Molecular characterization of measles virus strains circulating in Cameroon during the 2013-2016 epidemics
Franck-Martin Obam Mekanda, Chavely Gwladys Monamele, Frédy Brice Simo Neng, Gilde Martial Yonga, Diane Ouapi, Véronique Penlap Beng, Christophe Batejat, Valerie Caro, Jean-Claude Manuguerra, Maurice Demanou

To cite this version:
Franck-Martin Obam Mekanda, Chavely Gwladys Monamele, Frédy Brice Simo Neng, Gilde Martial Yonga, Diane Ouapi, et al. Molecular characterization of measles virus strains circulating in Cameroon during the 2013-2016 epidemics. PLoS ONE, Public Library of Science, 2019, 14 (9), pp.e0222428. 10.1371/journal.pone.0222428 . pasteur-02921762

HAL Id: pasteur-02921762
https://hal-pasteur.archives-ouvertes.fr/pasteur-02921762
Submitted on 25 Aug 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution 4.0 International License
Molecular characterization of measles virus strains circulating in Cameroon during the 2013-2016 epidemics

Franck-Martin Obam Mekanda¹,², Chavely Gwaldys Monamele¹, Frédy Brice Simo Nemg¹, Gilde Martial Yonga¹, Diane Ouapi¹, Véronique Penlap Beng², Christophe Batéjat³, Valérie Caro³, Jean-Claude Manuguerra³, Maurice Demanou¹*¹

¹ WHO National Measles Reference Laboratory, Department of Virology, Centre Pasteur of Cameroon (CPC), Yaoundé, Centre, Cameroon, ² Faculty of Science, University of Yaoundé 1, Yaoundé, Centre, Cameroon, ³ Cellule d’Intervention Biologique d’Urgence (CIBU), Unité de Recherche et d’Expertise ‘Environnement et Risques Infectieux’ (ERI), Institut Pasteur, Paris, France

* demanou@pasteur-yaounde.org

Abstract

The first genotyping data on measles virus (MeV) strains in Cameroon dates from 1994, while other studies were realized in 2001 and 2011 with the establishment of MeV virological surveillance. However, the genetic data of MeV strains circulating in Cameroon remains fragmented and concentrated in certain regions, hence the need for an update. The objective of this study was to have recent data on MeV genotypes circulating in Cameroon. Ninety throat swabs collected during recent measles outbreaks were analyzed by MeV genotyping RT-PCR using the nucleoprotein gene N. The resulting sequences were analyzed on the basis of 450 nucleotides with MEGA 7 software. Overall genome analysis was performed on 40/90 sequences. The strains were from all ten regions and all belonged to cluster 1 of genotype B3. The genotype B3 has been circulating in Cameroon for long periods of time; efforts must be made in immunization for its elimination.

Introduction

Measles is a highly contagious and potentially fatal viral infection of the child and young adult caused by measles virus (MeV): a 15.9 kb enveloped, non-segmented, negative-stranded RNA virus belonging to the Paramyxoviridae family and the Morbillivirus genus [1]. This virus is transmitted from human to human via sputum and droplets from the respiratory tract [2].

Clinically a person is declared a suspect case of measles when he has a generalized maculopapular rash and fever, with cough and/or coryza, and/or conjunctivitis [3]. Once the disease progresses, the immunosuppression caused by measles virus (MeV) exposes the patient to bacterial super infections causing otitis media, pneumonia, or gastrointestinal infections. The main cause of measles-related morbidity and mortality is due to association of these symptoms with other factors such as malnutrition and vitamin A deficiency [4].
Before the introduction of measles immunization in the 1960s, almost everyone contracted measles usually during childhood with an estimated annual incidence of 130 million cases and an early mortality of 2.5 million each year. Since the introduction of the vaccine, the incidence of measles as well as the associated early mortality rate decreased. From 2000 to 2016, a 84% drop in mortality was noted from about 550,100 to 89,780 deaths, global annual reported incidence decreased by about 87% from 145 to 19 cases per million persons, estimates of the first dose of measles-containing vaccine (MCV1) coverage increased globally from 72% to 85% and the number of countries providing second dose of measles-containing vaccine (MCV2) nationally through routine services increased from 98 (51%) to 164 (85%) in which 24 African countries [5]. Despite this progress, epidemics have continued in some regions. In 2016, of the 89,780 deaths attributed to measles, 85% occurred in Africa and Asia [5].

To date, some countries where measles has been declared eliminated have confirmed new cases in recent years, such as, South Korea with 107 new cases, of which 04 imported in 2013 [6]. The WHO European Region which was already close to eliminating measles confirmed a total of 21,315 cases between 2016 and 2017 with 35 deaths [7]. Measles remains a global public health problem hence the need to strengthen immunization, surveillance and health monitoring.

The MeV is monotypic, but the genetic variability of the genes encoding the viral hemagglutinin (H) and the nucleoprotein (N) makes it possible to be genetically characterized in 8 clades (A-H) and 24 genotypes (A, B1-B3, C1-C2, D1-D11, E, F, G1-G3, H1-H2) [8]. Genotype B3 endemic in several African countries can be further divided into 3 clusters (B3.1, B3.2, and B3.3) [9].

As part of strategic plan for measles control and elimination, the World Health Organization (WHO) has recommended that member states implement virological surveillance for circulating MeV strains [10]. Molecular characterization of strains is a key tool in the study of measles transmission pathways, evaluation of endemic measles eradication and indirect evaluation of the vaccination system in place [11,12]. When high quality surveillance system associated with absence of endemic MeV transmission exist in certain region or defined geographic area for ≥12 months of period of time, measles can be declared eliminated in this area or region [13].

The latest genetic data on MeV strains circulating in Cameroon have been known since 1994 [14] and were updated in 2001 [15] and 2011 [16], however they remain fragmented and are relatively old. The aim of this study was to update the MeV strains circulating in Cameroon. These results will enable to establish a genetic basis of virological surveillance in Cameroon and evaluate the country’s efforts to eradicate this disease.

**Material and methods**

**Ethics statement**

This study was carried out as part of measles surveillance in Cameroon at Centre Pasteur of Cameroon (CPC), the National Reference Laboratory (NRL) for measles since 2011. There was thus no need for an ethical clearance. However, all participants read an information notice and gave a verbal consent before enrollment and sample collection.

**Clinical samples**

Ninety (90) throat swabs collected during the 2013, 2014, 2015 and 2016 measles outbreaks were collected by the surveillance team of the Expanded Program on Immunization (EPI) and transported to the NRL while respecting the cold chain. Once in the laboratory, samples were stored at -20 °C prior to analyses.
Laboratory methods

RNAs were extracted directly from the swabs using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions. RNA extracts were then analyzed by MeV genotyping RT-PCR in search of 634 nucleotides of the N-gene with forward primer MeV214 (5’-TAACAATGATGGAGGGTAGG-3’) and reverse primer MeV216 (5’-TGGAGCTATGCCAGGTAAGT-3’). The SuperScript™ III one-step system with Platinum™ Taq High Fidelity enzyme was used for amplification of the target gene fragment. The RT-PCR mixture was composed of 17 μl DNAse/RNase-free water, 25 μl of 2X buffer, 1 μl of MeV214 primer, 1 μl of MeV216 primer, 1 μl of the enzyme, and 5μl of the RNA extract. RT-PCR was programmed for 40 cycles including a reverse transcription step of 30 minutes at 50°C and 15 minutes at 95°C, a denaturation step of 30 seconds at 94°C, primers hybridization of 30 second at 55°C, an initial elongation of one minute at 72°C, a final elongation of 10 minutes at 72°C and storage at 4°C. The RT-PCR products were revealed by 1% agarose gel electrophoresis after 30 minutes migration for 250 volts. All RT-PCR positive products were sequenced using the MeV214 and MeV216 primers and the resulting sequences were edited and assembled into a single consensus using the CLC Mainworkbench 5.5 software.

Phylogenetic analyses

Phylogenetic analyses were performed on 450 nucleotides of the N gene according to WHO recommendations for measles virus strains genotyping [17]. All consensus sequences obtained from the CLC Mainworkbench 5.5 software were first aligned with the 26 reference sequences and subsequently by 11 other sequences of genotype B3 available in GenBank using the Clustal W algorithm in MEGA 7 software. The best fit-model of nucleotide substitution was selected in MEGA 7 and was found to be the Kimura two-parameter gamma model among 24 different nucleotide substitution models. Phylogenetic trees were generated with the Maximum likelihood algorithm using Kimura two-parameter distance model in MEGA 7. Evolutionary rates among sites were modeled using the Gamma distribution and the attribution of clades, genotypes and clusters was based on the clusters formed between the studied sequences and the reference sequences of known clades. All sequences obtained in this study were named according to the WHO recommendations and submitted to Measles Nucleotide Surveillance (MeaNS) and GenBank under accession number MH255950 to MH255989.

Results

Overall, 41 of 90 samples (45.55%) were RT-PCR positive for the genotyping assay and 40 of these sequences could be used for phylogenetic analyses. Table 1 summarizes the sociodemographic data of the subjects from which the sequenced samples were obtained. All strains were collected from 2013 through 2016: 08 in 2013, 20 in 2014, 05 in 2015 and 07 for 2016. The average age of the affected subjects was 8.6 years with extremes of 7 months and 20 years, the majority of viruses were obtained from children under 10 years of age with a predominance of those aged 7 months to 5 years (29/40). The male sex was the most predominant gender (27/40) and 08 (20%) patients had a history of vaccination against measles.

The sequences obtained came from all regions of Cameroon: 01 from Adamawa, 02 from the Center, 08 from the East, 07 from the Far North, 02 from the Littoral, 04 from the North, 03 from the North West, 04 from the South, 04 from the South West and 05 from the West (Fig 1, Table 1).

Phylogenetic analyses showed that all sequences obtained in this study clustered with the MVi/Ibadan.NGA/0.97/1 genotype B3 sequence with a bootstrap value greater than 90%
Fig 2). A phylogenetic tree of the sub-group to which the Cameroon strains belong is shown in Fig 3. All strains belonged to cluster B3.1 with a bootstrap value greater than 90%.

Discussion

Cameroon integrated measles surveillance in 1974 through sentinel sites in the city of Yaoundé [18]. The first genetic data on MeV date from 1994 with the identification of genotype B1

Table 1. Characteristics of patients in whom MeV strains were collected in Cameroon from 2013 to 2016.

| Genbank number | Strain name | Patient age (months) | Sex | Patient location (Region-District) | Last immunization date |
|----------------|-------------|----------------------|-----|-------------------------------------|------------------------|
| MH255950       | MVs/Olamze.CMR/2.13/ | 36 | M | South-Olamze |                         |
| MH255951       | MVs/Garoua Boulai.CMR/10.13/ | 12 | M | East-Garoua Boulai |                         |
| MH255952       | MVs/Loun.CMR/14.13/ | 72 | M | Littoral-Loun |                         |
| MH255953       | MVs/Cite des Palmiers.CMR/25.13/ | 10 | F | Littoral-Cite des Palmiers | 15/05/2013 |
| MH255954       | MVs/Olamze.CMR/5.13/ | 156 | M | South-Olamze |                         |
| MH255955       | MVs/Mifi.CMR/7.13/ | 72 | M | West-Mifi |                         |
| MH255956       | MVs/Foumbot.CMR/7.13/ | 12 | M | West-Foumbot |                         |
| MH255957       | MVs/Foumbot.CMR/6.13/ | 144 | F | West-Foumbot |                         |
| MH255958       | MVs/Maroua Rural.CMR/8.14/ | 12 | F | Far North-Maroua Rural |                         |
| MH255959       | MVs/Yoko.CMR/9.14/ | 36 | M | Centre-Yoko |                         |
| MH255960       | MVs/Mbouda.CMR/10.14/2/ | 36 | F | West-Mbouda |                         |
| MH255961       | MVs/Mbouda.CMR/10.14/ | 72 | F | West-Mbouda |                         |
| MH255962       | MVs/Bibemi.CMR/10.14/ | 36 | F | North-Bibemi |                         |
| MH255963       | MVs/Foundong.CMR/9.14/ | 48 | M | North West-Foundong | 14/09/2010 |
| MH255964       | MVs/Maroua Urbain.CMR/11.14/ | 36 | M | Far North-Maroua Urbain |                         |
| MH255965       | MVs/Maroua Urbain/11.14/2/ | 60 | F | Far North-Maroua Urbain |                         |
| MH255966       | MVs/Maroua Urbain.CMR/11.14/3/ | 60 | M | Far North-Maroua Urbain |                         |
| MH255967       | MVs/Kumba.CMR/26.14/ | 7 | M | South West-Kumba |                         |
| MH255968       | MVs/Betare Oya.CMR/38.14/ | 60 | F | East-Betare Oya |                         |
| MH255969       | MVs/Betare Oya.CMR/38.14/2/ | 12 | M | East-Betare Oya |                         |
| MH255970       | MVs/Betare Oya.CMR/38.14/3/ | 96 | F | East-Betare Oya |                         |
| MH255971       | MVs/Kumba.CMR/43.14/ | 12 | M | South West-Kumba |                         |
| MH255972       | MVs/Ngaoundere Rural.CMR/46.14/ | 168 | M | Adamawa-Ngaoundere Rural |                         |
| MH255973       | MVs/Touboro.CMR/46.14/ | 240 | M | North-Touboro |                         |
| MH255974       | MVs/Bertoua.CMR/51.14/ | 12 | M | East-Bertoua |                         |
| MH255975       | MVs/Nwa.CMR/3.14/ | 8 | M | North West-Nwa |                         |
| MH255976       | MVs/Nwa.CMR/3.14/2/ | 60 | M | North West-Nwa | 01/02/2009 |
| MH255977       | MVs/Garoua Boulai.CMR/8.14/ | 24 | F | East-Garoua Boulai |                         |
| MH255978       | MVs/Guidiguis.CMR/3.15/ | 36 | F | Far North-Guidiguis |                         |
| MH255979       | MVs/Guidiguis.CMR/3.15/2/ | 84 | M | Far North-Guidiguis |                         |
| MH255980       | MVs/Biyem Assi.CMR/8.15/ | 10 | M | Centre-Biyem Assi |                         |
| MH255981       | MVs/Yokadouma.CMR/8.15/ | 36 | F | East-Yokadouma | 21/10/2012 |
| MH255982       | MVs/Kumba.CMR/18.15/ | 36 | M | South West-Kumba | 01/11/2012 |
| MH255983       | MVs/Lagdo.CMR/10.16/ | 11 | M | North-Lagdo |                         |
| MH255984       | MVs/Lagdo.CMR/12.16/ | 12 | M | North-Lagdo |                         |
| MH255985       | MVs/Kumba.CMR/18.16/ | 24 | F | South West-Kumba | 02/02/2015 |
| MH255986       | MVs/Zoetele.CMR/38.16/2/ | 168 | M | South-Zoetele | 01/02/2003 |
| MH255987       | MVs/Zoetele.CMR/38.16/ | 36 | M | South-Zoetele | 01/12/2013 |
| MH255988       | MVs/Mora.CMR/45.16/ | 10 | M | Far North-Mora |                         |
| MH255989       | MVs/Bertoua.CMR/7.16/ | 108 | M | East-Bertoua |                         |

https://doi.org/10.1371/journal.pone.0222428.t001
strain Yaounde.CAE/12.83 "Y-14" in Yaoundé [14]. Virological surveillance of MeV strains in Cameroon was effectively implemented in 2011 at the National Measles Laboratory [16]. This study is the fourth to investigate MeV strains circulating in Cameroon [14–16] and the second from the virological surveillance program set up in 2011.
Fig 2. Phylogenetic tree of sequences studied with reference sequences. The studied sequences are in bold and represented by a black triangle, the reference sequences are demarcated with the genotype between hooks. Accession numbers and virus names are included on the tree. Phylogenetic analysis was performed based on 450 nucleotides of the N gene in MEGA version 7.0. Evolutionary history was inferred using the Maximum Likelihood method and distances were computed using the Kimura two-parameter model. The bootstrap test was set to 1000 replicates and bootstrap values above 70% are shown next to the main tree branches.
Fig 3. Sub genotyping phylogenetic tree. The studied sequences are represented by a black triangle. The tree was rooted with a sequence belonging to genotype D2. Accession numbers and virus names are included on the tree. Phylgetic analysis was performed based on 450 nucleotides of the N gene in MEGA version 7.0. Evolutionary history was inferred using the Maximum Likelihood method and distances were computed using the Kimura-two parameter model. The bootstrap test was set to 1000 replicates and bootstrap values above 70% are shown next to the main tree branches.

https://doi.org/10.1371/journal.pone.0222428.g003
The results show that the average age of the affected subjects is 8.6 years with extremes of 7 months and 20 years, suggesting that, measles remains a disease of children and young adults regardless of gender [19].

According to the phylogenetic analyses, all the sequences from Cameroon belonged to cluster 1 of genotype B3. The same cluster was detected in Cameroon in 2001 and from 2010 to 2011 [15,16]; in Nigeria and Ghana in 1998 [20]; in Sudan from 1997 to 2000 [21]; and in the Central African Republic in 2000 [22]. Cluster B3.1 strains remain endemic in Cameroon, where they have been circulating for about 17 years. This study is the first to characterize the MeV strains among Cameroonians in Adamawa, East and South regions. The East region borders the Central African Republic by Garoua Boulai and Betare Oya health districts and the South region borders Gabon and Equatorial Guinea by Olamze health district. Although there is no evidence of imported MeV strains in this study, the possibility of cross-contamination between Cameroonians and those bordering them is not excluded as the same genotype (B3) circulates in almost all of sub-Saharan Africa [21]. A previous study reported the circulation of this same genotype in the East region of Cameroon though it was among refugees from Central African Republic [23].

A small subset of the population (20%) from whom the samples were collected were vaccinated but no vaccine strain was detected. Keeping in mind that in Cameroon the first dose of measles-containing vaccine (MCV1) is administered at the age of 9 months [24], the patient in whom the MeV strains were detected presented rash at varying periods between 1 month and 12 years following immunization. These results reflect vaccination failures in these individuals probably due to non-development of vaccine induced protective immunity in some individuals, non-compliance to the CDC recommendations for uptake of two vaccine doses [25] or a poor conditioning of the vaccines [26].

The exclusive presence of cluster B3.1 strains in this study indicates the possible elimination of cluster B3.3 from Cameroon which was identified to circulate between 2010 and 2011 in the Far North and in the Centre regions. This suggests that, efforts made by the country for elimination of the disease are effective though should be re-enforced as the B3.1 genotype has been in circulation for over 17 years. Efforts should be put on childhood immunization since most affected children are not immunized. A second vaccine dose must as well be introduced into routine childhood immunization [13] which will allow the catch-up of failures of the first dose for a better follow-up of the recommendations for control and elimination of measles as proposed by WHO.

The study provided recent data on circulating measles viral strains throughout the country as the incidence of measles has increased worldwide. The limits of this work were the impossibility to completely sequence the studied genomes and the presence of limited samples in some years.

Acknowledgments

We would like to acknowledge the Cameroon health districts for collecting samples and the Expanded Program of Immunization for collaboration in the transport of samples to the laboratory. We also want to thank the Pasteur Institute in Paris for supporting in sequencing the samples.

Author Contributions

Conceptualization: Véronique Penlap Beng, Maurice Demanou.

Data curation: Franck-Martin Obam Mekanda, Gilde Martial Yonga.
Formal analysis: Franck-Martin Obam Mekanda, Chavely Gwladys Monamele, Frédry Brice Simo Nemg, Christophe Batéjat, Valérie Caro, Jean-Claude Manuguerra.

Investigation: Franck-Martin Obam Mekanda, Chavely Gwladys Monamele, Frédry Brice Simo Nemg, Diane Ouapi, Christophe Batéjat, Valérie Caro, Jean-Claude Manuguerra.

Resources: Maurice Demanou.

Supervision: Véronique Penlap Beng, Maurice Demanou.

Validation: Véronique Penlap Beng, Christophe Batéjat, Valérie Caro, Jean-Claude Manuguerra, Maurice Demanou.

Writing – original draft: Franck-Martin Obam Mekanda.

Writing – review & editing: Franck-Martin Obam Mekanda, Chavely Gwladys Monamele, Frédry Brice Simo Nemg, Gilde Martial Yonga, Diane Ouapi, Véronique Penlap Beng, Christophe Batéjat, Valérie Caro, Jean-Claude Manuguerra, Maurice Demanou.

References
1. Griffin DE. Measles virus. Fields Virology: Volume 1. Wolters Kluwer Health Adis (ESP); 2007. pp. 1551–1585. https://jhu.pure.elsevier.com/en/publications/measles-virus
2. Alla A, Waku-Kouomou D, Benjouad A, Elaouad R, Wild TF. Rapid diversification of measles virus genotypes circulating in Morocco during 2004–2005 epidemics. J Med Virol. 2006; 78: 1465–1472. https://doi.org/10.1002/jmv.20720 PMID: 16998886
3. Heymann DL, Murphy KR, Guyer B, Foster SO. Organisation Coverage by Sample Survey. Yale J Biol Med. 1979; 1979–1981.
4. Mahamud A, Burton A, Hassan M, Ahmed JA, Wagacha JB, Spiegel P, et al. Risk factors for measles mortality among hospitalized Somali refugees displaced by famine, Kenya, 2011. Clin Infect Dis. 2013; 57. https://doi.org/10.1093/cid/cit442 PMID: 23821730
5. Dabbagh A, Patel MK, Dumoland L, Gacic-dobo M, Mulders MN, Kretsinger K, et al. Progress Toward Regional Measles Elimination—Worldwide, 2000–2016. 2017; 66: 1148–1153.
6. Eom H, Park Y, Kim J, Yang J-S, Kang H, Kim K, et al. Occurrence of measles in a country with elimination status: Amplifying measles infection in hospitalized children due to imported virus. PLoS One. 2018; 13: e0188957. https://doi.org/10.1371/journal.pone.0188957 PMID: 29447169
7. Salvi C, Manager ER, Emergencies H, Diseases C, Negru L, Media C, et al. Europe observes a 4-fold increase in measles cases in 2017 compared to previous year. 2018; 2017–2019.
8. W.H.O. D of C of NTD. Weekly epidemiological record Relevé épidémiologique hebdomadaire. 2016; 89: 285–296.
9. Haddad-Boubaker S, Rezq M, Smeo MN, Ben Yahia A, Abudher A, Slim A, et al. Genetic characterization of clade B measles viruses isolated in Tunisia and Libya 2002–2009 and a proposed new subtype within the B3 genotype. Virus Res. Elsevier B.V.; 2010; 153: 258–264. https://doi.org/10.1016/j.viruses.2010.08.011 PMID: 20728482
10. Plan S. Global Measles and Rubella Strategic Plan. 2012; 44.
11. Mulders MN, Rota PA, Icenogle JP, Brown KE, Takeda M, Rey GJ, et al. Global Measles and Rubella Laboratory Network Support for Elimination Goals, 2010–2015. MMWR Morb Mortal Wkly Rep. 2016; 65: 438–42. https://doi.org/10.15585/mmwr.mm6517a3 PMID: 27148917
12. Rota PA, Bellini WJ. Update on the global distribution of genotypes of wild type measles viruses. J Infect Dis. 2003; 187 Suppl: S270-6. https://doi.org/10.1086/368042 PMID: 12721925
13. Patel MK, Gacic-dobo M, Strebel PM, Dabbagh A, Mulders MN. Progress Toward Regional Measles Elimination—Worldwide, 2000–2015. 2016; 65: 1228–1233.
14. Rota PA, Bloom AE, Vanchiere JA, Bellini WJ. Evolution of the Nucleoprotein and Matrix Genes of Wild-Type Strains of Measles Virus Isolated from Recent Epidemics. Virology. 1994. pp. 724–730. https://doi.org/10.1006/viro.1994.1066 PMID: 8291252
15. Waku Kouomou D, Nerrienet E, Moupouendoun J, Tene G, Whittle H, Wild TF. Measles virus strains circulating in Central and West Africa: Geographical distribution of two B3 genotypes. J Med Virol. 2002; 68: 433–446. https://doi.org/10.1002/jmv.10222 PMID: 12226833
16. Demanou M, Ratsitoarana R, Yonga M, Dosseh A, Anya B, Kobela M, et al. Molecular characterization of measles viruses that circulated in Cameroon between 2010 and 2011. Virol J. Virology Journal; 2013; 10: 1.

17. Focus E. WHO: Weekly epidemiological record Relevé épidémiologique hebdomadaire. Wkly Epidemiol Rec. 2016; 21: 265–284.

18. Heymann DL, Murphy KR, Kesseng Mayben G, Guyer B, Foster SO. MEASLES CONTROL IN YAOUNDE: JUSTIFICATION OF A ONE DOSE, NINE MONTH MINIMUM AGE VACCINATION POLICY IN TROPICAL AFRICA. Lancet. Elsevier; 1983; 322: 1470–1472. https://doi.org/10.1016/S0140-6736(83)90813-9

19. Delaporte E, Siegrist C, Wyler CA, Gervais A. Rougeole : diagnostic et prise en charge d’une maladie toujours d’actualité. Rev Med Suisse. 2008; 19: 920–924.

20. Hanses F, Truong AT, Ammerlaan W, Ikusika O, Adu F, Oyefolu AO, et al. Molecular epidemiology of Nigerian and Ghanaian measles virus isolates reveals a genotype circulating widely in western and central Africa. J Gen Virol. 1999; 80: 871–877. https://doi.org/10.1099/0022-1317-80-4-871 PMID: 10211955

21. El Mubarak HS Van De Bildt MWG, Mustafa OA Vos HW, Mukhtar MM Ibrahim SA, et al. Genetic characterisation of wild-type measles viruses circulating in suburban Khartoum, 1997–2000. J Gen Virol. 2002; 83: 1437–1443. Available: http://www.microbiologyresearch.org/docserv/fulltext/jgv/83/6/0831437a.pdf?expires=1525683365&id=id&accname=guest&checksum=2B0AEC6A352C143F908E0875A0286248 PMID: 12029159

22. Gouandjika-Vasilache I, Waku-Kouomou D, Mé D, Beyrand C, Guye F, Ngoay-Kossy JC, et al. Cocirculation of Measles Virus Genotype B2 and B3.1 in Central African Republic During the 2000 Measles Epidemic. J Med Virol J Med Virol. 2006; 78. https://doi.org/10.1002/jmv.20648 PMID: 16721862

23. Ndombo PK, Ndze VN, Mbarga FD, Anderson R, Acho A, Ebu J, et al. Molecular characterisation of measles virus strains among refugees from Central African Republic in Cameroon in 2014. 2018;

24. Gateff C. Evaluation of a mass measles immunization campaign in yaoundé, cameroun. Trans R Soc Trop Med Hyg. 1976; 70: 206–212. https://doi.org/10.1016/0035-9203(76)90040-7 PMID: 982514

25. MMR Vaccination | What You Should Know | Measles, Mumps, Rubella | CDC [Internet]. [cited 9 May 2019]. https://www.cdc.gov/vaccines/vpd/mmr/public/index.html

26. Secondary failure rates of measles vaccines: a metaanalysis . . . : The Pediatric Infectious Disease Journal [Internet]. [cited 29 Mar 2018]. https://journals.lww.com/pidj/Abstract/1996/01000/Secondary_failure_rates_of_measles_vaccines_a_14.aspx