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Short communication

Phylogenetic analysis of rubella viruses found in Morocco, Uganda, Cote d'Ivoire and South Africa from 2001 to 2007☆

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Abstract

Background: Rubella virus (RV) causes a mild disease, but maternal infection early in pregnancy often leads to birth defects known as congenital rubella syndrome (CRS). Rubella remains poorly controlled in Africa.
Objectives: To identify RV genotypes found in Africa to help establish a genetic baseline for RV molecular epidemiology.
Study design: Urine and nasopharyngeal specimens were collected between 2001 and 2004 during measles surveillance in Morocco, Uganda and South Africa, and from two persons in the United States who contracted rubella in Cote d'Ivoire and Uganda in 2004 and 2007, respectively. RV RNA was obtained directly from specimens or from RV-infected cell cultures, amplified by reverse transcriptase polymerase chain reaction, and the resulting DNAs sequenced. Sequences were assigned to genotypes by phylogenetic analysis with RV reference sequences.
Results: Nine RV sequences were assigned as follows: 1E in Morocco, 1G in Uganda and Cote d'Ivoire, and 2B in South Africa.
Conclusions: Information about RV genotypes circulating in Africa is improved which should aid in control of rubella and CRS in Africa.

Keywords: Rubella virus; Genotype; Molecular characterization; Sequences

1. Introduction

Rubella virus (RV) infection during the early stages of pregnancy can lead to serious birth defects, known as congenital rubella syndrome (CRS). Although a vaccine-preventable disease, rubella still accounts for an estimated 100,000 CRS cases annually worldwide (Robertson et al., 2003). Rubella remains endemic in most of Africa; as of 2004, only Morocco, Tunisia, Libya, and Egypt included rubella vaccine in their national immunization programs (World Health Organization (WHO), 2006a). For most of Africa, little surveillance data for rubella or CRS are available (Bloom et al., 2005).

RV is a positive sense, single-stranded RNA virus in the genus *Rubivirus* within the Togaviridae family. Although known RVs are a single serotype, sufficient genetic variation exists to allow the molecular epidemiology of wild-type RVs to be used in control and elimination efforts (Icenogle et al., 2006). A systematic nomenclature for wild-type RVs (WHO, 2005, 2007) divided rubella viruses into two clades containing nine genotypes (designated by clade and a letter

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designation: 1B, 1C, 1D, 1E, 1F, 1G, 2A, 2B and 2C), and four provisional genotypes (1a, 1h, 1i, and 1j).

General summaries of RV genotypes found in the world, including limited information from Africa, have been reported (WHO, 2005, 2006b); the sequence information, phylogenetic analysis, and virus circulation information reported here applies to four countries in Africa.

2. Materials and methods

2.1. Specimens

Four urine specimens from Morocco and two from South Africa were collected from rubella IgM-positive persons with rash illness. A nasal aspirate from Uganda was obtained from a suspected measles case from which measles virus could not be isolated. A throat swab and a urine specimen collected in NH, USA were from an infant with CRS whose mother had been infected with rubella early in her pregnancy in Cote d'Ivoire (Centers for Disease Control and Prevention (CDC), 2005; Plotinsky et al., 2007). A throat swab specimen was collected in 2007 in MA, USA, from a person who developed rash illness 1 week after returning from a trip to Uganda.

2.2. Laboratory methods

Virus isolations and RNA extractions were done as previously described (Frey et al., 1998). The presence of virus in cultures of Vero cells was detected by a reverse transcription-polymerase chain reaction (RT-PCR) which amplified a 185-nucleotide (nt) fragment of the E1 coding region. The detailed description of this 185-nt RT-PCR including its sensitivity, specificity, and a comparison of it with other assays including an immunofluorescent assay has been described (Zhu et al., 2007). A 1124-nt fragment of the E1 coding region containing the 739-nt WHO-recommended sequence window (nts 8731–9469) was amplified from positive cultures using the Titanium One Step kit (BD Bioscience, San Jose, CA) and the resultant DNA used for sequencing. The sensitivity of the 1124-nt reaction was determined to be 3000 copies of rubella RNA using known copy number transcribed RNA as the template. The 1124-nt reaction mix consisted of 1× kit buffer, 1× dNTP mix, 20 units RNase inhibitor, 1× thermostabilizing reagent, 1× GC-melt, 1× RT-Titanium *Taq* enzyme mix, 0.45 μM of primers RV8656 (5′ CCCCACCGACACCGTGATGAG) and RV3′ (5′ TTTTTTTTTTTTTTTTTTCTATACAGCAACAGGTGC), and 1/50 of the extracted RNA. The parameters for amplification were 50 °C for 1 h, 94 °C for 5 min, 40 cycles of 94 °C for 30 s, 65 °C for 30 s, and 68 °C for 1 min.

For RNA extracted directly from clinical specimens, either 250 μl of specimen and Tri-Reagent for liquid samples (Molecular Research Center, Cincinnati, OH) or 140 μl of specimen and the QIAamp Viral Mini Kit (Qiagen, Valencia, CA) was used. The small amounts of RV RNA present in clinical specimens made nested RT-PCR reactions necessary for amplification of sufficient DNA for sequencing. Two pairs of primers were chosen to amplify a 722-nt fragment of the E1 coding region containing a 601-nt region to be sequenced (nts 8869–9469) using the Titanium One Step kit as described above. One-tenth of the extracted RNA was added to the 50-μl reaction mix containing 0.45 μM of primers RV8812 (5′ CAACACGCCGCACGGACAAC [Bosma et al., 1995]) and RV3′. After amplification, 3 μl of the first round product was transferred to the second round PCR reaction containing 0.45 μM of primers RV8823 (5′ ACGGACAACTCGAGGTCC) and RV9545 (5′ TGGTGTGTGTGCCATAC); the 1-h reverse transcription segment was omitted. Sensitivity of this nested set was determined to be 30 RNA copies, using transcribed RV RNA.

2.3. Sequencing and data analysis

RT-PCR products, purified with the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI) were sequenced using Big Dye fluorescent dye terminators (Applied Biosystems (ABI), Foster City, CA). Reaction

Table 1
Wild-type rubella viruses from Africa

WHO name/genotype[a]	Country of origin	Age at onset	Specimen type	IgM results	Accessions Number
RVs/Berkane.MAR/24.02[1E]	Morocco	7 years	Urine	Positive	EF588973
RVs/Oujda.MAR/23.04[1E]	Morocco	7 years	Urine	Positive	EF588971
RVs/Taroudant.MAR/22.04[1E]	Morocco	5 years	Urine	Positive	EF588972
RVs/Tetouan.MAR/29.04[1E]	Morocco	10 years	Urine	Positive	EF588974
RVi/UGA/20.01[1G][b]	Uganda	2 years	Nasal aspirate	Unknown	EF588978
RVi/Boston.MA.USA/13.07[1G]	Uganda	38 years	Throat swab	Positive	EF588977
RVs/Westonaria.ZAF/47.03[2B]	South Africa	5 years	Urine	Positive	EF588976
RVs/Vereeniging.ZAF/2.04[2B]	South Africa	46 years	Urine	Positive	EF588975
RVi/Lebanon.NH.USA/3.05[1G]	Ivory Coast	Birth[c]	Throat swab, urine	Positive	EF588979

[a] Date of specimen collection is indicated by week and year (e.g., 24.02).
[b] This virus is a WHO approved reference virus for genotype 1G. Other indicated genotypes are based on figure.
[c] Specimens collected 3 months after birth; CRS infants can remain virus positive for up to 1 year of age (Bellini and Icenogle, 2007).