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Overview of laboratory-confirmed measles cases in Ivory Coast between 2003 and 2022

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Virological surveillance of measles virus (MeV) is a key component of measles elimination program. The goals of this study were to describe the epidemiology of measles infection and to evaluate MeV genotype strains circulating in Cote d'Ivoire. ELISA tests and viral isolation were performed on biological samples from suspected measles patients. Viral RNA was also extracted either directly from clinical specimens or from cell culture lysate. Fragment containing the 450 nucleotides sequence encoding the 150 carboxy-terminal amino acids of the N protein (N450), was amplified by RT-PCR and sequenced by Sanger sequencing. Of 28,418 serum samples and 122 oral fluid samples tested by ELISA, 6507 (22.89%) and 80 (65.5%) were positive. Of 516 Viral RNA extracts used for genotyping RT-PCR; 82 were positive. N450 sequences were obtained for 79 strains. Phylogenetic analysis of these sequences showed that they all belonged to genotype B3. This study provides a baseline for molecular epidemiology of MeV in Cote d'Ivoire. The dynamic of several clusters of MeV highlights the importance of continuing MeV surveillance. These data help elucidate the burden of measles infection and will inform the vaccination strategic plan and document measles elimination in Cote d'Ivoire.

Key words: Measles epidemiology, genotyping, Cote d'Ivoire, Africa.

INTRODUCTION

Measles is a highly contagious viral infection caused by the measles virus (MeV), which remains a significant cause of death among young children and a leading cause of vaccine-preventable disease in childhood worldwide, despite the availability of a safe and effective vaccine (Rota et al., 2016). MeV is transmitted via the respiratory route, causing systemic disease (de Vries et al., 2012). The virus infects a primary target cell, leading to systemic spread and clinical signs appearing after 9-19 days. The prodromal stage begins with fever, malaise, cough, coryza, conjunctivitis, and Koplik's spots on the buccal mucosa. A maculopapular skin rash then develops, starting behind the ears and spreading to the face, trunk, and extremities. MeV infection is usually self-limiting due to immune system clearance of infected cells (Laksono et al., 2016).

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From 2000 to 2016, annual measles incidence decreased by 88%, from 145 cases per 1 million population to 18. However, incidence increased to 120 cases per million in 2019 and then decreased by 82% to 21 in 2020 and by 22% to 17 in 2021, with considerable regional variation (Minta, 2022). Measles remains common in many developing countries, particularly in Africa and Asia. Over 95% of measles deaths occur in low-income countries with weak health infrastructures (World Health Organization [WHO], 2019).

MeV is a negative-sense, single-stranded virus belonging to the Morbillivirus genus of the Paramyxoviridae family. Although other members of the genus infect various animal species, MeV exclusively infects humans and nonhuman primates. MeV is serologically monotypic, but genetic variation in the genome, especially in the hemagglutinin (H) and nucleoprotein (N) genes, can be observed using molecular epidemiologic techniques (WHO, 2001). MeV strains are currently classified into 8 clades (A-H) divided into 24 genotypes. Between 2003 and 2019, 16 genotypes were detected worldwide. However, in 2020 and 2021, only three genotypes (B3, D4, and D8) were detected (Anonymous, 2012; Williams et al., 2022).

In 2012, the World Health Assembly endorsed the Global Vaccine Action Plan, with the objective of eliminating measles in five regions by 2020. Considerable efforts are being made in a growing number of countries to meet WHO measles control targets. African and South-East Asian regions have implemented the strategy of measles mortality control and elimination based on a set of activities. The first activity is to achieve and maintain high levels of population immunity by providing high vaccination coverage with two doses of measles- and rubella-containing vaccines through routine immunization activities and supplemental immunization activities (SIAs) to reduce the size of susceptible populations. To achieve immunity for interruption of measles transmission, a single or combined measles-rubella campaign must reach at least 95% of the target population (Moss and Griffin, 2006). The second activity is to monitor disease using effective surveillance and evaluate programmatic efforts to ensure progress.

Laboratory-based surveillance for measles, including confirmation of cases and genetic characterization of circulating measles strains, provides a valuable tool for measuring the effectiveness of measles control programs (Mulders et al., 2001; WHO, 2001). In the African region, 176,785 confirmed measles cases were reported through case-based surveillance from 2013 to 2016 (Masresha et al., 2017). The number of confirmed measles cases declined 60%, from 71,529 in 2013 to 28,279 in 2016. During 2013 to 2016, 60% of reported measles cases occurred among children aged 9-59 months. Recently, Sub Saharan Africa faces a spike of Measles where a total of 17 500 cases of measles have been reported in the region as of January 2022, signifying an increase of 400% compared to cases reported in 2021 (WHO Regional Office for Africa, 2023). Measles genotype data from only a few countries were published during the last decade. A study of measles resurgence in southern Africa in 2009-2011 showed that measles genotype B3 was predominant, and was found in seven countries: Botswana, eSwatini, Lesotho, Malawi, Namibia, South Africa and Zimbabwe (Shibeshi et al., 2014). Measles genotype B2 was found in Namibia, genotype D4 in Botswana, and genotype D8 in South Africa (Shibeshi et al., 2014). A report on measles elimination progress in the African region showed that during 2013-2016, 249 MeV genotype results were reported to WHO from 14 (30%) countries and all were genotype B3 (Masresha et al., 2017). Genotype B3 was also found in Senegal in 2004-2013 (Dia et al., 2015) and more recently in Gabon in 2017 (Lekana-Douki et al., 2019).

In Cote d'Ivoire, measles vaccine was introduced in the national immunization program in 1990. However, MeV continues to circulate, and the number of measles cases is persistently high and constantly increasing. Measles vaccine is administered to infants in the form of a single dose at 9 months of age. It is a live attenuated vaccine that should induce active immunity in 85 to 90% of susceptible subjects. Regular follow-up vaccination campaigns are carried out every 3 to 4 years, targeting children born since the last campaign, providing a second opportunity for measles vaccination to those who missed the first dose or who did not develop an immune response after the first vaccination.

Despite efforts to improve immunization coverage, vaccine coverage remained low, with rates below 50% in some years (DCPEV, 2011). Case-based surveillance of measles was implemented in Cote d'Ivoire in 2005 (Aka et al., 2017).

Since then, samples have been collected from measles suspected cases so that suspected cases of measles can be confirmed every year. Very limited information is currently available on measles genotypes in Cote d'Ivoire. The goals of this study were to describe the epidemiology of measles infection and to evaluate the genotype MeV strains that exist in Cote d'Ivoire from 2003 to 2022.

MATERIALS AND METHODS

Sample collection

Blood samples have been collected over the last two decades (2003 to 2022) from patients who met the measles case definition, using the measles surveillance guidelines from the WHO Regional Office Africa (WHO/AFRO) as part of Cote d'Ivoire national measles surveillance program. A suspected measles case was defined as any person presenting a generalized rash and fever of >38ºC, and cough, coryza, or conjunctivitis, or a patient in whom a clinician suspected measles. Approximately 5 mL of blood was drawn by venipuncture into a sterile anticoagulant-free tube. The samples were collected within 30 days of rash onset from suspected cases. Samples were either directly shipped to the measles National

Reference Laboratory (NRL) at the Institute Pasteur of Cote d'Ivoire, or after the blood sample was first centrifuged, the sera were aliquoted and stored at +4ºC until shipment to the NRL for testing. In addition to blood, throat swabs were taken during outbreak investigation. Oral fluids were also collected in parallel to serum sample in five districts using the Oracol collection device (Malvern Medical Development, Malvern, UK) following the manufacturer's instructions as part of a study to evaluate the use of oral fluid as an alternative biologic specimen for laboratory diagnosis of measles and rubella (Hutse et al., 2010; Rota et al., 2011a).

Serological diagnostics

Measles-specific immunoglobulin M (IgM) was detected by ELISA in serum samples using two different kits, Dade Behring Enzygnost Anti-Measles-Virus/IgM Kit (SIEMENS, Erlangen, Germany) and EUROIMMUN Anti Measles Virus ELISA (IgM). Serological tests were performed in oral fluid samples using the MicroImmune anti-IgM antibody capture EIA kit (MicroImmune, Brentford, United Kingdom). The three assays were performed according to the manufacturer's instructions. IgM positive samples screened from patients confirmed that there is measles.

Isolation of MeV in tissue culture

MeV isolation was performed by inoculation of throat swab samples on Vero/hSLAM cells in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2% fetal bovine serum per the Laboratory-based Surveillance of Measles, Rubella and Congenital Rubella Syndrome laboratory manual (World Health Organization Regional Office for Africa, 2007).

Cytopathic effect (CPE) was observed within 2 to 7 days. Cells were harvested when the CPE was at a maximum and the isolated viruses were stored at -80ºC.

Molecular analysis

Viral RNA was extracted either directly from clinical specimens (oral fluids and throat swabs) or from tissue culture lysat using QIAamp viral RNA mini kit (Qiagen, Courtaboeuf, France) in accordance with the manufacturer's protocol. For MeV genotyping, the 3'terminal region of the nucleoprotein (N) gene was amplified by reverse transcription PCR (RT-PCR) using the Qiagen One-Step RT-PCR kit (Qiagen). The following primers, MeV216 forward (5'-TGGAGCTATGCCATGGGAGT-3') and MeV214 reverse (5'-TAACAATGATGGAGGGTAGG-3') (Costales et al., 2022), were used to amplify a 634bp fragment containing the N-450 sequencing window required for phylogenetic analyses (Costales et al., 2022).

Sequencing and phylogenetic analysis

The PCR products were separated by gel electrophoresis and visualized using a UV transilluminator. The PCR products from RT-PCR positive reactions were purified for sequencing using the Invitrogen Charge Switch kit (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. The purified PCR products were then sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The PCR sequencing products were cleaned with the Agencourt CleanSeq kit (Agencourt Bioscience Corporation, Beverly, Massachusetts, US). Sequencing was performed using an ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The sequences were processed, aligned, and analyzed phylogenetically using the Molecular Evolutionary Genetics Analysis (MEGA) software version 7 (Tamura et al., 2007). Phylogenetic trees were constructed by comparing measles sequences from Côte d'Ivoire to the WHO measles reference sequences and measles named strains available in the WHO measles sequence database (MeaNS) (Rota et al., 2011b), using the neighbor-joining algorithm. Measles sequences previously reported from Côte d'Ivoire (MVs/Divo.CIV/25.04/1 and MVs/Divo.CIV/25.04/3) were also included in the analysis. The sequences were submitted to the MeaNS database.

RESULTS

Serological analysis of measles cases

Between 2003 and 2022, a total of 28,418 serum samples were analyzed by ELISA for the presence of anti-measles virus (MeV) specific IgM. The results showed that 22.89% (6,507/28,418) of the samples were positive for measles IgM, while 76.30% (21,684/28,418) were negative, and 0.8% (227/28,418) was indeterminate (Table 1). In addition to serum samples, 122 oral fluid samples were tested for measles IgM detection by ELISA. Of these, 65.5% (80/122) were positive, 30.3% (37/122) were negative, and 4% (5/122) were equivocal.

Demographic parameters of measles cases

Among the patients with confirmed measles, 3490/6507 (53%) were males and 2995/6507 (46%) were females, for an M: F sex ratio of 1.16 (Table 2). Information on sex was unknown in 1% measles-positive cases. The distribution of laboratory-confirmed measles cases among age groups showed that children were the most frequently impacted group. The age group from 1–4 years old was the most affected, with 2745/6507 (42%) of measles positive cases, followed by the 5–9-year-old 1381/6507 (21%), the <1 year old age group 701/6507 (11%), by >15 years old 668/6507 (10%) and 10-14 496/6507 (8%) age groups (Table 2). Age was unknown for 39% of measles-positive cases.

Vaccine coverage and seasonality of measles infections

Vaccine coverage varied between the lowest rate of 49% in 2011 and the highest rate at 94% in 2018. Yearly distribution of measles cases showed that the highest positivity rate was in 2003 (68%) followed by 2005 (55%) and 2010 (50%). Measles cases were found every year; The annual incidence rate, taking into account population data from the World Bank for children aged 0 to 59 months, make it possible to distinguish three periods of high circulation of measles during the two decades of monitoring: 2003-2005, 2009-2012 and 2018-2022. The first period shows a peak in 2003 with an incidence rate

Table 1. Results of laboratory testing of serum samples from suspected measles cases, Cote d'Ivoire 2003-2022.

Year	Total samples tested for measles IgM	Measles IgM test results			Measles IgM positive (%)
		Positive	Negative	Indeterminate	
2003	631	426	204	1	68
2004	261	128	121	12	49
2005	208	115	80	13	55
2006	139	11	125	3	8
2007	285	4	276	5	1
2008	428	12	404	12	3
2009	421	174	228	19	41
2010	914	461	396	57	50
2011	785	330	419	36	42
2012	1007	115	873	19	11
2013	562	43	516	3	8
2014	619	44	569	6	7
2015	668	36	629	3	5
2016	1253	45	1202	6	4
2017	2028	151	1870	7	7
2018	2101	276	1810	15	13
2019	2690	315	2369	6	12
2020	2599	555	2044	0	27
2021	4833	1759	3073	1	36
2022	5986	1507	4476	3	25
Total	28418	6507	21684	227	23

Table 2. Demographic characteristics of study population in Cote d'Ivoire 2003-2022.

Variable	Categories	Total samples tested for measles IgM	Measles IgM test results			Measles IgM positive (%)
			Positive (%)	Negative	Indeterminate	
Sex	Male	15336	3490	11740	106	12
	Female	13030	2995	9914	121	11
	Unknown	52	22	30	0	0
	Total	28418	6507	21684	227	23
Age group (year)	< 1	2961	701	2255	6	11
	1-4	11658	2745	8847	67	42
	5- 9	6535	1381	5093	61	21
	10 -14	2698	496	2174	28	8
	≥ 15	2966	668	2262	36	10
	Unknown	1600	516	1053	29	8
	Total	28418	6507	21684	227	23

of 24 cases of measles per million. In the second epidemic period, the highest incidence rate is observed in 2010. This rate is 22 cases of measles per million. From 2018 to 2022, the highest incidence rate peaks at 64 measles cases per million in 2021 (Figure 1a). Monthly distribution of measles cases during the high circulation periods showed that measles cases were found all year round, with greater activity from January to March (Figure 1b).

Identification of measles virus genotypes

Between 2003 and 2022, RNA samples were used to amplify PCR fragments for genotyping. The RNA samples were extracted from various sources, including measles IgM-positive serum samples (45), IgM-positive oral fluid (OF) samples (346), throat swab samples (52), and cell culture lysate. RT-PCR genotyping was successful for 82 RNA samples, comprising 2 sera, 52

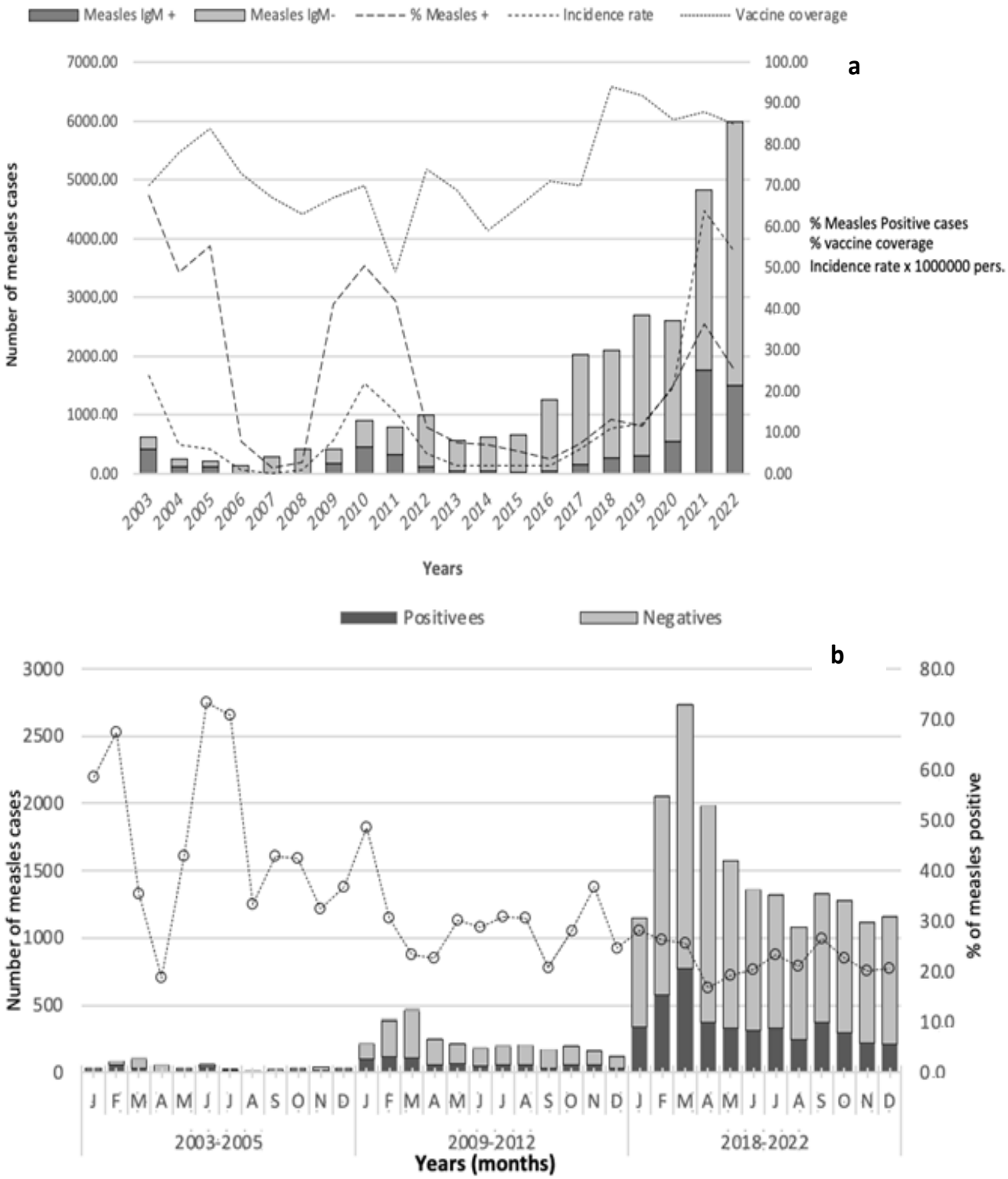

Figure 1. (a) Vaccine coverage and distribution of measles cases by year, Cote d'Ivoire 2003-2022. (b) Monthly distribution of measles cases during the high circulation periods (2003-2005; 2009-2012; 2018-2022) in Cote d'Ivoire.

OF, 25 throat swab, and 3 virus isolates. Measles virus (MeV) sequences were obtained for 79 samples. Comparison of the Côte d'Ivoire measles sequences with the WHO reference sequences revealed that all belonged

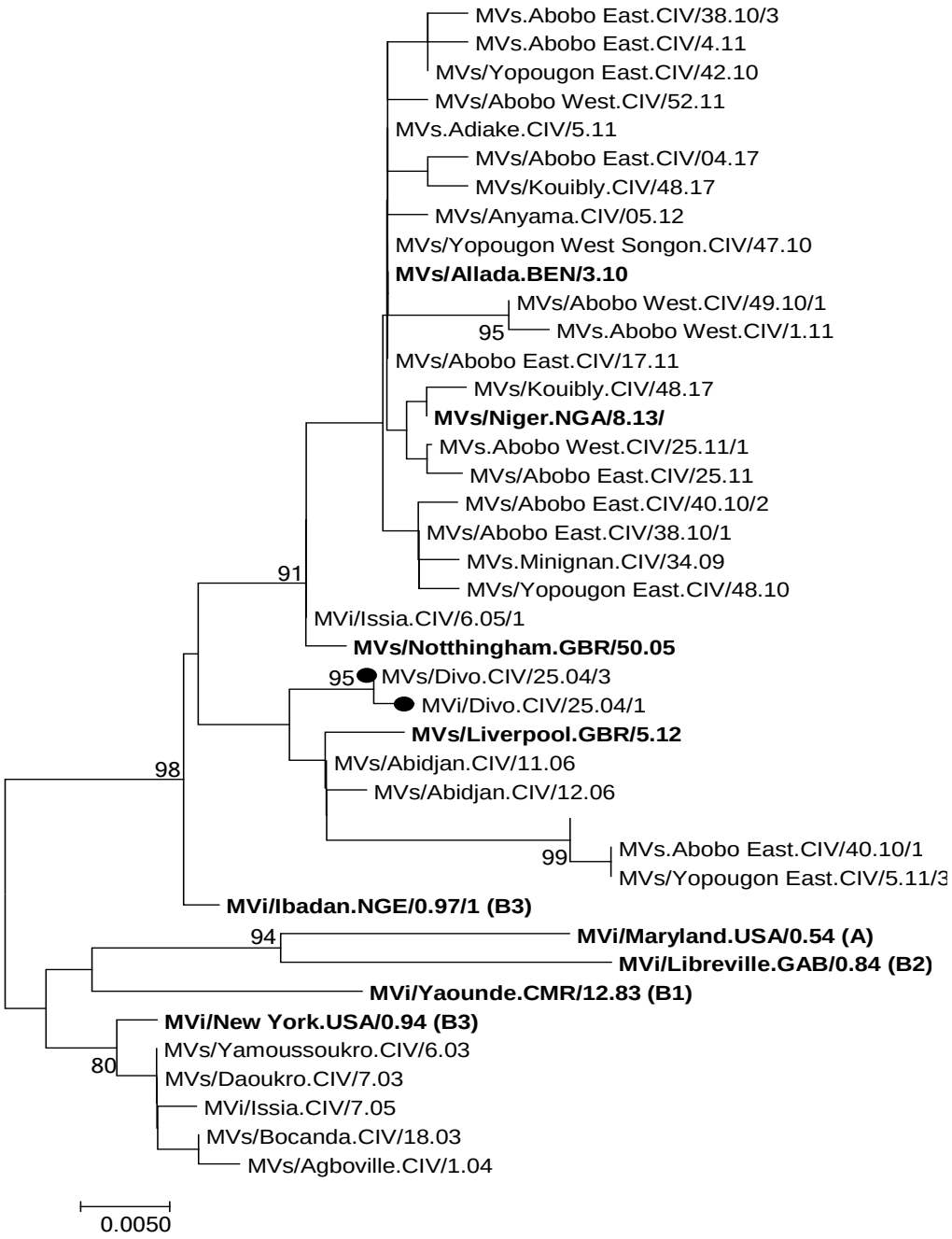

Figure 2. Phylogenetic analysis of Cote d'Ivoire measles strain based on N-450 sequences. Only 32 representative sequences of the 79 Cote d'Ivoire sequences obtained in this study were used to construct this phylogenetic tree. WHO reference sequences are indicated by genotype in parenthesis (in bold). WHO "named strains" are also indicated in bold. Bootstrap values (>80%) are indicated.

to genotype B3 (Figure 2). Phylogenetic tree analysis showed that 83.5% (66/79) of the sequences were closely related to the WHO reference strain MVi/Ibadan.NGE/0.97/1 (B3), while the remaining 16.5% (13/79) were closely related to the reference strain MVi/NewYork.USA/0.94 (B3). Comparison of the Côte d'Ivoire strains with the WHO "named strains" revealed close identity with MVs/Allada.BEN/3.10, Mvs/Niger.NGA/8.13, MVs/Notthingham.GBR/50.05, and MVs/Liverpool.GBR/5.12. Geographic distribution analysis showed that the majority (67%, 53/79) of the measles strains was found in Abidjan city, and 9.5%

(8/79) were found in Agboville city, both located in the southeastern part of the country. The remaining strains were mostly found in the central and western parts of the country. Temporal analysis of the circulating measles strains revealed that the measles strains found in 2003 clustered with the WHO reference strain MVi/NewYork.USA/0.94. However, during 2004-2005, there was a co-circulation of measles strains belonging to both clusters MVi/NewYork.USA/0.94 and MVi/Ibadan.NGE/0.97/1. The latter cluster became the dominant strain from 2006 to 2017.

DISCUSSION

This report presents information on the epidemiology of measles infections and genotypes of measles strains found in Cote d'Ivoire from 2003 to 2022. Despite the introduction of measles vaccine in Cote d'Ivoire 30 years ago, measles cases continue to be regularly reported and cause outbreaks. In Cote d'Ivoire, the positivity rate of measles infection was 23% based on serum sample ELISA testing, which is equivalent to the 24% (11,305/47,021) recently reported for the year 2020 in the African region (WHO, 2020). A similar positivity rate was reported in Senegal, where the measles positivity rate was 21.4% during 2004 to 2013 (Dia et al., 2015) and in the Central African Republic, with a 24% positivity rate (Lekana-Douki et al., 2019). This low positivity rate may be because these studies are based on laboratory-confirmed cases only and did not consider clinically suspected, nor epidemiologically linked cases. The low positivity rate also suggests that the case definition for measles surveillance is too sensitive and potentially insufficiently specific for measles, as many other diseases (such as rubella, Parvo virus B19, pediatric enteroviral infections) have the same clinical symptoms of rash and fever. However, over the 2 decades there have been periods of high circulation of measles viruses, with positivity rates ranging from 25 to 57%.

A higher positivity rate was found in OF samples (65.5%) compared to 23% in serum samples. Several studies have shown that sensitivity and specificity for detecting MeV-specific IgM in OF is almost equivalent to serum (Centers for Disease Control and Prevention (CDC), 2008; WHO, 2008). The high positivity rate found with OF samples in this study is due to the fact that OFs were only used to collect sample during investigation of measles outbreaks in order not only to make the diagnosis but also to search for circulating virus genotypes. There has been a considerable increase in the number of samples from suspected measles cases sent to the laboratory for confirmation since 2018. This situation is linked to the strengthening of surveillance at the national level through the Field Epidemiology Training Program (FETP) (Division of Global Health Protection CDC, 2016), launched in 2016, and the training of stakeholders following the catch-up campaign against measles and rubella.

Over these two decades, vaccination coverage had not reached the 95% threshold set as the goal of the elimination program (Masresha et al., 2017). The highest coverage reported in Cote d'Ivoire was 94% in 2018. This may explain the continued circulation of MeV in Cote d'Ivoire. Low measles vaccine coverage has been reported in other African countries such as the Central African Republic, where the highest coverage was 49% during 2007 to 2015 (Farra et al., 2019) and Senegal, where the highest measles vaccine coverage was 84% during 2004 to 2013 (Dia et al., 2015). Many other countries in the sub-Saharan region are far from the 95% anti-measles vaccine coverage goal, with at least two doses needed to achieve the level of immunity required to stop transmission of the virus in the population as recommended by WHO (2012). In addition, many countries, was affected by the COVID-19 pandemic, which resulted in a drop in vaccination coverage and a resurgence of measles epidemics (Aborode et al., 2021; WHO, 2023).

Measles vaccination strategies should be reinforced in Cote d'Ivoire to appropriately cover the unvaccinated population. Measles cases are detected throughout the year with an annual and monthly incidence that varies from year to year. However, during periods of high circulation, virus circulation was more marked in January, February and March, as shown by a study carried out in Senegal between 2010 and 2021 (Jallow et al., 2022). In Côte d'Ivoire, the period from January to March corresponds to the short dry season. Several other countries, such as Burkina Faso and Although countries such as Burkina Faso (Sahuguède et al., 1989) and the Central African Republic (Farra et al., 2019) have demonstrated a correlation between a high incidence of measles cases and the dry season.

Children between 1 and 4 years old were the most affected, accounting for 42% (2745/6507) of all confirmed measles cases. This is similar to the findings in the Central African Republic, where measles cases in the 1 to 4 year old group represented 43% of cases (Farra et al., 2019) but less than in the Democratic Republic of the Congo, where 60% of confirmed measles cases were in children less than 5 years old (Scobie et al., 2015). These results suggest that children from 1 to 4 years are most susceptible to measles infection. Supplementary immunization activities should be offered more often and aim to attain high coverage to reduce the number of susceptible children in this age group as well as among the whole population.

Measles genotype B3 was found in Cote d'Ivoire. This genotype is known to be endemic in sub-Saharan Africa. During this 2003–2022 study period, measles genotype B3 was reported in many other African countries, including Senegal in 2004 to 2013, 2010 to 2021 (Dia et al., 2015; Jallow et al., 2022), Namibia in 2009 to 2011

(Shibeshi et al., 2014), Cameroon in 2013 to 2016 (Mekanda et al., 2019; Ndombo et al., 2018) and Gabon in 2017 (Lekana-Douki et al., 2019).

Measles genotype B3 has also been found worldwide, including in Italy in 2011 to 2013 (Magurano et al., 2017), Taiwan in 2012 to 2014 (Cheng et al., 2016), and in India in 2011 to 2015 (Vaidya and Chowdhury, 2017). Sequence comparisons indicated that multiple clusters were found in Cote d'Ivoire, suggested that there were many sources of MeV causing outbreaks in the country.

Conclusion

The persistent circulation of measles in Côte d'Ivoire over the past two decades is a pressing concern, with periodic outbreaks linked to fluctuating and low vaccine coverage. This study's findings can help clarify the burden of measles infections in the country and inform Côte d'Ivoire's vaccination strategic plan. The predominance of genotype B3 suggests continuous regional transmission, highlighting the need to strengthen immunization programs and genomic surveillance. Enhancing these efforts is crucial to mitigating future outbreaks and ultimately eliminating measles in Côte d'Ivoire

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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