# Clinical-Epidemiological Evaluation of Respiratory Syncytial Virus Infection in Children Attended in a Public Hospital in Midwestern Brazil

**Tatiany Calegari[1], Divina A.O. Queiroz[1], Jonny Yokosawa[2], Hélio L. Silveira[3], Lourenço F. Costa[1], Thelma F.M. Oliveira[1], Lysa N. Luiz[1], Renata C. Oliveira[1], Francisco C. Diniz[3], Lívia M.G. Rossi[1], Cláudio J Carvalho[1], Ana Cláudia Lima[1] and Orlando C Mantese[3]**

*Laboratory of Virology - Biomedical Institute - Federal University of Uberlândia[1]; Uberlândia, MG; NG Biotecnologia Ltda[2]; São Paulo, SP; Medicine School - Federal University of Uberlândia; Uberlândia, MG, Brazil*

**Respiratory syncytial virus (RSV) is responsible for annual respiratory infection outbreaks in infants and young children worldwide, frequently causing bronchiolitis and pneumonia. We evaluated clinical and epidemiological features of acute respiratory infections (ARIs) caused by respiratory syncytial virus (RSV) in children less than five years old. Nasopharyngeal aspirate samples from children with ARI symptoms, attended at the 'Hospital das Clínicas' – Federal University of Uberlândia, MG, Brazil, were collected and tested for RSV by the immunofluorescence assay (IFA). Patients' clinical and epidemiological data were also obtained. From April 2000 to June 2003, 317 nasopharyngeal samples were collected from children less than 54 months old. Seventy-six samples (24.0%) were positive for RSV, with 53% (40/76) obtained from male patients. Hospitalization occurred in 50% (38/76) of the cases, with an average period of 10.6 days, in most cases (87%, 33/38) occurring in children less than 12 months of age. Although an association between this age group and the presentation of more severe clinical symptoms was observed, such as bronchiolitis in 51% (27/53) of the patients and pneumonia in 19% (10/53), no patients died. RSV was found from February to August, with the highest incidence in May. Conclusions: RSV is an important agent that causes ARIs; the clinical manifestations varied from mild to severe and patients frequently required hospitalization; RSV mostly affected children less than one year old.**
**Key Words: Respiratory syncytial virus, respiratory infection, children.**

Children younger than five years old, who become ill and use the health care system, frequently suffer from respiratory infections. Acute respiratory infection (ARI) is the most common manifestation, and it is responsible for high morbidity and mortality rates, especially in developing countries [1-3]. A study conducted in Sao Paulo city in 1996 revealed that 27.7% of hospitalized children within that age group had ARI [1].

Received on 14 Novemver 2004; revised 17 March 2005.
Address for correspondence: Dr. Orlando C Mantese, MD, PhD. Universidade Federal de Uberlândia – Faculdade de Medicina Avenida Pará, 1979. Uberlândia, MG. Zip code: 38.405-320. Brazil E-mail: orlando@ufu.br Phone/fax: (55-34) 3232 2736.

**The Brazilian Journal of Infectious Diseases** 2005;9(2):156-161


Viruses are considered the most important agents in ARI of the lower respiratory tract (LRT) that require hospitalization [4,5]. Respiratory syncytial virus (RSV), one of the most important respiratory viruses, is responsible for annual epidemic ARI outbreaks in infants and pre-scholar children, worldwide [6], frequently causing bronchiolitis and pneumonia, mostly in infants less than six months old [7]. In São Paulo city, a prospective study revealed that 62.7% of hospitalized children had LRT infection, among which 56.4% had a virus as the etiological agent; RSV was identified in 52.4% of these cases [8].

It has been suggested that upon completion of the second year of life RSV has infected virtually all children at least once and 10-20% of them would have been reinfected [9]. Moreover, in the United States, RSV is responsible for 1% child hospitalization, 70% of which during the first year of life [10]. In São Paulo state,

from 1979 to 1993, respiratory infections were responsible for 18.4% of deaths in children younger than five years old [11]. Another study in Brazil indicated that RSV infection was the main cause of mortality in this same age range, especially among infants less than 11 months old [12].

In temperate countries, RSV outbreaks have a defined seasonality, occurring mainly during fall and winter [13]. In Brazil, the few studies that have been carried out have revealed that the RSV infection period varies according to the region: in Rio de Janeiro city, it occurs from March to May [14], from April/May to July/August in São Paulo city [15], from May to July in Salvador, Bahia state [16], and from April to May in Uberlândia, Minas Gerais state [17,18].

RSV is transmitted by respiratory secretion on hands, on fomites [19], and in contaminated aerosols [9]. The incubation period can last five days [19] and the duration of viral shedding varies according to the intensity of the disease and the host immune state. Infants with LRT infection shed RSV for 5-12 days [9]. Initial symptoms are fever, runny nose, coughing and wheezing [6]. With progression of the disease, there is an increase in wheezing, coughing and dyspnea, with chest hyperextension, chest retractions, tachypnea and cyanosis [9]. Some infants also develop pharyngitis [9]. RSV has also been found to be responsible for acute otitis media in children [20,21].

Although RSV may cause severe infections in healthy young children, the infections are more severe in premature children, in those with immune deficiency, and in those with a subjacent illness, such as bronchopulmonary dysplasia or congenital heart disease [15,22,23]. Other risk factors for RSV infections include early weaning, exposition to cigarette smoke, age younger than six months, another sibling attending school or pre-school living in the same house, low social-economic status, being of black race and being male [10].

We analyzed clinical and epidemiological features of RSV infections in children less than five years of age attended at the Hospital de Clínicas of the Universidade Federal de Uberlândia (HC/UFU) from April, 2000 to June, 2003.

## Material and Methods

### Sample collection

Between April, 2000, and June, 2003, at the 'Hospital das Clínicas' of Federal University of Uberlândia (HC/UFU), 317 nasopharyngeal secretion samples were obtained from children less than five years of age with acute respiratory infection. This study was approved by the Ethics and Research Council of UFU and a signed consent was obtained from each child's parent or foster parent. Samples were collected at the Pediatric Emergency Service (PES), Pediatric Ward (PW), Pediatric Intensive Care Unit (PICU) and the Neonatal Intensive Care Unit (NICU).

The HC/UFU is a public university hospital that cares for people who live in Uberlândia city and 120 other cities and towns in the region, covering an estimated population of approximately two million people. The hospital is a 481-bed facility, and it is a regional medical reference center; most of the beds are occupied by patients who require complex treatment. Among these beds, there are 110 available for children: 60 for neonates, 41 in PW and 9 in PICU. In Uberlandia city, there are other primary care hospitals that use the HC/UFU as a reference hospital.

### Patients

The following information was obtained: name, age, gender, date and place in the facility where the sample was collected, clinical diagnosis, progression or not to hospitalization, duration of hospitalization and whether the case was fatal. In order to be eligible for inclusion in the study, the patient was required to have ARI symptoms that had started within five days before he/she was admitted to the hospital. Characteristic ARI symptoms included runny nose, coughing, wheezing, difficulty in breathing, with or without fever. Only the first five patients admitted to the hospital on a week day (Monday through Friday) were included in the study. The clinical diagnosis was in accordance with the 10th Review on International Classification of Diseases (ICD-10 – World Health Organization, 1994).

Cold, flu and upper respiratory tract infections (URTI) were grouped under URTI, pneumonia and bronchopneumonia under pneumonia, and laryngotracheobronchitis, tracheobronchitis and bronchitis under bronchitis. Bronchiolitis, considered a separate diagnosis according to ICD-10, was grouped under pneumonia in this study for statistical analysis.

Nasopharyngeal samples: secretion was aspirated after instillation of 0.5mL of sterile 0.9% NaCl in each nostril; the samples were transferred to a sterile vial. They were kept on ice and processed within four hours after collection [15,24]. Specimens were divided into three aliquots, and only one of them was used for indirect immunofluorescence assay.

Indirect immunofluorescence assay (IFA): samples were tested for the presence of RSV with the Respiratory Panel I Viral Screening and Identification Kit (Chemicon International, Inc., Temecula, CA).

Statistical analysis

The data was analyzed with the program SPSS (Statistical Package for Social Sciences) 8.0 for Windows (SPSS Inc., Chicago, IL) for obtaining absolute frequencies and for comparing variables. Mean, standard deviation, median and mode values were obtained for age and duration of hospitalization. The chi-square ($\chi^2$) test was used for comparison of proportions; the significance level was established as 0.05 (5%).

## Results

Samples from 317 children with ARI symptoms were obtained; 24% (76/317) of them were positive for RSV by IFA. Their age varied from less than one to 54 months old (mean of 11.0 ± 12.6 months, median of six months and mode of one month). Approximately 70% (53/76) of the RSV-infected children were younger than 12 months old and 53% (40/76) were male.

About 81% (62/76) of the samples were collected in PES, 15% (11/76) in PICU and 3.9% (3/76) in NICU. The clinical diagnostics were: 39.5% (30/76) of the cases presented bronchiolitis, 34% (26/76) URTI, 15% (11/76) pneumonia and 12% (9/76) bronchitis/laryngotracheobronchitis.

Fifty percent (38/76) of the infected children were hospitalized from one to 63 days (mean 10.6 ± 13.8 days, median of four days and mode of one day). Approximately 60% (23/38) of the hospitalized patients stayed at the hospital for less than seven days, 18% (7/38) from eight to 14 days and 21% (8/38) more than 14 days. All the hospitalized children survived.

In terms of annual distribution, 2.6% (2/76) of all RSV cases occurred in 2000, 44% (33/76) in 2001, 9.2% (7/76) in 2002 and 45% (34/76) in 2003. Regarding seasonality, 11% (8/76) of cases happened in February, 28% (21/76) in March, 20% (15/76) in April, 32% (24/76) in May, 9.2% (7/76) in June and 1.3% (1/76) in August.

Patients were also grouped according to age and clinical diagnosis (Table 1), with a predominance of bronchiolitis (36%, 27/76) and pneumonia (13%, 10/76) in children younger than 12 months old. The correlation between age group and disease progression to hospitalization was determined (Table 2); Most of the hospitalizations (87%, 33/38) involved children younger than 12 months old. Bronchiolitis and pneumonia were the principal clinical diagnosis in hospitalized children (Table 3).

## Discussion

Given that HC/UFU is a regional medical reference center that cares for patients who generally require complex treatments, the samples that were collected for this study involve only a portion of the children with ARI. Most probably, these samples were from children with especially severe symptoms. Though many children presented the criteria for inclusion in this study, samples from only 317 patients were collected, due to the limit established for processing and storage of samples (five samples/working day). This limitation was one of the factors that contributed to the variations in the quantity of samples collected throughout the years,

**Table 1.** Distribution of patients with acute respiratory infections according to age and clinical diagnosis

| | Clinical diagnosis | | | | |
|---|---|---|---|---|---|
| **Age (in months)** | **Bronchiolitis** | **URTI*** | **Bchtis/Ltbchtis**** | **Pneumonia** | **Total** |
| <1-12 | 27 | 14 | 2 | 10 | 53 |
| 13-54 | 3 | 12 | 7 | 1 | 23 |
| Total | 30 | 26 | 9 | 11 | 76 |

*URTI – Upper respiratory tract infection.**Bchtis/Ltbchtis – Bronchitis and laryngotracheobronchitis.$\theta^2$ Pearson= 20.91; P= 0.0001.

**Table 2.** Distribution of patients with acute respiratory infections according to age and progression or not to hospitalization

| | Progression | | |
|---|---|---|---|
| **Age (in months)** | **Hospitalization** | **No hospitalization** | **Total** |
| <1-12 | 33 | 20 | 53 |
| 13-54 | 5 | 18 | 23 |
| Total | 38 | 38 | 76 |

$\theta^2$ Pearson= 10.54; P= 0.001.

**Table 3.** Distribution of patients with acute respiratory infections according to progression to hospitalization and clinical diagnosis

| | Clinical diagnosis | | | | |
|---|---|---|---|---|---|
| **Hospitalization** | **Bronchiolitis** | **URTI*** | **Bchtis/Ltbchtis**** | **Pneumonia** | **Total** |
| Yes | 24 | 2 | 2 | 10 | 38 |
| No | 6 | 24 | 7 | 1 | 38 |
| Total | 30 | 26 | 9 | 11 | 76 |

*URTI – Upper respiratory tract infection.**Bchtis/Ltbchtis – Bronchitis and laryngotracheobronchitis. $\theta^2$ Pearson= 39.56; P= 0.0001.

with high RSV rates (detection/no. samples collected) in 2001 and 2003. The detection of RSV in 24% of the samples in our study was similar to the rates observed by others [16,25]. Furthermore, the slightly higher incidence of RSV infections in males that we found (52.6%) had also been reported previously [7,8,14, 15]. RSV has been referred to as the main agent responsible for severe symptoms, such as bronchiolitis and pneumonia [2,4,13], especially during the first year of life [8,10,12,16]. In our study, 54% of the children that tested positive for RSV had bronchiolitis and pneumonia and 90% were younger than one year old, suggesting that illnesses caused by RSV might be severe for these children and thus require a fast and effective medical intervention.

In fact, most of the infection cases caused by RSV were cared for at the PES of the HC/UFU. There was a highly significant correlation between children younger than one year old with bronchiolitis and pneumonia diagnosis (Table 1, P=0.0001) and progression to hospitalization (Table 2, P=0.001). Also, a correlation was observed between the progression to hospitalization and children younger than one year old (Table 3, P=0.0001). Thus, among children less than one year old, those with bronchiolitis or pneumonia had a higher possibility of having RSV infection and of needing hospitalization than those with other clinical symptoms.

In other studies [2,15,26], RSV was referred to as the main viral pathogen associated with pediatric hospitalization. However, the 50% rate of hospitalization that we found is in contrast with the indexes of 2% found by others for developed countries [13,27] and of 13% and 29.5%, for cases treated outside of hospital facilities and those cared for in emergency units, respectively [12]. It is possible that this discrepancy is due to the fact that most of the children in our study (especially those cared for at PES) were transferred from basic health units, after initial care.

With regard to the annual distribution of RSV cases in Brazil, no uniform seasonality has been observed [12,14-16], probably due to the weather variations in the different geographic regions. RSV occurs from February to August, with a peak in May. It is important to define the seasonality of respiratory viruses, since it is possible to predict the etiology of some illnesses based on symptoms and on virus seasonality, even though clinical manifestations of various respiratory syndromes are normally of low intensity [28].

We were able to demonstrate the presence and importance of RSV in respiratory infections. Annual outbreaks particularly affect younger children; these children have moderate to severe clinical symptoms of ARI that require medical care and often need to be hospitalized. However, monitoring is necessary in a broader population and for a longer period of time in order to better delineate the clinical and epidemiological behavior of RSV in this age range and in this region.

## Acknowledgements

We thank Dr. Dean D. Erdman of the Respiratory and Enteroviruses Branch, Centers for Disease Control and Prevention (CDC, Atlanta, GA) for providing the IFA kit and the Laboratório de Imunologia of Instituto de Ciências Biomédicas (UFU) for lending equipment for this study. We also thank the staff of HC/UFU, where the samples were collected.

## References

1. Caetano J.R.M., Bordin I.A.S., Puccini R.F., Peres C.A. Fatores associados à internação hospitalar de crianças menores de cinco anos, São Paulo, SP. Rev Saúde Pública **2002**;36(3):285-91.
2. Rodrigues J.C., Filho L.V.F.S., Bush A. Diagnóstico etiológico das pneumonias – uma visão crítica. J Pediatr (Rio J) **2002**;78 Suppl 2:S129-40.
3. Benguigui Y. Acute respiratory infections control in the context of the IMCI strategy in the Americas. Rev Bras Saude Mater Infant **2003**;3(1):25-36.
4. Davies H.D., Matlow A., Petric M., et al. Prospective comparative study of viral, bacterial and atypical organisms identified in pneumonia and bronchiolitis in hospitalized Canadian infants. Pediatr Infect Dis J **1996**;15(4):371-5.
5. Law B.J., Carbonell-Estrany X., Simoes E.A.F. An update on respiratory syncytial virus epidemiology: a developed country perspective. Respir Med **2002**;96 Suppl B:S1-7.

6. Black C.P. Systematic review of the biology and medical management of respiratory syncytial virus infection**.** Respir Care **2003**;48(3):209-31; discussion 231-3.
7. Queiroz D.A.O., Durigon E.L., Botosso V.F., et al. Immune response to respiratory syncytial virus in young Brazilian children. Braz J Med Biol Res **2002**;35(10):1183-1193.
8. Miyao C.R., Gilio A.E., Vieira S., et al. Infecções virais em crianças internadas por doença aguda do trato respiratório inferior. J Pediatr (Rio J) **1999**;75(5):334-44.
9. McIntosh K. Vírus sincicial respiratório. In: Behrman R.E., Kliegman R.M., Arvin A.M. (Eds). Nelson Tratado de Pediatria. 15. ed. Rio de Janeiro: Guanabara Koogan; **1997**. p. 1050-2.
10. Farhat C.K., Cintra O.A.L., Tregnaghi M.W. Vacinas e o trato respiratório – o que devemos saber? J Pediatr (Rio J) **2002**;78 Suppl 2:S195-204.
11. Sistema de Informação sobre Mortalidade 1979-1993. Dados de declaração de óbito (Data on CD-ROM). Fundação de Saúde, Brasília, Junho **1995**.
12. Durigon E.L., Takahashi V.N.V.O., Soares P.B.M., Botosso V.F. Vírus respiratório sincicial humano: revisão e levantamento dos dados brasileiros. [S.l.]: Abbott **2001**; 16 p. Apostila.
13. Handforth J., Friedland J.S., Sharland M. Basic epidemiology and immunopathology of RSV in childen. Paediatr Respir Rev **2000**;1(3): 210-4.
14. Nascimento J.P., Siqueira M.M., Sutmoller F., et al. Longitudinal study of acute respiratory diseases in Rio de Janeiro: occurrence of respiratory viruses during four consecutive years. Rev Inst Med Trop São Paulo **1991**;33(4):287-96.
15. Vieira S.E., Stewien K.E., Queiroz D.A.O., et al. Clinical patterns and seasonal trends in respiratory syncytial virus hospitalizations in São Paulo, Brazil. Rev Inst Med Trop São Paulo **2001**;43(3):125-31.
16. Moura F.E.A., Borges L.C., Portes S.A.R., et al. Respiratory syncytial virus infections during an epidemic period in Salvador, Brazil. Viral antigenic group analysis and description of clinical and epidemiological aspects. Mem Inst Oswaldo Cruz **2003**;98(6):739-43.
17. Costa L.F., Silveira H.L., Oliveira T.M., et al. Incidence of respiratory virus in children less than 5 years old in Uberlândia city, MG, Brazil – January 2001 to May 2003. Virus Reviews and Research, São Paulo **2003**; 8 Suppl 1:245-6.
18. Carvalho C.B., Yokosawa J., Durigon E.L., et al. Circulation of respiratory syncytial virus and other respiratory viruses in children nasopharyngeal specimens in 2001 in the Triângulo Mineiro region, MG. Virus Reviews and Research **2002**; Special Edition: 30-1.
19. Collier L., Oxford J. Human virology. 2. ed. New York: Oxford University; **2000**. p. 79-80.
20. Rezende V.A., Almeida E.R., Bento R.F., et al. Estudo da flora bacteriana e viral na otite média secretora e rinofaringe na infância. Revista Brasileira de Otorrinolaringologia **1999**;65:10-17.
21. Monobe H., Ishibashi T., Nomura Y., Shinogami M., Yano J. Role of respiratory viruses in children with acute otitis media. Int J Pediatr Otorhinolaryngol **2003**;67(7):801-6.
22. Soldatou A., Davies E.G. Respiratory virus infections in the immunocompromised host. Paediatr Respir Rev **2003**;4(3):193-204.
23. Welliver R.C. Review of epidemiology and clinical risk factors for severe respiratory syncytial virus (RSV) infection. J Pediatr **2003**;143(5 Suppl):S112-7.
24. Queiroz D.A.O. Característica da resposta imune ao vírus respiratório sincicial (VRS), em espécimes de crianças lactentes, durante as fases aguda e/ou convalescente [PhD dissertation]. São Paulo (SP): Universidade de São Paulo; **1999**.
25. Viegas M., Barrero P.R., Maffey A.F., Mistchenko A.S. Respiratory virus seasonality in children under five years of age in Buenos Aires, Argentina – a five-year analysis. Journal of Infection **2004**;49(3):222-8.
26. Lichenstein R., King Jr J.C., Lovchik J., Keane V. Respiratory viral infections in hospitalized children: implications for infection control. South Med J **2002**;95(9):1022-5.
27. Collins P.L., McIntosh K., Chanock R.M. Respiratory Syncytial virus. In: Fields B.N., Knipe D.M., Howley P.M. (Eds). Fields Virology. Third edition. New York: Lippincott-Raven Publishers, **1996**, v. 1, p. 1337.
28. Monto A.S. Epidemiology of viral respiratory infections. Am J Med **2002**;112 Suppl 6A:4S-12S.