Journal of Medical Virology 61:275–279 (2000)

Respiratory Syncytial Virus: Changes in Prevalence of Subgroups A and B Among Argentinian Children, 1990–1996

Guadalupe Carballal,[1]* Cristina Videla,[1] María D. Sequeira,[3] Alicia Mistchenko,[2] Paula V. Requeijo,[1] and Juan Arbiza[4]

[1]*Laboratorio de Virología, Centro de Educación Médica e Investigaciones Clínicas N. Quirno (CEMIC), Buenos Aires, Argentina*
[2]*Hospital R. Gutierrez Virología, Buenos Aires, Argentina*
[3]*Instituto Nacional de Epidemiología E. Coni, Santa Fe, Argentina*
[4]*Sección Virología, Facultad de Ciencias, Montevideo, Uruguay*

The frequency of respiratory syncytial virus (RSV) and the distribution of subgroups A and B strains during 7 consecutive years (1990–1996) were examined in two cities of Argentina. Nasopharyngeal aspirates from 1,304 children less than 2 years of age hospitalized with acute lower respiratory infection were studied by indirect immunofluorescence. RSV was detected in 352 cases (26.9%), and the peak activity was observed in midwinter. Subgroup characterization was performed with two monoclonal antibodies against the F protein on nasopharyngeal aspirate smears. Of 195 samples, 174 (89.2%) were identified as subgroup A strains and 21 (10.8%) as subgroup B. Both strains cocirculated during 5 of 7 years studied with subgroup A predominating. Subgroup A occurred at least 8 times as often in all years except for 1994–1995. Children infected by subgroup A were younger than those infected by subgroup B ($P < 0.05$). The association of subgroup A infection with bronchiolitis and subgroup B with pneumonia was statistically significant ($P < 0.03$). ***J. Med. Virol. 61: 275–279, 2000.***

KEY WORDS: respiratory syncytial virus infections; prevalence of subgroups a and b; acute lower respiratory infections in Argentinian children

INTRODUCTION

Respiratory syncytial virus (RSV) is a major pediatric respiratory tract pathogen. It has a worldwide distribution, and in temperate climates epidemic outbreaks are detected in small children every winter [Collins et al., 1996]. Originally, two subgroups A and B were recognized with monoclonal antibodies. The greatest antigenic variation between strains of RSV subgroups was detected in the surface G glycoprotein, but changes were also observed in the F, NP, and M proteins. Extensive antigenic variation of the G epitopes even among strains of the same subgroup has been described with panels of monoclonal antibodies or by reverse transcription-polymerase chain reaction (RT-PCR) and restriction fragment analysis with endonucleases [Mufson et al., 1985; García et al., 1994; Collins et al., 1996; Gottschalk et al., 1996].

In the northern hemisphere, both subgroups cocirculate within a community during the same RSV season. Usually, subgroup A predominates, but in some epidemics subgroup B is detected more frequently. In some countries (e.g., Finland), a clear pattern of alternating predominance between the A and B subgroups, with a 2-year cycle were reported [Waris, 1991]. The antigenic differences between the two subgroups are likely to have an influence on the subsequent susceptibility to reinfection. Reinfection by members of the opposite subgroup being more frequent than homologous reinfection [Mufson et al., 1987].

The importance of the two subgroups in the epidemiology of RSV for the southern hemisphere is not yet completely understood. In Buenos Aires, Argentina, RSV was found to be the major viral pathogen in children less than 4 years of age with acute lower respiratory infection detected in 18–36% of the cases, depending on the age, season, and hospitals studied. The

Grant sponsor: Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina (CONICET); Grant number: 3-012100/88; Grant sponsor: Secretaría de Ciencia y Tecnología (SECYT); Grant number: 92-3010201; Grant sponsor: Fundación Rocca, Buenos Aires, Argentina.

*Correspondence to: G. Carballal, Laboratorio de Virología, CEMIC, Billinghurst 2447 (1425), Buenos Aires, Argentina.
E-mail: gcarballal@cemic.edu.ar

Accepted 2 December 1999

seasonal distribution of RSV exhibited a clear peak during the coldest months of the year, and the outbreaks typically lasted 6 months (May to October) [Weissenbacher et al., 1990; Sequeira et al, 1997; Videla et al., 1998].

The aims of the present study were to determine the RSV frequency, the prevalence of antigenic subgroups A and B, and the clinical features of children less than 2 years of age admitted to the hospital due to acute lower respiratory infection in two cities of Argentina during 7 consecutive years (1990–1996).

MATERIALS AND METHODS

Population

A total of 1,304 children less than 2 years of age, hospitalized due to acute lower respiratory infection in Buenos Aires (the capital city) from 1990 to 1996 and in Santa Fé city from 1993 to 1996, were included in the study. Children eligible for the study were those with clinical diagnosis of respiratory infection of the lower tract and a disease duration of 7 days or less at admission. Children with a history of asthma, human immunodeficiency virus (HIV), or cystic fibrosis, or with nosocomial infections were ineligible.

Acute lower respiratory infection was defined as an illness resulting in two or more of the following signs and symptoms: tachypnea, cough, rales, chest retraction, wheezing, and stridor. The clinical diagnosis of pneumonia, bronchiolitis, and so forth, was made by means of both physical examination and chest radiographic studies according to the World Health Organization (WHO) guidelines [PAHO, 1983]. At admission, a complete clinical history, physical examination, chest radiography, and nasopharyngeal aspirate were obtained. Informed consent was obtained from parents of infants participating in this study.

Virologic Studies

Nasopharyngeal aspirates were collected with a soft sterile catheter by suction with a vacuum pump into a mucus trap and immediately sent on ice in transport media (Hank's balanced salt solution [HBSS] supplemented with 2% fetal calf serum [FCS] and antibiotics) to the laboratory. Nasopharyngeal aspirate smears were tested for RSV antigen by indirect immunofluorescence using specific monoclonal antibodies (Chemicon, USA) and a fluorescein-labeled anti-mouse IgG (Sigma).

RSV Antigenic Typification

In a retrospective study, 195 nasopharyngeal aspirate slides with three spots from children with RSV diagnosis, kept at – 20°C, were studied. Only appropriately preserved smears with enough cells were selected for this study. The typification was performed with two monoclonal antibodies against the glycoprotein F provided by Dr. J.A. Melero from the Instituto de Biología Molecular (Majadahonda, Spain). The monoclonal 47 F was specific for glycoprotein F1 from subgroups A and B, while the monoclonal 2 F detected only subgroup A [García Barrero et al., 1989]. The reaction was revealed with a fluorescein-labeled anti mouse-IgG (Sigma). Readings were performed with a Zeiss microscope fitted with epifluorescence equipment and a mercury lamp.

TABLE I. Subgroups A and B of RSV in Argentinian Children With Acute Lower Respiratory Infection

	RSV positive/studied		Subgroup A		Subgroup B	
Year	n	%	n	%	n	%
1990	27/88	30.6	7/7	100	0/7	0
1991	77/180	42.8	29/30	96.7	1/30	3.3
1992	30/129	23.2	25/26	96.2	1/26	3.8
1993	40/176	22.7	32/32	100	0/32	0
1994	48/143	33.6	8/15	53.3	7/15	46.7*
1995	49/245	20.0	22/31	71.0	9/31	29.0†
1996	81/343	23.6	51/54	94.4	3/54	5.5‡
Total:	352/1,304	26.9	174/195	89.2	21/195	10.8

RSV, respiratory syncytial virus.
*Fisher's exact test; $P < 0.05$ frequency of subgroup B for 1994 vs the rest of the years, except for 1995.
†Fisher's exact test; $P < 0.0008$ frequency of subgroup B for 1995 vs 1993.
‡Fisher's exact test, $P < 0.001$ frequency of subgroup B for 1995 vs 1996.

Data Analysis

Proportions were compared by Fisher's exact test and χ^2 test. Means of age were compared using the *t*-test.

RESULTS

Frequency of RSV

During the 7 consecutive years studied, RSV was detected in 352 of 1,304 (26.9%) samples from children with acute respiratory infection. RSV epidemics were detected every year; detection rate ranged from 42.8% in 1991 to 20% in 1995 (Table I).

In 1991, the high RSV frequency (42.8%) was statistically significant ($P < 0.0005$) relative to the rest of the years studied, except for 1990. Furthermore, RSV activity was first observed in April of 1991, while it started in June for 1990, 1992, 1993, and 1994 and May in 1995 and 1996. A progressive increase in the number of RSV cases in autumn followed, with peaks in midwinter (June, July, and August). Little or no activity was observed during spring or summer.

No differences were observed in the seasonal distribution of RSV between the cities of Buenos Aires and Santa Fe. The latter is located at 500 km north of Buenos Aires, and its climate is more humid.

Frequency of A and B RSV Subgroups

Out of 352 RSV positive smears, 195 (55%) were available for further subgroup characterization. The prevalence of the two subgroups varied over the 7 years studied. From 1990 to 1996, subgroup A strains widely predominated over subgroup B (89.2% vs 10.8%) (Table I), irrespective of total RSV frequencies. The proportion of subgroup A strains to subgroup B was approximately 8 : 1.

The 1994 season was unique in that the lowest number of subgroup A strains were detected (53.3%) and the highest frequency of subgroup B strains (46.7%) were observed. Subgroup B strains were not detected

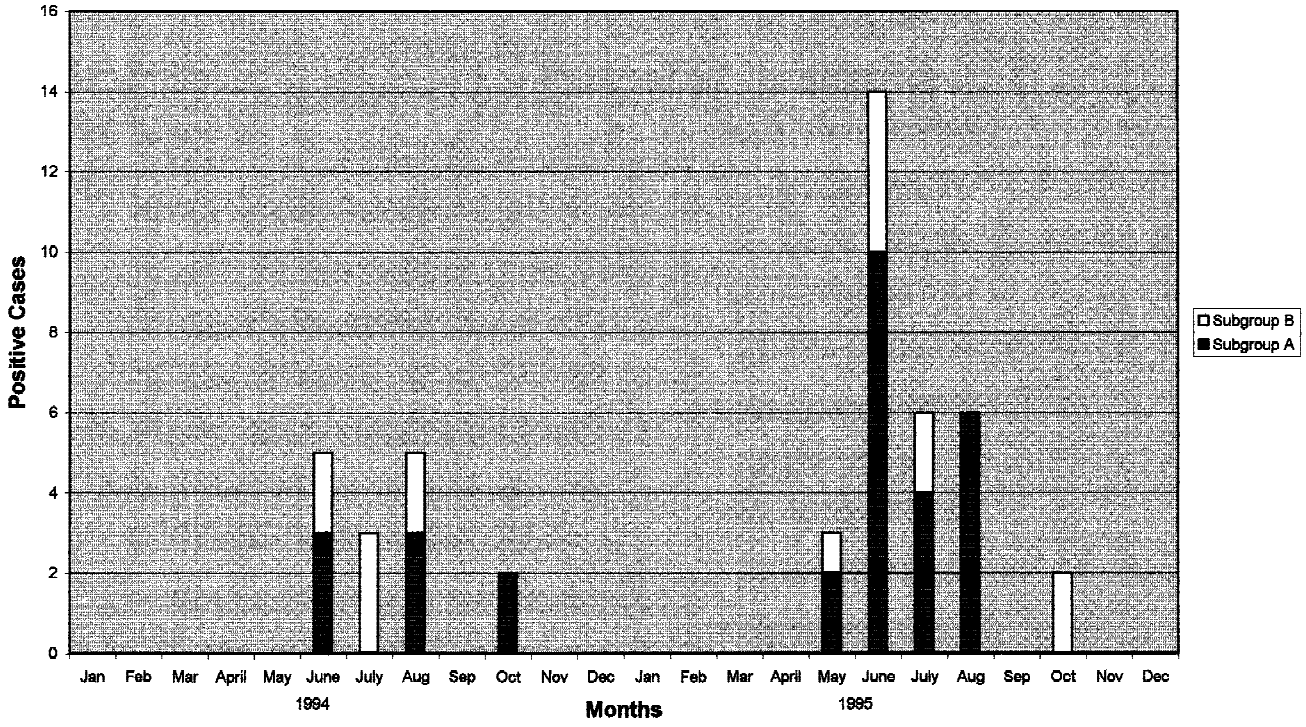

Fig. 1. Monthly distribution of RSV subgroups A and B during 1994 and 1995

during 1990 and 1993, and very low numbers were observed in 1991 and 1992. A significant increase was recorded for 1994 and 1995 in relation to 1993 ($P < 0.0001$ and $P < 0.0008$) while its frequency decreased again in 1996. No differences were noted in the prevalence of RSV subgroups between the hospitals of Buenos Aires and Santa Fe cities from 1993 to 1996, when children from both cities were included in the study.

Figure 1 shows the monthly distribution of the subgroups during 1994 and 1995, a period during which subgroup B was detected more frequently. The cocirculation of both subgroups during some of the coldest months of the year was observed for that period. In 1994, both subgroups cocirculated during June and August, but only subgroup B strains were found in July. In 1995, subgroups A and B were detected together during May, June, and July. In August, only subgroup A strains were observed, while in mid-spring, two subgroup B viruses were detected.

Clinical Features

The mean age of children infected with RSV from subgroup A was significantly lower (4.5 months ± 4.2) than that of children infected with subgroup B (7.3 months ± 5.9) ($P < 0.05$) (Table II). For subgroup A, males were more often infected than were females (1.9: 1 vs 1.2:1).

At admission, 81 of 118 children with subgroup A strain infection (68.6%) had clinical diagnosis of bronchiolitis, and 37 (31.4%) of pneumonia. By contrast, 9 of 14 (64.3%) children with subgroup B infection had pneumonia, while only 5 (35.7%) exhibited bronchiolitis (Table II). The association of subgroup A with clinical diagnosis of bronchiolitis and subgroup B with pneumonia was statistically significant ($\chi^2 = 4.61$, $P < 0.03$).

TABLE II. Features of Children With Acute Lower Respiratory Infection and RSV Diagnosis

Features	Subgroup A	Subgroup B	*P*-value
Age (months)	n = 135	n = 20	
Mean ± SD	4.5 ± 4.2	7.3 ± 5.9	<0.05*
Range	0–18	1–24	
Gender	n (%)	n (%)	
Male	88/135 (65.2)	11/20 (55)	ns
Female	47/135 (34.8)	9/20 (45)	
Ratio male/female	1.9:1	1.2:1	
Clinical diagnosis at admission[a]	n (%)	n (%)	
Bronchiolitis	81/118 (68.6)	5/14 (35.7)	<0.03†
Pneumonia	37/118 (31.4)	9/14 (64.3)	

[a]These data were available for 132 children of the 195 in whom NPAs were typified.
*t-test t = 2.2; $P < 0.05$.
†$\chi^2 = 4.61$; $P < 0.03$.

DISCUSSION

This report describes experience of RSV subgroup infections in Argentinian children during 7 consecutive years (1990–1996). During the study period, the frequency of RSV observed (26.9%) was similar to previous reports for small children from Argentina [Weissenbacher et al., 1990; Sequeira et al., 1997; Videla et al., 1998].

RSV exhibited a marked seasonality with a clear

peak in winter. A wide predominance of subgroup A strains of RSV was observed in children with acute respiratory infection from both, Buenos Aires and Santa Fé and the cocirculation of both subgroups was detected in 5 of the 7 epidemic periods studied (Table I). Subgroup A strains occurred eight times as often as subgroup B strains during all studied years, except for 1994 and 1995, when subgroup B increased. Subgroup B was observed with limited frequency during 1991, 1992, and 1996, and no cases were detected during 1990 and 1993.

According to McIntosh et al. [1993b], when a sufficient number of strains are examined ($n \geq 75$) for a certain geographic area and epidemic period, subgroups A and B can both be detected. Therefore, the small number of samples available for 1990 and 1993 in our study may explain why subgroup B was undetected. However, in 1994, when only 15 strains were typified, the frequencies of both subgroups were similar (subgroup A = 53% vs subgroup B = 47%). The frequency of subgroup B during 1994 was statistically significant in relation to the rest of the studied years, except for 1995. Thus, 1994 and 1995 were unique in that the highest prevalence of subgroup B strains was observed (Table I).

Our results are consistent with those found in the northern hemisphere where the cocirculation of both variants has been detected during the same epidemic, with a predominance of subgroup A in different outbreaks [McIntosh et al., 1993b].

By contrast, the first study performed in Buenos Aires in children admitted to hospital with acute lower respiratory infection showed a predominance of subgroup B during three epidemic periods (1985, 1986, and 1987), with 70 of 117 cases (76.9%) of subgroup B, while subgroup A was observed in 20.9% [Salomon et al., 1988]. In neighboring Uruguay, subgroup A strains of RSV predominated in the epidemics of 1985 and 1986, accounting for 65.7% of all typed specimens. However, in 1987, subgroup B surpassed subgroup A, being detected in 82.4% of samples studied [Russi et al., 1989b].

In Brazil, both group A and group B isolates were identified in all 7 years studied (1982–1988) but their relative importance appeared to vary from year to year. Coincidently with findings from Argentina and Uruguay, subgroup B strains were most common in Brazil during 1987 [Siqueira et al., 1991]. Therefore, 1987 was unique in that subgroup B strains were more prevalent in three countries of the southern hemisphere.

Children infected by subgroup A were younger than those infected by subgroup B (Table II), as also shown for the United States [Taylor et al., 1989] and Uruguay [Russi et al., 1989a]. In children infected by subgroup A, a higher frequency of bronchiolitis was observed, while pneumonia predominated in subgroup B infections. These findings could suggest a higher severity of subgroup B infections, although the number of cases analyzed was low, and diagnoses were made at admission.

Consistency is lacking in the clinical severity of disease caused by the two RSV subgroups. Mufson et al. [1988] also observed that fewer children with subgroup B developed bronchiolitis compared to children with subgroup A infections. McConnochie et al. [1990] determined a predominance of subgroup A for 1985–1987 in the United States and observed that children infected by this subgroup had a more severe disease than those infected by subgroup B. Because most of the studies on RSV types were carried out in hospitalized children, the author questions whether the predominance of subgroup A is a reflex of a greater virulence of this variant, or it represents a real higher frequency of subgroup A in the community. Similar findings were observed by Taylor et al. [1989] and Salomon et al. [1991].

By contrast, Hall et al. [1990] found no difference in the proportion of A and B subgroups between children with minimal respiratory signs and inpatients during 5 years in the United States. A greater clinical severity for subgroup A infections, specifically the A 2 subgroup was suggested, since those children required intensive care more often.

Coincidently, when clinical and progression data from 85 inpatients to the R Gutierrez Hospital, Santa Fé city, included in this study, were examined children with subgroup A infection required oxygen therapy or assisted ventilation more frequently (84% vs 55% for A and B infections, respectively, $P < 0.055$). For infants aged 0–6 months, this difference was increased (95% vs 62% for subgroups A and B, respectively, $P < 0.035$). These data, which cannot be explained by differences in the mean age or the proportion of children with underlying conditions, suggest a greater virulence for subgroup A strains [Imaz et al., 1999].

In Australia, McIntosh et al. [1993a] also observed a predominance of subgroup A from 1989 to 1991, but no differences in the severity of disease between both subgroups were detected. It was postulated that subgroup A probably prevents further infections with subgroup B. By contrast, a recent study performed in Denmark using PCR and nucleic acid restriction analysis found that genotype B-1122 produced more severe disease than genotype A 2311 in infants aged 0–11 months old, as assessed on the length of hospitalization and use of respiratory support as severity index [Hornsleth et al., 1998].

Future studies of the antigenic variants observed within the two major RSV subgroups employing expanded panels of monoclonal antibodies, RT-PCR, sequencing, or RNase A mismatch cleavage method [Cristina et al., 1991] may be useful to elucidate differences in virulence.

The predominance of subgroup A during 7 consecutive years (1990–1996) was shown for children hospitalized with acute lower respiratory infection in Argentina and the cocirculation of both subgroups was detected in 5 of 7 epidemic years studied.

Future studies over a longer period in inpatients with infection of the lower respiratory tract and also in outpatients with mild respiratory infections from dif-

ferent age groups and from diverse geographic areas of our country, as well as studies on strain differences in pathogenicity will be required to a better understanding of RSV epidemiology in Argentina.

ACKNOWLEDGMENTS

We thank the technical assistance of Cristina Juarez, Carmen Ricarte, and Beatriz Ebekian.

REFERENCES

Collins PL, McIntosh K, Chanock R. 1996. Respiratory syncytial virus. In: Fields BN, Knipe DM, Howley PM, editors. Virology. 6th ed. Philadelphia: Lippincott-Raven. p 1313–1347.

Cristina J, Moya A, Arbiza J, Russi J, Hortal M, Albo C, Garcia Barreno B, Garcia O, Melero J, Portela A. 1991. Evolution of the G and P gene of human respiratory syncytial virus (subgroup A) studied by RNase A mismatch cleavage method. Virology 184:210–218.

García O, Martin M, Dopazo J, Arbiza J, Frasbasile S, Russi J, Hortal M, Perez Brena P, Martinez J, García Barrero B, Mellero JA. 1994. Evolutionary pattern of human respiratory syncytial virus (subgroup A): cocirculating lineages and correlation of genetic and antigenic changes in the G glycoprotein. J Virol 68:5448–5459.

Garcia-Barreno B, Palomo C, Peña C, Delgado T, Perez-Breña P, Melero J. 1989. Marked differences in the antigenic structure of human respiratory syncytial virus F and G glycoproteins. J Virol 63:925–932.

Gottschalk J, Zbinder R, Kaempt L, Heinzer I. 1996. Discrimination of Respiratory Syncytial virus subgroups A and B by reverse transcription-PCR. J Clin Microbiol 34:1–43.

Hall CB, Walsh E, Schnabel KC, Long CE, McConnochie KM, Hildreth SW, Anderson LJ. 1990. Occurrence of Groups A and B of respiratory syncytial virus over 15 years: associated epidemiologic and clinical characteristic in hospitalized and ambulatory children. J Infect Dis 162:1283–1290.

Hornsleth A, Klug B, Nir M, Johansen J, Hansen K, Christensen L, Larsen L. 1998. Severity of respiratory syncytial virus disease realted to type and genotype of virus and to cytokine values in nasopharyngeal secretions. Pediatr Infect Dis J 17:1114–1121.

Imaz MS, Sequeira MD, Videla C, Veronessi I, Cocoglio R, Zerbini E, Carballal G. 1999. Clinical and epidemiologic characteristic of respiratory syncytial virus Subgroups A and B infections in Santa Fé, Argentina. J Med Virol (in press).

McConnochie K, Hall C, Walsh E, Roghmann KJ. 1990. Variation in severety of Respiratory Syncytial virus infections with subtype. J Pediatrics 117:52–62.

McIntosh E, De Silva L, Oates R. 1993a. Clinical severity of respiratory syncytial virus group A and B infection in Sydney, Australia. Pediatr Infect Dis J 12:815–819.

McIntosh K, Halomen P, Ruuskanen O. 1993b. Report of a workshop on respiratory viral infections: epidemiology, diagnosis, treatment and prevention. Clin Infect Dis 16:151–164.

Mufson MA, Orvell C, Rafnar B, Norrby E. 1985. Two distinct subtypes of human respiratory syncytial virus. J Gen Virol 68:3125–3135.

Mufson MA, Belshe RB, Orvell C, Norrby E. 1987. Subgroup characteristics of respiratory syncytial virus strains recovered from children with two consecutive infections. J Clin Microbiol 25:1535–1539.

Mufson MA, Belshe RB, Orvell C, Norrby E. 1988. Respiratory Syncytial virus epidemics: variable dominance of subgroup A and B strains among children, 1981–1986. J Infect Dis 157:143–147.

Pan American Health Organization. 1983. Acute respiratory infections in children. RD 21/1. Washington, DC: PAHO, Regional office of the World Health Organization.

Russi J, Chiparelli H, Montano A, Etorena P, Hortal M. 1989a. Respiratory Syncytial virus subgroups and pneumonia in children. Lancet 28:1039–1040.

Russi J, Delfraro A, Arbiza J, Chiaparelli H, Orvell C, Grandien M, Hortal M. 1989b. Antigenic characterization of respiratory syncytial virus associated with Acute Respiratory Infections in Uruguayan children from 1985 to 1987. J Clin Microbiol 27:1464–1466.

Salomon H, Russi J, Grandien M, Orvell C, Avila M, Hortal M, Weissembacher M. 1988. Variantes antigénicas del virus respiratorio sincicial en Argentina y Uruguay. Rev Argentina Microbiol 20: 147–150.

Salomon H, Avila MM, Cerqueiro MC, Orvell C, Weissembacher M. 1991. Clinical and epidemiologic aspects of respiratory virus antigenic variants in argentinian children. J Infect Dis 163:1167.

Sequeira MD, Zerbini E, Imaz MS, Veronessi Y, Latini O, Cociglio R, Pierini J. 1997. Etiología de las infecciones agudas del tracto respiratorio inferior en niños menores de cinco años en Santa Fé. Medicina (Buenos Aires) 57:191–199.

Siqueira M, Nascimento J, Anderson L. 1991. Antigenic characterization of respiratory syncytial virus group A and B isolates in Rio de Janeiro, Brazil. J Clin Microbiol 29:557–559.

Taylor CE, Morrow S, Scott M, Young B, Toms G. 1989. Comparative Virulence of respiratory syncytial virus Subgroups A and B. Lancet 8:777–778.

Videla C, Carballal G, Misirlian A, Aguilar M. 1998. Acute lower respiratory infections due to respiratory syncytial virus and adenovirus among hospitalized children from Argentina. Clin Diagn Virol 10:17–23.

Waris M. 1991. Pattern of respiratory syncytial virus epidemics in Finland: two year cycles with alternating prevalence of subgroup A and B. J Infect Dis 163:464–469.

Weissembacher M, Carballal G, Avila M, Salomón H, Harisiadi J, Catalano M, Cerqueiro MC, Murtagh P. 1990. Etiologic and clinical evaluation of acute lower respiratory tract infections in young Argentinian children: an overview. Rev Infect Dis 12/8:S889–S998.