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# First report of the genomic characterization of rubella viruses circulating in Cameroon

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## Abstract

Rubella is an acute and contagious viral infection whose gravidity resides in infection during pregnancy, which can result in miscarriage, fetal death, stillbirth, or infants with congenital malformations. This study aimed to describe the genome of rubella viruses (RUBVs) circulating in Cameroon. Throat swabs were collected from health districts as part of the measles surveillance program from 2010 to 2016 and sent to the Centre Pasteur of Cameroon. Samples were amplified by genotyping reverse transcription polymerase chain reaction (RT-PCR) in the search of two overlapping fragments of the gene that encodes the E1 envelope glycoprotein of RUBV. PCR products were sequenced and phylogenetic analysis was performed with MEGA 6 software. Overall, 9 of 43 samples (20.93%) were successfully amplified and sequenced but only eight sequences could be exploited for phylogenetic analysis with respect to the required fragment length of 739 nucleotides. Analysis of viral sequences from Cameroon with other epidemiologically relevant sequences from around the world showed that all RUBVs belonged to lineage L1 of genotype 1G. Cameroon sequences clustered with viruses from West Africa including Nigeria, Ivory Coast, and Ghana with a percentage similarity of 95.4% to 99.2%. This study will enable an update on the molecular epidemiology of RUBV in Cameroon and help in monitoring circulating RUBV for a better implementation of elimination strategies.

### Keywords

Cameroon; genome characterization; genotype 1G; lineage L1; rubella virus

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

## 1 | INTRODUCTION

Rubella is an acute and contagious viral infection usually causing mild fever and rash in children and adults.[1] The disease is transmitted by direct contact or droplet contact with respiratory secretions from infected people.[1,2] The seriousness of rubella is because infection during pregnancy, especially during the first trimester, can result in miscarriage, fetal death, stillbirth, or infants with congenital malformations, known as congenital rubella syndrome (CRS).[1] Reported rubella cases have declined due to increased coverage with vaccine, which is very efficient in controlling the infection.[1] Global estimates of disease burden suggest that approximately 100000 infants are born with CRS each year with the highest rates observed in Africa and South-East Asia where vaccine coverage is the lowest.[3]

Rubella virus (RUBV) is an enveloped single-stranded, nonsegmented, and linear RNA with positive polarity belonging to the *Togaviridae* family and the sole member of the genus *Rubivirus*.[2] RUBVs have a single serotype that does not cross-react with other *Togaviridae*.[2] However, it displays enough genetic variability to be distinguished as two clades (1 and 2), which include 13 genotypes (1a, 1B, 1C, 1D, 1E, 1F, 1G, 1H, 1I, 1J, 2A, 2B, and 2C); 1a is provisional.[4] A recent report by Rivailler et al[5] suggested a more precise grouping of RUBVs into lineages which utilized both genetic diversity and geographic information; statistically justified lineages within genotypes 1G, 1E, and 2B were identified. Previous studies from African countries predominantly identified viruses of the genotype 1G particularly in West and East Africa with limited data from Central Africa.[5–8]

The World Health Organization's (WHO) Measles and Rubella Laboratory Network has recommended the collection of RUBV genotype data to support control and elimination programs globally.[9,10] This recommendation has been successfully implemented in countries that have eliminated endemic rubella and CRS, whereas in Africa this implementation is still lagging. In Cameroon, surveillance of rubella or CRS is not yet implemented. However, laboratory testing of rubella-specific immunoglobulin M (IgM) antibodies has been integrated in the measles case-based surveillance system since 2004.[11] Only one study has reported on the epidemiology of rubella in the country,[11] whereas molecular data on circulating RUBVs have never been reported. The aim of this study was to describe the RUBV genotypes circulating in Cameroon, which were collected through the measles surveillance program from 2010 to 2016.

## 2 | MATERIALS AND METHODS

### 2.1 | Ethics statement

This study was carried out in the WHO's Measles and Rubella Reference Laboratory in the framework of the measles surveillance program, which has an activity for the genetic characterization of measles and RUBVs. There was no need for an ethical clearance.

### 2.2 | Clinical samples

Throat swabs obtained from the measles surveillance program from 2010 to 2016 were sent to the Centre Pasteur of Cameroon, the National WHO Measles and Rubella Reference Laboratory. For this study, swabs were selected based on the serologic results: only swabs

from a case with a positive or an indeterminate result for rubella IgM antibodies were chosen for genome characterization. Overall, 43 swabs were selected; 33 had a positive serology (IgM) to rubella and 10 had an indeterminate serological result.

### 2.3 | Laboratory techniques

RNA extraction was performed on the 43 swabs using the RNA QIAamp Viral Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. All RNA extracts were amplified by a reverse transcription polymerase chain reaction (RT-PCR) for two overlapping fragments of a portion of the gene that encodes the E1 envelope glycoprotein of RUBV. Primers 8633F (5′-AGC GAC GCG GCC TGC TGG GG-3′) and 9112R (5′-GCG CGC CTG AGA GCC TAT GAC-3′) were used to amplify a 480 nucleotide (nt) fragment, while primers 8945F (5′-TGG GCC TCC CCG GTT TG-3′) and 9577R (5′-CGC CCA GGT CTG CCG GGT CTC-3′) were used to amplify a 633nt fragment. SuperScript III One-Step RT-PCR Enzyme Kit System with Platinum Taq High Fidelity (Invitrogen, Carlsbad, CA) was used. One reaction mixture was constituted of 17 μL of DNAse- and RNAse-free water, 25 μL of 2× buffer, 1 μL of each primer at 20 μM, 1 μL of SuperScript III One-Step RT-PCR Platinum Taq enzyme and 5 μL of RNA extract. The total reaction mixture of 50 μL was run in the thermocycler using the following thermal cycling conditions: reverse transcription for 30 minutes at 50°C and 15 minutes at 95°C, 40 cycles of denaturation-annealing-elongation for 30 seconds at 94°C, 30 seconds at 55°C, and 1 minute at 72°C, followed by a final elongation for 10 minutes at 72°C and storage at 4°C.

All RT-PCR products were analyzed with 1.5% agarose gel electrophoresis and the positive products were sequenced by the Sanger's method by GENEWIZ laboratory (https://www.genewiz.com/Public/Services/Sanger-Sequencing), a partnership solution for sequencing. Sequences obtained were then edited and assembled into a single consensus sequence of 739 nt required by the WHO using the CLC Main Workbench 5.5 software (CLC bio A/S, Aarhus, DK).

### 2.4 | Phylogenetic analysis

Phylogenetic analysis was performed using the 739 nt consensus sequences.[4] The sequences were aligned by the ClustalW algorithm in MEGA 6 software[12] with the reference sequences available in the Rubella Nucleotide Surveillance (RubeNS) database and other epidemiologically relevant RUBVs.[5–7] The phylogenetic tree was constructed by the neighbor-joining algorithm and distance estimation was performed with the Kimura 2 parameter model in MEGA 6. Lineage designation of RUBVs from Cameroon was assigned by clustering with viruses of known lineages. We also estimated the evolutionary divergence of Cameroonian RUBVs with respect to other relevant sequences using the Maximum Composite Likelihood model in MEGA 6. The sequences obtained in this study were named according to WHO recommendations and submitted to RubeNS and GenBank.[4]

## 3 | RESULTS

Overall, 9 of the 43 samples (21%) were successfully amplified and sequenced, and the complete sequences of the 739 nt targeted were obtained from eight samples. The sequenced

viruses came from four regions of Cameroon: three from the South-West, two from the Far North, two from the Littoral, and one from the North-West regions (Figure 1 and Table 1).

Table 1 summarizes the socio-demographic data and the rubella IgM results of the cases from Cameroon yielding sequences of RUBV. All samples were obtained from children below 10 years of age, the majority being between 3 and 5 years. Female was the predominant gender (5 of 8). Of the 8 sequenced samples, 7 of 8 had IgM antibodies to RUBV, while one sample was from a person with an indeterminate serological status. All samples were collected between 2010 and 2015: one in 2010, two in 2012, four in 2013 and one in 2015.

Phylogenetic analysis of viral sequences from Cameroon with the 32 reference sequences available in the RubeNS database showed that all sequences formed a high bootstrap grouping with genotype 1G and genotype 1H sequences (Figure 2). These sequences were further analyzed with epidemiologically relevant sequences of genotypes 1G and 1H: 2 reference sequences of genotype 1H, 21 1G sequences from West Africa, 20 1G sequences from viruses collected from East Africa, and 10 European and American 1G sequences available in GenBank.[5,7] Cameroonian RUBV sequences were clustered with those from West Africa including Nigeria, Ivory Coast, and Ghana (Figure 3). As recently reported by Rivailler et al,[5] a more precise grouping of RUBV sequences is possible and Cameroonian RUBV sequences were found to belong to lineage L1 of genotype 1G.

Estimates of evolutionary divergence of the Cameroonian RUBVs with respect to other relevant sequences of genotype 1G were evaluated using the Maximum Composite Likelihood model in MEGA 6. The highest percentage similarity was observed within Cameroonian RUBVs at 96.6% to 99.5% followed by sequences from West Africa at 95.4% to 99.2% (Table 2). The lowest percentage similarity was observed with East African RUBVs.

## 4 | DISCUSSION

This study is the first to provide data on the molecular epidemiology of circulating RUBVs in Cameroon. All sequences obtained in this study were from children below 10 years of age with nearly even gender split. Nimpa Mengouo et al[11] also reported that the highest prevalence of RUBV was in children below 15 years with females representing more than half of positive cases.

Phylogenetic analysis showed that all eight Cameroonian viruses belonged to lineage L1 of genotype 1G.[5] This lineage has been shown to be endemic in West Africa in contrast to the lineage 1G-L2 which has been shown to be endemic in East Africa. In this study, a high percentage similarity between Cameroonian RUBVs and West African RUBVs was found (95.4%−99.2%), with a percent similarity to East African RUBVs of 92.6% to 97.1%. This reinforces the observation by Rivailler et al that L1 and L2 are geographically separated in Africa. Because the literature is not consistent on the terminology of lineages, the classification proposed by Rivailler et al[5] was used here because of its precision concerning the geographical distribution of the lineages.

Two other studies in Africa reported a mixed circulation of viruses of several genotypes. Pukuta et al[8] reported the circulation of four genotypes of RUBV (1B, 1E, 1G, and 2B) from 2007 through 2013 in the Democratic Republic of Congo, while Namuwulya et al[7] reported the circulation of RUBVs of genotypes 1E and several lineages of genotype 1G in Uganda from 2003 through 2012.[7] Similarly, there could be multiple genotypes circulating in Cameroon.However, the limited number of samples in which genome characterization was performed does not allow a strong conclusion concerning this possibility. Moreover, sequenced viruses were obtained from only 4 of the 10 administrative regions of Cameroon.

It is possible that Cameroonian RUBVs, were imported from the border regions of Nigeria. However, because this is the first report of genotypic characterization of RUBVs in Cameroon, more reports are required to ascertain the source of the circulating viruses. Genotyping should be performed routinely for a better understanding of the molecular epidemiology of RUBVs in Cameroon.

Samples were collected between 2010 and 2015 with the majority obtained during the years 2013 and 2012, which also correspond to the years with a high prevalence of the disease. Over 11% of suspected measles cases which were IgM negative for measles for these two years tested positive for rubella IgM.[11] According to the framework for verifying elimination of measles and rubella by the working group of the Strategic Advisory Group of Experts on Immunization, endemic RUBV transmission is defined as the existence of continuous transmission of indigenous or imported RUBV that persists for greater than or equal to 12 months in any defined geographic area.[13,14] The RUBVs detected in Cameroon in the present study are likely endemic viruses. For future verification of rubella elimination in Cameroon, interruptions in the transmission of these viruses will need to be confirmed by effective virologic surveillance systems.[9]

In most African countries, including Cameroon, rubella has remained endemic and difficult to control due to low vaccination coverage or lack of rubella-containing vaccine, lack of a well-defined surveillance program for rubella and CRS, and a paucity of molecular data on the circulating RUBVs. This study initiates the development of a sequence database for RUBVs circulating in Cameroon and should enable stakeholders to monitor changes in circulating RUBVs for a better implementation of RUBV elimination strategies.

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Centre Pasteur Cameroon

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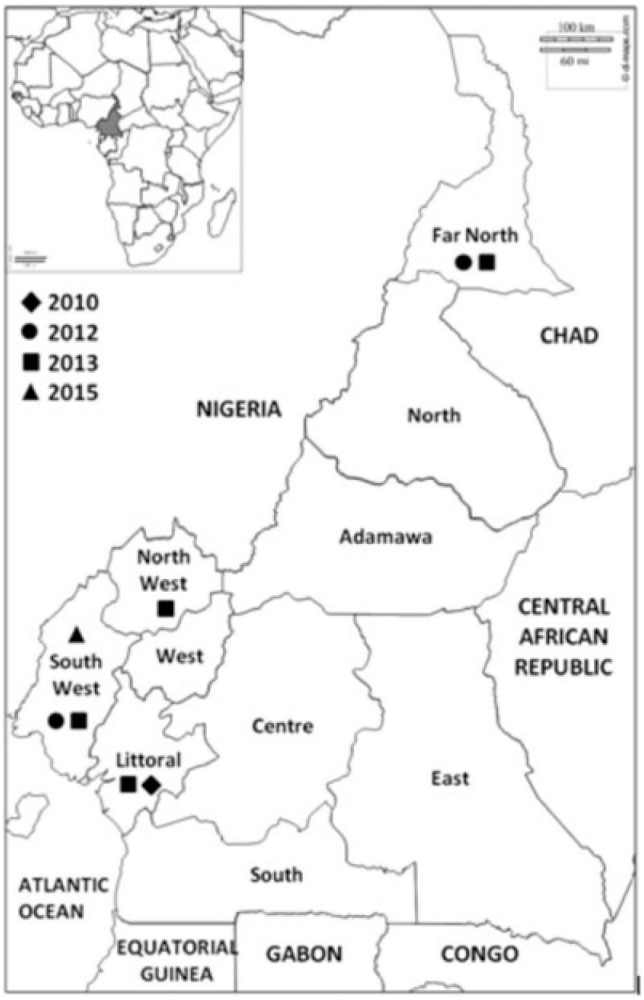


**FIGURE 1.**

Distribution of Cameroon strains by date and region of collection. ♦ represents viruses collected in 2010, ● represents viruses collected in 2012, ■ represents viruses collected in 2013, and ▲ represents viruses collected in 2015

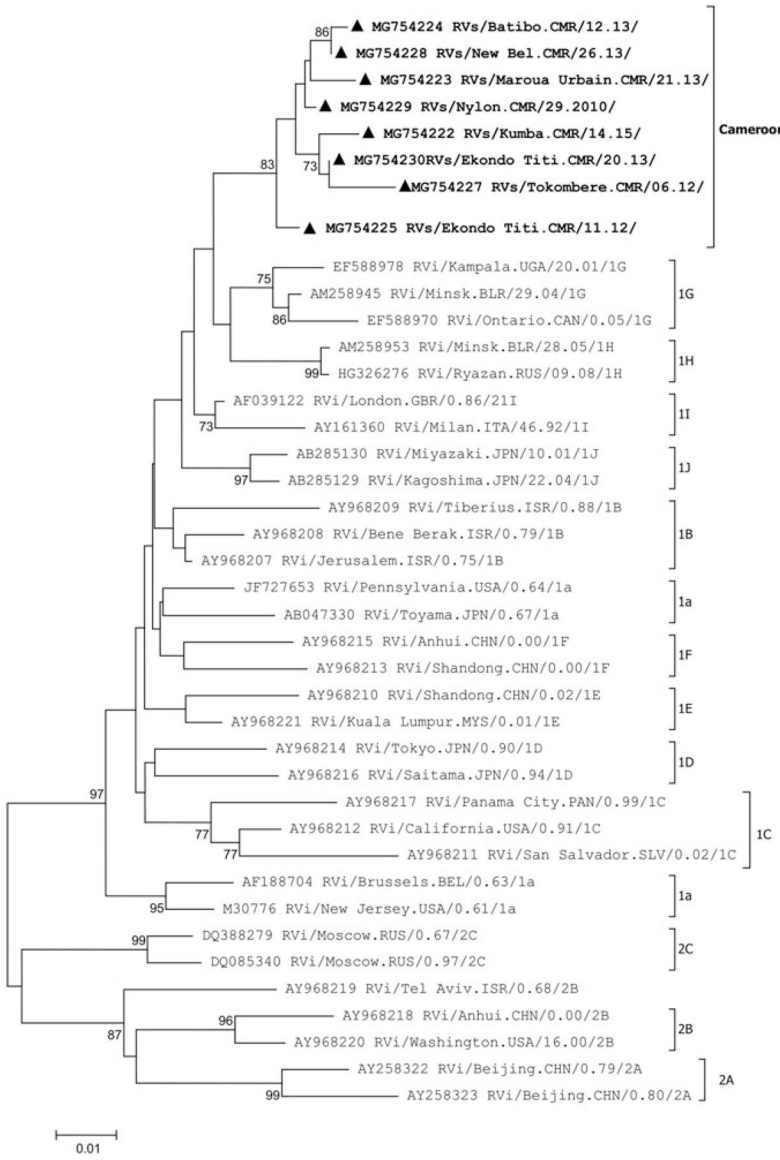


**FIGURE 2.**

Phylogenetic analysis of the Cameroon strains and reference sequences from RubeNS. Cameroon sequences are represented by triangles (▲). Accession numbers and virus names are included on the tree. Phylogenetic analysis was performed based on 739 nucleotides of the E1 gene in MEGA version 6.0. Evolutionary history was inferred using the neighbor-joining method and distances were computed using the Kimura 2 parameter model. The bootstrap test was set to 1000 replicates and bootstrap values above 70% are shown next to the main tree branches. RubeNS, Rubella Nucleotide Surveillance

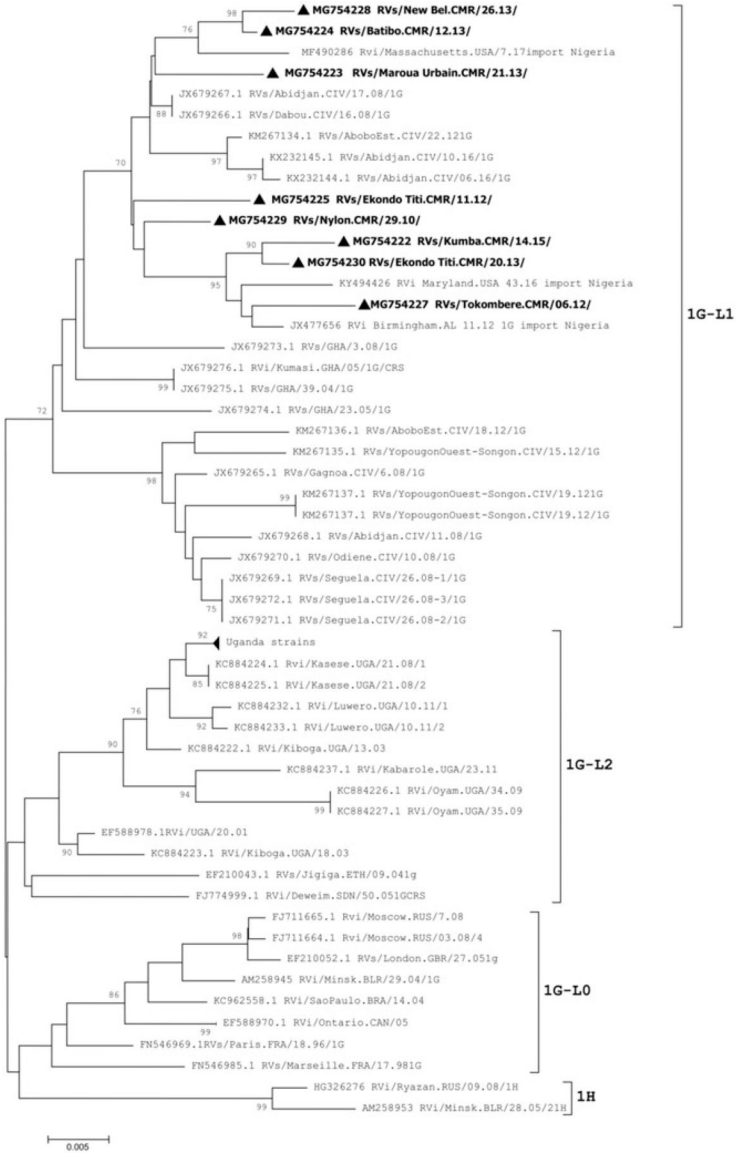


**FIGURE 3.**

Phylogenetic analysis of Cameroon strains and epidemiologically relevant strains of genotype 1G. Cameroon sequences are represented by triangles (▲). Accession numbers and virus names are included on the tree. Phylogenetic analysis was performed based on 739 nucleotides of the E1 gene in MEGA version 6.0. Evolutionary history was inferred using the neighbor-joining method and distances were computed using the Kimura 2 parameter model. The bootstrap test was set to 1000 replicates and bootstrap values above 70% are shown next to the main tree branches

**TABLE 1**

Characteristics of Cameroonian RUBVs collected from 2010 to 2016

| WHO names (RubeNS) | Collection date | Region | Age, y | Sex | IgM status |
|---|---|---|---|---|---|
| RVs/Kumba.CMR/14.15/ | 30/03/2015 | South-West | 3 | F | Positive |
| RVs/New Bel.CMR/26.13/ | 27/06/2013 | Littoral | 2 | M | Positive |
| RVs/Maroua Urbain.CMR/21.13/ | 22/05/2013 | Far North | 9 | F | Positive |
| RVs/Ekondo Titi.CMR/20.13/ | 14/05/2013 | South-West | 7 | F | Positive |
| RVs/Batibo.CMR/12.13/ | 20/03/2013 | North-West | 5 | F | Positive |
| RVs/Ekondo Titi.CMR/11.12/ | 14/03/2012 | South-West | 5 | M | Indeterminate |
| RVs/Tokombere.CMR/06.12/ | 06/02/2012 | Far North | 3 | F | Positive |
| RVs/Nylon.CMR/29.2010/ | 22/07/2010 | Littoral | 8 | M | Positive |

Abbreviations: F, female; M, male, IgM, immunoglobulin M.

**TABLE 2**

Estimates of evolutionary divergence between Cameroon rubella viruses (RUBVs) and other relevant RUBVs of genotype 1G

| Sequences | Lineages | Distances | Percentage similarity |
|---|---|---|---|
| Cameroon | 1G-L1 | 0.005–0.034 | 96.6–99.5 |
| West Africa | 1G-L1 | 0.008–0.046 | 95.4–99.2 |
| East Africa | 1G-L2 | 0.029–0.074 | 92.6–97.1 |
| European and American | 1G-L0 | 0.03–0.054 | 94.6–97.0 |

Analyses were conducted using the Maximum Composite Likelihood model in MEGA 6