirmation that MV is still circulating within the Lagos enviroment and probably other states in Nigeria, which is corroborated by the report of the Lagos State Government on 22nd of February 2016 that measles was responsible for an outbreak of FRI in Southwestern Nigeria [21]. The outbreak began in January 2016 and resulted in the deaths of over 20 children in the Ikate, Lekki area of Eti-Osa LGA of Lagos State [21]. This was alluded to the facts that children from the affected communities in Lagos state had not been immunized against measles during the state's vaccination campaign [21]. Also, massive migration into Lagos state has made it difficult for the government to keep track of all communities in need of health services, leading to some of these communities being missed, overlooked and under-vaccinated [21]. Further reports confirmed that from 4 January to 23 September 2016, a total of 3,905 suspected cases of measles were reported in four conflict affected states in Nigeria: 846 in Borno, 2,510 in Yobe, 273 in Adamawa and 276 cases in Gombe state. Of these, 129 cases were laboratory confirmed [22]. Another outbreak responsible for 23 deaths and over 300 cases, was also reported on February 25, 2017 in Sokoto State, in the north of Nigeria [23,24,25].

The detection of measles cases in this study among the non-immunized under 5 years old population is a confirmation of the fact that measles virus is currently circulating, and this age group would remain vulnerable to measles if not protected with minimum doses of measles vaccine. This finding was corroborated in another study by Grais et al. [26], in outbreaks report in sub-

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Saharan Africa including Nigeria that half of all deaths in children less than five years of age were attributed to measles. It further highlighted that prompt recognition and response to measles outbreaks, in addition to appropriate case management, is critical to reducing measles morbidity and mortality and preventing further transmission of measles virus. In this study, the proportion of the unvaccinated among the confirmed measles cases was 88.89% with all of the cases being recorded among children less than 5 years of age. This finding is similar to the finding in another study by Masresha et al. [27], which put the median proportion of unvaccinated children by state, among the confirmed measles cases between 71% and 85% in the northern states for the period between 2012 and 2016, while it was 32.5% – 56% in the southern states. It further stated that the proportion of confirmed measles cases in the age group less than 5 years' ranges between 65% and 83% of the annual number of confirmed cases [27].

A further finding of this current study was that 11.11% of the total confirmed measles cases being recorded were among the immunized population. The likely reasons include the fact that there could be vaccine potency issue due to compromise in the vaccine cold chain system or if the available vaccine does not confer full protection against probable circulating wild strain that could be different from vaccine strain or the fact that booster vaccination is required, as one dose is not likely to have provided full protection or malnutrition issues, which could likely lead to poor seroconversion. The 11.11% confirmed cases arising from previously vaccinated in this study is similar to the finding by Baptiste *et al* [28], which confirmed that the percentage of confirmed measles cases among those vaccinated with one or two doses of measles vaccines was high in Nigeria. Specifically, previous findings confirm that the range could be as high as 10-15% in infants vaccinated at age nine months, and could even be higher as the case was in the finding by Faneye et al. [29], which put the percentage as 55.3% among the immunized. The findings in the current study could be an alert signal that further studies will be expedient.

Evidence from this study showed that the highest (55.7%) reported suspected cases of Measles were within children aged 0 – 5 years, with all laboratory confirmed cases still within the same age group. This supports the findings of other researchers that measles is a childhood disease, causing great fatality within the first 5 years of life [28,32,36]. The high prevalence in this category of children might be attributed to unsustained vaccination programs whose primary target were children aged less than one year [28], leaving other children greater than one year vulnerable to measles infection particularly if they missed the initial immunization schedule before year one. Therefore, immunization of children by nine month and before 5 years of age might be helpful and contribute to the reduction and possible eradication of measles in our environment.

Although, more females tested positive than males with a ratio of 3:1, every child whether male or female less than 5 years of age is still vulnerable as there was no significant gender difference among those who were infected [26]. Also, the detection of MV in only two (Eti-Osa and Lagos Mainland) LGAs in Lagos may not mean that other LGAs were totally free of the virus. There was no significant difference among the LGAs sampled and thus the improvement in the vaccination coverage and sustenance should not be targeted at Eti-Osa and Lagos Mainland alone but should cover all LGAs, particularly the hard-to-reach areas in Lagos State. This is necessary as most LGAs in the state are overpopulated due to uncontrolled influx of people from other states and neighbouring countries into Lagos State.

The observed detection of MV in this study also highlights that the acclaimed state and local immunization days (SIDs, LIDs) as well as supplementary immunization activities (SIA) in Lagos, needed to be heightened and monitored effectively for them to have major impact on the control and elimination of measles outbreak in the state. Kremer with his co-workers earlier observed in their study that the SIA have so far had no significant effect in the interruption of the circulation of measles virus in Lagos and Nigeria at large [37].

CONCLUSION

Despite the fact that measles is a vaccine preventable disease, it remains a public health concern particularly amongst children less than 5 years in Nigeria. One of the best means of providing prompt diagnosis for measles is the RT-PCR technique. Continuous surveillance and access to prompt diagnosis will limit the spread of the disease through proper management of cases and enabling other interventions such as mop up immunization where required. All these are important actions for the successful eradication of measles in Nigeria. Protecting the Nigerian children against measles will make a significant contribution to reducing child mortality, which is a key millennium development goal. The progress in reducing measles-related deaths in Nigeria is therefore a complementary success towards attaining the millennium development goal.

ABBREVIATIONS
EDTA: Ethylenediamine Tetraacetic acid
FRI: Febrile Rash Illness
H: Haemagglutinins
LGA: Local Government Area
LIDs: Local Immunization Days
MV: Measles Virus
NP: Nucleoprotein
OF: Oral Fluid
RNA: Ribonucleic acid
RT-PCR: Reverse Transcription Polymerase Chain Reaction
SIA: Supplementary Immunization Activities
SIDs: State Immunization Days
WHO: World Health Organization

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