Molecular analysis of rubella virus in travelers suspected of measles infection in São Paulo, Brazil

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Summary

Objective: To identify measles virus genotypes in three cases of travelers suspected of measles infection. **Methods:** Samples (blood and urine) were collected for serology, virus isolation, and genotyping. Sera were analyzed for IgM antibodies against measles virus and rubella virus by enzyme-linked immunosorbent assay (ELISA) (Siemens – Marburg, Germany). Clinical samples (lymphocytes and urine) were inoculated into Statens Serum Institute rabbit corneal epithelial cell line- ATCC CL 60 (SIRC) and Vero Slam cells. RNA was extracted from clinical samples and cell culture was inoculated and processed by polymerase chain reaction (PCR) with oligonucleotides specific for measles virus (MV) and rubella virus (RV). **Results:** All patients showed IgM negative serology for MV and positive IgM for RV. RV belonging to genotypes 1B, 1C, and 1E were isolated from patients who came from Finland, Peru, and Germany, respectively. Genotype 1B has been found in Europe and on the East Coast of South America; 1C has been found in Peru and the West Coast of South America, and 1E, first identified in 1997, now appears to have worldwide distribution. **Conclusion:** Information about RV and MV genotypes circulating in São Paulo is essential for the control of measles, rubella, and congenital rubella syndrome (CRS) in Brazil.

Keywords: Measles virus; rubella virus; imported; genotype.

Resumo

Análise molecular do vírus da rubéola em turistas suspeitos de infecção por sarampo em São Paulo, Brasil

Objetivo: Identificar o genótipo do vírus do sarampo em três viajantes suspeitos de infecção por sarampo. **Métodos:** Amostras (sangue e urina) foram coletadas para sorologia, isolamento viral e genotipagem. As sorologias para pesquisa de IgM para o vírus do sarampo e da rubéola foi realizada utilizando-se o kit de ELISA (Siemens – Marburg, Alemanha). As amostras clínicas (linfócito e urina) foram inoculadas na SIRC (Statens Serum Institute rabbit corneal epithelial cell line-ATCC CL 60) e nas células Vero Slam. O RNA foi extraído das amostras clínicas e das células inoculadas e processadas por PCR, utilizando oligonucleotideos específicos para sarampo e rubéola. **Resultados:** Todos os pacientes apresentaram sorologia IgM negativa para sarampo e positivo para rubéola. Os vírus da rubéola isolados dos pacientes que vieram da Finlândia, Peru e Alemanha pertencem aos genótipos 1B, 1C e 1E, respectivamente. O genótipo 1B foi encontrado na Europa e na costa oriental da América do Sul, o genótipo 1C foi encontrado no Peru e na costa oeste da América do Sul e o genótipo 1E, identificado pela primeira vez em 1997, agora aparenta ser um genótipo com distribuição mundial. **Conclusão:** O conhecimento dos genótipos de sarampo e rubéola que circulam em São Paulo é essencial para o controle do sarampo, rubéola e síndrome da rubéola congênita.

Unitermos: Sarampo; rubéola; importado; genótipo.

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Introduction

Measles is one of the most highly transmissible human diseases. In Brazil the goal was to interrupt the transmission of endemic measles virus (MV) by the end of 2000 using strategies recommended by the Pan American Health Organization (PAHO), which included vaccination activities, intended to achieve high population immunity, together with sensitive surveillance for suspected measles cases, incorporating effective virological and serological surveillance[1]. Until measles is eradicated globally, the Americas face the risk of importation and secondary cases[2]. Across the world, migratory movements and travels to areas with low vaccine coverage have compromised eradication programs.

São Paulo is the largest state in Brazil, with a population of 37 million inhabitants. From 2001 to 2006, no indigenous measles cases were reported and only three imported cases, two from Japan in 2001 and 2002 (genotype D5) and one from another Asian country in 2005 (genotype D5), were registered in São Paulo[3,4]. Molecular characterization of rubella virus (RV) and MV isolates has been successfully used to determine epidemiological links between cases and the geographic origin of imported viruses. During the period of 2005-2006, three individuals who had recently arrived from Finland, Peru, and Germany, respectively, were suspected of measles due to rash and fever, and were reported to the health service quarantine staff. The results showed that these patients were infected with RV. Rubella is a common cause of childhood rash and fever, whose public health importance relates to the teratogenic effects of primary rubella infection in pregnant women. In 2003, the PAHO adopted a resolution calling for the elimination of rubella and congenital rubella syndrome (CRS) in the Americas by the year 2010[5]. Three cases of imported rubella diagnosed at the Adolfo Lutz Institute, São Paulo, Brazil are described.

Methods

Patients and samples

The first case was a 29-year-old male, unvaccinated, living in São Paulo, who had previously traveled to Finland to work on September 30th, 2005. He returned ten days later and developed fever (39°C), sore throat, coryza, cough, and rash after three days.

The second case was a 31-year-old woman, not pregnant, of unknown immunization status, living in Peru, who had come to São Paulo on October 3th, 2005. After one day, she developed fever (38°C), sore throat, coryza, cough, and rash.

The third case was a 28-year-old, male, Brazilian, unvaccinated, living in Germany, who had come to São Paulo, Brazil with his wife and two children on May 27th, 2006. After two days, he developed fever (39°C) and headache, and after three days, conjunctivitis and rash. None of the individuals mentioned had been in contact with visitors from Brazil with rubella-like illness in the preceding days.

Samples (blood and urine) were collected for serology, virus isolation, and genome detection. Peripheral blood was separated with Ficoll-Hypaque gradients and suspended in Dulbecco's Minimum Eagle Essential Medium (DMEM, Invitrogen Life Technologies – Carlsbad, CA, USA) supplemented with 2% fetal bovine serum (FBV). The urine was collected in a sterile vessel and neutralized with sodium bicarbonate to produce pH 7.0 as measured with indicator paper.

Serology

Serum rubella IgM antibody was determined with a commercial Enzygnost anti-rubella virus IgM (Siemens — Marburg, Germany) according to the instructions provided by the manufacturer.

Isolation ad cell culture

The Statens Serum Institute rabbit corneal epithelial cell line- ATCC CL 60 (SIRC) and Vero Slam cell lines containing 1 x 10^6 cells/mL were grown in T25 flasks in DMEM/RPMI supplemented with 10% inactive fetal calf serum (FCS, Invitrogen Life Technologies – Carlsbad, CA, USA), 20 mM L-glutamine. The confluent cells were inoculated with 500 µL of each sample for one hour at room temperature. After one hour, each cell line received 5mL of medium with 2% FCS and was incubated at 37°C. Cell cultures were observed for CPE daily during seven days as previously described[6]. The cells inoculated were harvested by centrifugation and RNA was extracted from the cell pellet. The growth of the virus in cultures of SIRC and Vero Slam cells was detected by a reverse transcription-polymerase chain reaction (RT-PCR)[7,8].

Uninfected and infected cultures were also prepared and were treated identically to the inoculated cells.

RNA extraction and reverse transcription

Nucleic acid extraction from 200 µL from samples and inoculated cell culture were extracted using TRI reagent (Molecular Research Center — Cincinnati, Ohio, USA). RV and MV-RNA were detected by the previously described RT-PCR[7,8]. Both cDNA synthesis and PCR followed a strict procedural condition to prevent contamination, including redundant negative controls and segregated environments for pre- and post-amplification procedures. After PCR amplification, the presence of a product was confirmed by agarose gel electrophoresis and ethidium bromide staining.