ANALYSIS OF CLIMATIC FACTORS IMPACT ON RSV INFECTION DISTRIBUTION IN CHILDREN ATTENDING CHILDCARE AT NORTHWEST REGION OF SÃO PAULO, BRAZIL

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ABSTRACT

Respiratory syncytial virus (RSV) was detected in samples collected from children from 0 to 6 years of age with acute respiratory infection, attending public childcare on Northwest region of São Paulo, Brazil. RSV distribution was associated to seasonal climatic variables as temperature, rainfall and relative air humidity. We utilized samples of nasopharyngeal aspirate collected during the period of July 2003 to September 2005. RT-PCR was the chosen method for viral identification. Results showed that from the 817 samples (collected from 179 children), 7.7% (63/817) were RSV positive. In 2003, RSV was detected from July until October. In 2004, RSV infections occurred in March, May, June, July, October, November, and December. In 2005, RSV was detected in March, April, May, August, and September. RSV circulation patterns in childcare children showed seasonal distribution associated to decreases in temperature and relative air humidity. RSV was detected in childcare children as an important viral agent causing respiratory infections, with varying patterns of circulation into the cohort during the study period. Moreover, RSV distribution showed to be associated with the dry season on Northwest region of São Paulo, Brazil.

Keywords: respiratory virus, acute respiratory infection, daycare children, seasonal distribution

INTRODUCTION

Respiratory Syncytial Virus (RSV) is one of the main agents responsible for infections of the respiratory tract in children younger than 5 years old (Kim et al. 2007). RSV is an enveloped RNA virus, classified into the genus Pneumovirus and Paramixoviridae family (Santos et al. 2002; Hacking & Hull, 2002; Arbiza et al. 2005). Seasonality is the main epidemiological feature of RSV, with annual outbreaks observed at regular intervals that vary according to the climate (Lee et al. 2007). This factor complicates the global impact analysis from such infections, and regional epidemiological studies are necessary.

In regions with temperate climate, a higher number of infections are associated to the winter season.

In tropical regions, differences in the ability to cause infections and the virus infectivity have been described (Moura et al. 2006). Many climatic factors have been reported to influence the seasonal RSV distribution in Brazil (Gardinassi, et al. 2012), among them, there are several variations of temperature, rainfall and relative air humidity throughout the year (Moura et al. 2006). Despite the peaks of respiratory viral infection, in South America, occur mostly in the autumn and winter (Vieira et al. 2001), in Brazil, RSV seasonality has differences depending on the geographical region.

Brazil is a country with great territorial extension and presents regional climate diversity. While in the North Region, rainfall represents the main determinant meteorological factor for viral respiratory infections, changes in temperature have more significant roles in areas such as South and Southeast (Cintra et al. 2001).
In the Brazilian Southern Region, where the climate is predominantly temperate, RSV outbreaks are also related to the winter months (June, July and August). In cities from Southeast Region, like São Paulo and Rio de Janeiro, which presents a subtropical climate, outbreaks of respiratory viral infection begin in late March or early April, presenting peaks in May. Different from these regions, in Fortaleza, which is localized on Northeast Region, RSV epidemics are distributed throughout the year, but an increase in the number of cases is also observed at the months of April, May and June (Moura et al. 2006).

Therefore, the purpose of this study was to investigate RSV infection in children aged between 0 to 6 years with acute respiratory infections, who attended a public childcare in Northwest region of São Paulo, Brazil. In addition, we aimed to conduct an epidemic survey in the city of São José do Rio Preto, correlating RSV seasonal distribution with climatic factors such as temperature, rainfall and relative air humidity.

**MATERIAL AND METHODS**

**Study population**

The study was performed with nasopharyngeal aspirate samples (817) obtained from 179 children aged between 0 and 6 years attending the public childcare “Maria Inês Arnal” of São José do Rio Preto-SP. Only children that presented acute respiratory infections were included in the cohort. ARI was characterized by single or combined occurrence of the following physical signs and symptoms: cough, pharyngitis, rhinorrhea, nasal congestion, headache, low grade fever, facial pressure and sneezing. The period of sample collection was initiated in July 2003 and ended in September 2005.

**Clinical Specimens**

A single nurse was responsible for collecting the samples during the whole study. Nasopharyngeal washes were obtained after instillation of 0.5ml of sterile PBS (Phosphate Buffered Saline - NaCl, Na2HPO4, NaH2PO4) into each nostril with immediate aspiration through a sterile neonatal canula inserted into the child's nasopharynx. The sample was transferred to a sterile vial and immediately transported to the laboratory, processed and frozen at −80°C in Trizol LS (Invitrogen, Carlsbad, CA) for later RNA extraction and RT-PCR testing.

**RNA Extraction**

An amount of 200µL from homogenized chloroform (Merck, GER) were added to the aliquots of each sample containing Trizol-LS (Invitrogen, USA) and the mixture was incubated on ice for 5 minutes. The material was centrifuged to 13400g for 15 minutes at 4°C. Then, the supernatant was transferred to another tube with 400µL of isopropanol (Sigma, USA), which was homogenized and incubated for 15 minutes on ice. After incubation on ice, samples were centrifuged again at 13400g, for 15 minutes at 4°C. The supernatant was desipsed and added 800µL of 75% ethanol (Merck, GER) to tubes containing the precipitate. Then the material was centrifuged to 6000g for 8 minutes at 4°C. The supernatant was desipsed and the precipitated RNA was diluted in 48µL of water MiliQ treated with DEPC (diethyl pyrocarbonate – Sigma, USA), and added 2µL of RNase OUT (Invitrogen, USA).

**Reverse Transcription**

Reverse transcription was performed using High Capacity cDNA Archive Kit - Applied Biosystems (USA), as instructed by the manufacturer. For each sample, reactions of 100µL were prepared by adding 50µL RNA diluted in water RNase free, 10 µL of 10X Random Primers, 10µL of 10X RT Buffer, 4µL of 25X dNTPs, 5µL of Multiscribe (Reverse Transcriptase) and 21µL of water MiliQ treated with DEPC (diethyl pyrocarbonate – Sigma, USA). The reaction was submitted to GeneAmp® Thermocycler PCR System 9700 (Applied Biosystems) at 25° C for 10 minutes, 37°C for 120 minutes, for action of reverse transcriptase, kept at 4°C for a short period of time and then stored in the freezer -20°C.

**PCR Amplification**

The amplification reactions were performed with 1X Buffer (Biotools, SPN), 1mM of MgCl2 (Biotools, SPN), 0.2mM of dNTPs (Applied Biosystems, USA), 0.2mM of each specific primers (Table 1) and 2 units of DNA polymerase (Biotools, SPN). On step 1, PCR reaction (Zheng et al. 1996; Peret et al. 2000), FV and GAB (Table 1) primers were used to 5µL of cDNA, adding up MiliQ water to a final reaction volume of 50µL. GAPDH gene (Suwannakarn et al. 2008) was used as an internal control of reactions. When there was no amplification in PCR reaction, samples were subjected to step 2 PCR reaction.

On step 2 PCR reaction (Peret et al. 2000; Peret et al. 1998), the initial conditions of amplification reaction were maintained, using F1AB and GAB (Table 1) primers and 1.0µL of PCR product, adding up MiliQ water to a final reaction volume of 50µL.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Reaction</th>
<th>Genomic Region</th>
<th>Annealing</th>
<th>oligonucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td>FV (-)</td>
<td>Step 1 PCR</td>
<td>F protein</td>
<td>163 – 186</td>
<td>GTT ATG ACA CTG GTA TAC CAA CC</td>
</tr>
<tr>
<td>GAB (+)</td>
<td>Step 1 and 2 PCR</td>
<td>G protein</td>
<td>504 – 524</td>
<td>YCA YTT TGA AGT GTT CAA CTT</td>
</tr>
<tr>
<td>F1AB (-)</td>
<td>Step 2 PCR</td>
<td>F protein</td>
<td>3 – 22</td>
<td>CAA CTC CAT TGT TAT TTG CC</td>
</tr>
</tbody>
</table>

Table 1. List of primers used for amplification reactions of RSV samples in the step 1 and 2 of PCR reaction, showing for each primer the position of annealing in genome and the region that was amplified.
Reactions were submitted to the GeneAmp® Termocycler PCR System 9700 (Applied Biosystems). The cycling condition was 95°C for 5 minutes, then 40 cycles of 94°C for 60 seconds, 55°C for 60 seconds, 72°C for 60 seconds and 72°C for 7 minutes for polymerization. Analysis of the amplified products was performed by 1% agarose gel electrophoresis, using Ethidium Bromide (0.5µg/mL) to observe bands under UV light.

Statistical analysis

Statistical analysis was performed by the Chi-square test using the Minitab Statistical Software for Windows, version 12.22, and differences were considered significant if p<0.05.

Meteorological Data of São José do Rio Preto

Data on temperature and rainfall were supplied by the Agriculture and Supply Secretary, represented by the Integral Technical Assistance Coordination - CATI, collected daily the temperature and the rainfall measures made by the Seeds, Seedlings and Matrices Department of Seeds Production Nucleus from São José do Rio Preto. Data of relative air humidity were obtained from SOMAR Meteorology, through daily measures at São José do Rio Preto Airport by REDEMET (Air Command Meteorological Network).

Ethics

A Written Consent signed by the parents or legal responsible was obtained for each child. This study was approved by Research Ethics Unesp/IBILCE by opinion n° 062/2001 on June 11, 2001 in São José do Rio Preto, Brazil.

RESULTS

The studied population was composed by 231 children, aged 1 to 78 months (mean 30.85 months), 44.5% female and 55.5% male, who attended the public child care “Maria Inês Arnal” from Northwest region of São Paulo, Brazil from July 2003 until September 2005. Among these children, 179 presented at least one respiratory infection episode, from whom were collected 817 samples of nasopharyngeal aspirate. RSV infection investigated by RT-PCR revealed that 7.7% (63/817) of total samples were positive (Fig. 1).

Analyzing seasonal distribution of respiratory syncytial virus detected in 2003 (63/817), RSV was found in July (4/28), August (5/34), September (9/34), and October (2/22). There are no records from respiratory infections from January to June because the collection period initiated at July (Fig. 1). Despite of respiratory infections detection at November and December, no RSV infections were reported on these months (Fig. 1).

In 2004, RSV infections occurred on March (5/31), April (1/50), May (4/64), August (12/49), and September (6/48). Although respiratory infections occurred on June and July, no RSV infection was reported (Fig. 1).

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The association between RSV circulation pattern on childcare children with monthly average of minimal temperature (in °C), maximal relative air humidity (in %), and with monthly rainfall volume (in mm) presented on sample collection days is shown on figure 2.

We observed the existence of four rainfall peaks during the studied period; these peaks were not associated with RSV infection. Although there was no evidence of significant association between RSV presence and driest periods, it was clearly noted that there is a
tendency to increase virus detection on periods with no rain precipitation, which is confirmed by the analysis of monthly rainfall volume (mm) and RSV detection on samples collected in those periods (p=0.565). It was also observed that RSV infection occurs simultaneously with the decreases on minimum temperature and relative air humidity, which can be better observed by increases in the peaks of RSV infection in 2003 and 2005.

Thus, analyzing RSV detection rate, it was verified that RSV positivity was associated to decreases on temperature (p=0.049) and relative air humidity (p=0.002), which is the period that corresponds to the dry season in Northwest region of São Paulo, Brazil. In the city of São José do Rio Preto, rainy season usually begins on October, with increases in temperature and relative air humidity rates, during until March, with concomitantly decreases in temperature and relative air humidity rates.

**DISCUSSION**

Acute respiratory infections (ARI) are the main problem of public health in children worldwide, and most of these infections are caused by viral agents. In developing countries, laboratorial tests for identification of virus which cause these infections are not available (D’Ellia et al. 2005). However, it has been reported advances on the epidemiological knowledge of these infections in Brazil, mainly due to studies performed at Brazilian university hospitals (Salomão Junior et al, 2011; Gardinassi et al, 2012).

Respiratory syncytial virus (RSV) is the main viral agent that causes ARI in childhood and presents annual outbreaks in infants (Calegari et al. 2005). Aiming to detect RSV presence in non-hospitalized children from Northwest region of São Paulo, Brazil this study was performed on a population composed by childcare children.

Studies performed on childcare children have been done mainly in Scandinavia, United States and England (McCutcheon & Fitzgerald, 2001) and such studies are not common in Brazil. These studies evidence that children who attended childcare from the beginning of infancy present higher risks of respiratory infections comparing to children that did not attend childcare (Lu et al. 2004).

Exposures to repeated respiratory infections in infancy, period in which the lungs are not mature, have been related to a higher susceptibility to late respiratory problems (McCutcheon & Fitzgerald, 2001). This represents a high cost to public economy and great repercussions on children life quality, therefore surveys on these infections etiologies are extremely necessary in Brazil.

Concerning the circulation pattern of respiratory syncytial virus, it is well known that seasonality is a main feature and annual epidemic outbreaks are observed in regular intervals. These epidemics have been reported to vary according to the local, and are influenced by climatic factors variation (Checon et al. 2002). Associations between respiratory viruses presence with temperature, rainfall and relative air humidity have been adopted in epidemiological studies worldwide (Viegas & Mitschenko, 2005).

In the period from July 2003 to April 2004, RSV detection occurred on July, August, September, October, and only one sample in March. Although RSV detection on childcare children was low in 2004, this virus was identified mostly on May, June, July, and October. In 2005, a greater occurrence of infections was observed, with peaks on May, August and September. This consecutive variation along the years on virus identification may be related to immune system features of each host and circulating RSV strains.

In this study, RSV circulation showed an alternated pattern of incidence into the population during the years of study, a pattern that was also observed in other studies (Gardinassi et al, 2012). Comparing to 2004, a higher number of infections were reported in 2003 and 2005. Additional studies have been performed by this research group to elucidate respiratory infections etiology in cases that showed negative results to RSV. Absence of this virus could indicate the presence of other viral agents, as HMPV, FLU, PIVI and rhinovirus (Bonfim et al, 2011).

Analyzing RSV detection rate, it was verified that RSV positivity was associated to decreases in temperature and relative air humidity, which is the period that corresponds to the dry season in Northwest region of São Paulo, Brazil. Although there was no evidence of RSV infection associated to rains absence, it was clearly observed trends in that virus detection rate increases on periods with no rain precipitation.

Respiratory viral infections seasonality seems to present two components: host susceptibility and viral availability. Seasonal exposure to cold air has been suggested to cause weakness on nasal cavity, leaving deficiency on mucus production that acts against infections. As corporal temperature decreases in the course of a cooler environment, virus entry on low respiratory tract is favored. On the other side, viral stability also can be affected by meteorological factors (Eccles, 2002).

Enveloped virus as RSV and *Influenza virus type A* (FLUA) presents decreasing infectivity at hostile environment conditions such as high temperature, high solar radiation and low relative air humidity. This change in infection capacity is attributed to the natural instability of envelope structure, which is derived from plasmatic membrane and has its fluidity determined mainly by water quantity and temperature. Thus, RSV and FLUA occurrence is evidenced in periods when temperature and in relative air humidity rates decreases, being associated to dry seasons as winter (Viegas & Mitschenko, 2005).
Thomazelli et al. (2007) also observed an increase on RSV detection in dry seasons with low temperatures in São Paulo city. RSV presence has been associated to rainy seasons in studies performed in tropical and subtropical regions (Weber et al. 1998; Moura et al. 2006), however, typical climatic factors of each region could explain the results found by the present study.

In Brazil, it is observed a great climatic diversity, indicating the occurrence of regional climatic features. Consequently, RSV occurrence has been showed as non uniform between different Brazilian geographical regions. In São Paulo and Rio de Janeiro cities, infection outbreaks begin on late March or early April, with peaks from May to October. In the North of Brazil, RSV epidemics present peaks on May and June (Satraliotto, 2002). Therefore, it can be inferred that RSV infection in Brazil presents regional features that are influenced by the most marked and specific climatic factors from each region (Moura et al. 2006).

In conclusion, RSV was detected in childcare children as an important viral agent that causes respiratory infections. RSV incidence varied into population during the studied period, however, each year showed a RSV circulation pattern associated to decreases in temperature and relative air humidity, indicating that higher incidences of infection by RSV occur on dry seasons in Northwest region of São Paulo, Brazil.

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