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Unravelling respiratory syncytial virus outbreaks in Buenos Aires, Argentina: Molecular basis of the spatio-temporal transmission

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ABSTRACT

Respiratory syncytial virus (RSV) is the main viral cause of hospitalization due to acute lower respiratory tract infections in infants worldwide. Several vaccines against RSV are under research and development, which are about to be approved. We evaluated transmission patterns in different settings to determine age-specific vaccination targets from a viral perspective. We sequenced the G glycoprotein's ectodomain of a constant clinical sampling between two epidemic outbreaks in a limited geographical region and performed phylogeographic analyses. We described a spatio-temporal transmission between local strains, which were originated in the center of the analyzed area and then spread to others. Interestingly, that central area reported the highest population density of the region and also showed overcrowding. This information should be considered by public health systems to evaluate vaccination at all ages in those areas to decrease viral transmission and in lower density populations only susceptible children should be vaccinated.

1. Introduction

Respiratory syncytial virus (RSV) is known as the main viral cause of acute lower respiratory tract infections (ALRTI) in infants and children worldwide (Nair et al., 2010; Holberg et al., 1991). Viral transmission is established from one individual to another due to direct and indirect contact with nasal and oral secretions. Reinfections may occur throughout life (Glezen et al., 1986), being asymptomatic in adults but causing severe disease in the elderly population (Walsh and Falsey, 2012). In countries with temperate climates, as Argentina, RSV outbreaks occur mainly in the coldest months peaking annually from April to September, with a peak in June (Viegas et al., 2004).

RSV is a negative-sense, single stranded RNA virus from the *Pneumoviridae* family (Afonso et al., 2016), with a genome of approximately 15,200 nt in length (Collins and Karron, 2013). RSV genome presents 10 genes that codify for 11 proteins, three of which are located on the viral surface: small hydrophobic protein (SH), fusion glycoprotein (F) and the attachment glycoprotein (G). There is a single serotype of RSV, but there are two different genetic groups that consist of the antigenic groups A and B (Anderson et al., 1985; Cristina et al., 1990) which differ mostly genetic and antigenically in the sequence of the G glycoprotein (Kim et al., 2007). The G gene presents two hypervariable regions in its external domain, or ectodomain, which are the target to carry out molecular epidemiology and evolutionary studies of this virus. By those studies, it was recently described an unusual genetic event, a duplication of a segment located on the second hypervariable region of the G gene's ectodomain in strains of both antigenic groups. The first event was a 60 nt duplication described on B antigenic group, found between 1997 and 1999 in Buenos Aires, Argentina, named as BA genotype (Trento et al., 2003, 2006). The second one was a 72 nt duplication on A antigenic group from the GA2 genotype that circulated for the first time during 2010–2011 winter season in Ontario, Canada, named as ON1 lineage (Eshaghi et al., 2012; Duvvuri et al., 2015). Since their discovery, those strains have evolved

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acquiring different mutations in both duplicated segments, leading to the diversification of RSV. However, ON1 and BA have particularly replaced almost all genotypes and lineages from both antigenic groups globally (Trento et al., 2010). Some authors proposed the idea that the duplication brought them an evolutionary advantage over strains lacking it, giving them structural and antigenic changes, which alter immunogenicity and pathogenicity (Hotard et al., 2015). In Argentina, the molecular epidemiological studies of RSV have been limited to Buenos Aires City, where the ON1 lineage and BA lineages have been circulating during the last few years (Trento et al., 2006; Viegas et al., 2016).

There is only one prophylaxis method against RSV, named Palivizumab. It consists of a humanized monoclonal antibody against the F glycoprotein, and can be used to prevent RSV severe disease in extremely premature infants or those with congenital heart disease or chronic obstructive pulmonary disease, but its high cost and the need of several administrations makes it difficult to use. There are non-approved vaccines to protect against RSV, but currently several vaccine candidates are under research and development, and some of them are next to be released (Anderson et al., 2013; Higgins et al., 2016). Different age groups are targeted, as young children, adults and older adults. It has been proposed the vaccination of pregnant women to decrease transmissibility and to protect newborns by placental antibody transfer or the vaccination of the elderly population which also develops severe disease, but recent studies showed that vaccination of children less than 5 years of age is the most effective strategy to avert RSV in children and elderly (Yamin et al., 2016). Children are responsible for transmission because they present the highest infectious viral loads, which were found to increase disease severity as it was reported in children and adult volunteers (DeVincenzo et al., 2010; Wathuo et al., 2016), and also have greater frequency and duration of contacts between children of their own age group, enabling the infection with RSV within susceptible individuals (Hall et al., 1976).

Previously to a vaccine implementation, the epidemiological and molecular characteristics of the circulating viruses should be considered to know if the vaccine will be effective in a studied population. In addition, it is important to consider the transmission patterns of a studied virus in order to identify the most vulnerable population. Phylogeographic tools are useful for estimating these patterns, and at the same time to predict the location of the most recent common ancestor that gave origin to that group of viruses.

It has been shown that areas characterized by critical social determinants such as high overcrowding, stunting, and environmental factors such as in-house smokers, air humidity, temperature, air pollution might increase the RSV infection severity as well as favor the viral transmission (Viegas et al., 2004; Okiro et al., 2008; Caballero et al., 2015; Yamin et al., 2016). In recent years, there have been many studies attempting to describe the chains of transmission of RSV from the individuals' perspective, describing contacts between the susceptible population and their cohabitants, contacts in schools, etc (Read et al., 2012; Munywoki et al., 2015). The aim of this work was to describe the dynamic of RSV transmission from the viral perspective by performing phylogeographic analyses taking advantage of having a constant clinical sampling between two epidemic outbreaks and in a limited geographical region.

2. Materials and methods

2.1. Study area

For this study, the pediatric units from four public hospitals participated. Those hospitals are part of a localized region of Buenos Aires Province known as the VI Sanitary Region, which is located in the south of Buenos Aires City, Argentina. Those hospitals were: Hospital Interzonal General de Agudos “Evita” (Lanús district), Hospital Interzonal “Dr. Alberto Eurnekian” (Ezeiza district), Hospital Zonal General de Agudos “Evita Pueblo” (Berazategui district) and Hospital El Cruce “Dr. Néstor Carlos Kirchner” (Florencio Varela district), denoted with a white H in Fig. 1. The last hospital mentioned admits patients with severe disease from other hospitals of the region that are part of the VI Sanitary Region health service network: Hospital Zonal General “Dr. Arturo Oñativia” (Rafael Calzada – Almirante Brown district); Hospital General de Agudos “Evita Pueblo” (Berazategui district); Hospital Zonal de Agudos “Mi Pueblo” (Florencio Varela district); Hospital Zonal General de Agudos “Dr. Isidoro Iriarte” (Quilmes district); Hospital Subzonal Especializado Materno Infantil “Dr. Oller” (San Francisco Solano, Quilmes district); Hospital Zonal General de Agudos “Lucio Meléndez” (Adrogué district) (denoted with a green H in Fig. 1).

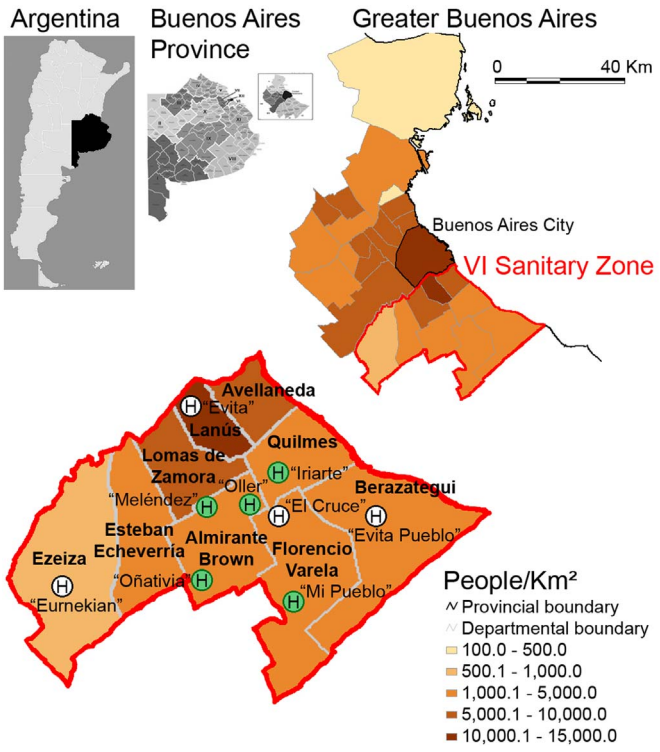

Fig. 1. Map of the studied area. The VI Sanitary Zone is delimited with red color and the districts names within are in bold. Population density of the Greater Buenos Aires from 2010 census is shown (http://www.sig.indec.gov.ar/censo2010/). The H surrounded by circles indicates the localization of hospitals: in white are shown the participating hospitals and in green are those that derived patients to the Hospital “El Cruce” (All hospitals names are between quotation marks).

2.2. Ethics statement

A written informed consent to participate in this study was obtained from parents or legal guardians of all patients, and the Medical Ethics and Research Committees from the four participating hospitals approved the study protocol. Additionally, a survey form was also obtained from each patient, referring to their location and socio-sanitary information such as age, sex, exact location, inhabitants in a household, housing characteristics, etc. To preserve the identity of the patients, every sample was codified before being analyzed, according to the Declaration of Helsinki and the *Habeas Data* law on protection of personal data (Law no. 25326, Argentina). The sample codification contained an acronym corresponding to the hospital where each sample was collected ("B" for the Berazategui district hospital, "C" for the El Cruce hospital, "E" for the Ezeiza district hospital and "L" for the Lanús district hospital) followed by an internal number.