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Viral etiology of acute respiratory infections among children in Porto Alegre, RS, Brazil

Etiologia viral das infecções respiratórias agudas em Porto Alegre, RS, Brasil

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Abstract *Although acute respiratory infections (ARIs) are a major cause of child morbidity and mortality in Southern Brazil, little information is available on their seasonality and viral etiology. This study was conducted on children under 5 years of age with ARI to assess viral etiology in the State of Rio Grande do Sul, from 1990 to 1992. A total of 862 nasopharyngeal secretion (NPS) samples were tested using indirect immunofluorescence. The results showed that 316 (36.6%) NPS samples were positive: 26.2% for RSV, 6% for adenovirus, 1.7% for influenzaviruses, 1.5% for parainfluenzaviruses, and 1.2% for mixed infection. The mean viral prevalence rates in out-patient services, emergency wards, and in-patient hospital wards were 26.7%, 53% and 42.3%, respectively. Respiratory syncytial virus (RSV) and adenovirus accounted for 91.4 % of the viral diagnoses. RSV was more frequent in children under one year of age at the three levels of health care and was prevalent in infants under six months. Adenovirus was the most prevalent pathogen in hospitalized children, in 1992. Influenza A virus showed an increased prevalence with age among out-patient children. This study shows the annual occurence of viral respiratory infections in the coldest months, with a significant annual variation in the frequency of RSV infection.*

Key-words: *Respiratory syncytial virus. Adenovirus. Acute respiratory infections. Child.*

Resumo *Embora as IRAs sejam importante causa de morbidade e mortalidade infantil no sul do Brasil, poucas e esparsas informações são disponíveis sobre sazonalidade e etiologia viral. Este estudo foi realizado em crianças menores de 5 anos de idade com IRAs para avaliar a importância da etiologia viral no Rio Grande do Sul, no período de 1990 a 1992. Foram processadas 862 secreções de nasofaringe, por imunofluorescência indireta. Os resultados mostraram que 316 (36,6%) amostras foram positivas: 26,2% para vírus respiratório sincicial (VRS), 6% para adenovírus, 1,7% para vírus influenza, 1,5% para vírus parainfluenza e 1,2% para infecção mista. As médias das prevalências virais nos serviços de ambulatório, emergência e hospitalizados foram de 26,7%, 53% e 42,3%, respectivamente. VRS e adenovírus foram responsáveis por 91,4% dos diagnósticos virológicos positivos. O VRS foi mais freqüente em menores de 1 ano, nos três níveis de atenção à saúde, sendo mais prevalente em menores de 6 meses. O adenovírus foi o patógeno mais prevalente em crianças hospitalizadas em 1992. O vírus influenza A mostrou uma tendência de acréscimo da positividade com o aumento da idade nas crianças de ambulatório. Este estudo mostrou a ocorrência anual de infecções respiratórias virais nos meses frios, com variação anual significativa na freqüência da infecção por VRS.*

Palavras-chaves: *Vírus respiratórios sinciciais. Adenovirus. Infecções respiratórias agudas. Criança.*

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Over 200 viral serotypes are associated with human respiratory diseases[15], with the most frequently reported types in newborns and children under five years of age being respiratory syncytial virus (RSV), parainfluenza type 3 virus (PF3), adenovirus (Adeno), influenza virus (FLU), and enterovirus[11 23 27].

There is evidence of marked seasonal variation in viral incidence, which is higher during the colder months in countries with temperate climates[1 27]. In countries with tropical climates the seasonality is variable according to the temperature–dependent local pattern, humidity or rainfall[3 11 12 26]. Differences in the prevalence of viral infections can also be observed between community groups[5 6 16].

Although recommended by the World Health Organization[49], until 1993, epidemiological studies on acute respiratory infections (ARIs) of viral etiology in the Brazilian pediatric population had only been performed in a few medical research centers in the Southeast[17 40 42] and North[14], showing different seasonal patterns. The Southern Region of Brazil has a distinct climate from the rest of the country, similar to the countries of the Southern Cone of South America (Argentina, Chile, Paraguay, and Uruguay). Several studies from these countries have reported seasonal trends and the relevance of viral ARIs[21 29 45 48].

In Rio Grande do Sul, Brazil's southernmost State, ARIs are the third most frequent cause of infant mortality, with non-homogeneous rates around the State and apparently with a higher frequency in the Porto Alegre metropolitan area[31 32]. In this area, respiratory diseases account for the greatest flow of patients to different levels of health care, with an increase in child mortality and hospitalization in the cold months, especially June and July[44]. As of 1989, no information was available on the frequency of viral respiratory diseases, although RSV had already been reported as the etiologic agent for bronchiolitis in Porto Alegre[22].

This is the first viral epidemiological study in Rio Grande do Sul, conducted on pediatric patients treated at various health care facilities located in the municipality of Porto Alegre from 1990 to 1992, aimed at a more in-depth evaluation of the relevance of acute viral respiratory diseases.

MATERIAL AND METHODS

Study Design. Porto Alegre is the capital of Rio Grande do Sul State, Brazil, located at 10m altitude, latitude 30.01 S, longitude 51.13 W, mean annual temperature 19ºC, area 497km², with a population of 1.5 million.

This study was conducted in two hospital from the city of Porto Alegre: Hospital da Criança Santo Antônio (HCSA) and Hospital de Clínicas de Porto Alegre (HCPA), in addition to an outlying urban public health clinic where out-patient NPS samples were obtained. At the HCSA, samples were collected from patients treated in the out-patient facility, emergency wards, in-patient wards, and intensive care unit (ICU). At the HCPA, samples were taken from patients in the in-patient wards and ICU. In addition to the Porto Alegre population itself, these hospitals also treat patients from neighboring municipalities and the interior of the States. Children were selected by pediatricians during the entire study.

Samples from hospitalized patients were taken from May to September 1990, May to December 1991, and throughout the entire year of 1992. Included in the study were 18 samples taken in 1992 from other hospitals in Porto Alegre. Emergency ward samples were taken from July to September 1990, July to December 1991, and throughout the entire year of 1992. In hospital, the out-patient samples were taken from May to October 1991 and May to November 1992, and in the outlying public health clinic were taken from May to September 1992.

Children eligible for the study were those under 5 years of age, with clinical diagnosis of acute upper and/or lower respiratory infection, and less than 7 days of evolution on the day of examination. Acute upper and/or lower respiratory infection was defined as an illness resulting in two or more of the following signs and symptoms: history of rapid breathing, cough, moaning, perioral cyanosis, fever, retraction, tachypnea, wheezing, bronchophony, and rales. Clinical diagnoses of pneumonia, bronchiolitis, etc., were made by physical examination and confirmed whenever necessary by chest x-ray. Presence of nasopharyngeal secretion for a sample was indispensable. Children were excluded from the study who presented exanthematic syndromes suggestive of rubella, measles, and chicken pox, as well as pertussis-like syndromes (whooping cough, cytomegalic inclusion disease, and others).

Information about number of death per mouth in children under one year of age, from 1990 to 1992, were obtained from the Health's Secretary, Porto Alegre, Brazil.

Meteorological data were obtained from the 8th Meteorology District, Porto Alegre, Brazil.

Indirect fluorescent antibody test (IFAT). Detection of RSV, adenovirus, influenza A virus, influenza B virus, and parainfluenza type 3 virus in cells shed by the respiratory tract was performed by IFAT, as previously[34,] using commercially available monoclonal antibodies (Chemicon).

Statistical analysis. Analysis of statistical significance was performed using the chi-square test, with Yates correction when necessary, as well as Pearson's correlation. Fisher's exact test was used for values under five. An alpha value equal to or less than 0.005 was considered statistically significant

Statistical analyses for prevalence of viral infection in this study only included the samples from 1991 and 1992.

RESULTS

From May 1990 to December 1992, a total of 862 NPS samples were tested for the four viruses by antigen detection. Of these, 386 were from hospitalized children, 150 from children treated in the hospital emergency ward, and 326 from children treated in the hospital out-patient facility and the outlying public health clinic. By hospitalized patients we refer to patients admitted to in-patient hospital wards and the ICU. The number of virus antigen-positive patients was 316 (36.6%): 226 (26.2%) for RSV, 52 (6%) for adenovirus, 15 (1.7%) for influenzaviruses, 13 (1.5%) for parainfluenzaviruses, and 10 (1.2%) for mixed infection. Acute lower respiratory infection was detected in 528 (55.3%) of the children, and 297 (31.2%) presented acute upper respiratory infection.

Of the 730 patients who reported their municipality of origin, 53% were from the municipality of Porto Alegre and 43% from municipalities in the State of Rio Grande do Sul.

Table 1- Number of nasopharyngeal secretion samples examined according to level of health care and percentage of viral antigen detection by gender in children (n = 862), Porto Alegre, Brazil.

	1990		1991*		1992		Total	
	n	%	n	%	n	%	n	%
Out-patient			47		279		326	
M	-		10	43.5	40	26.5	50	28.2
F	-		9	37.5	28	22.4	37	24.8
Emergency	16		88		46		150	
M	4	30.8	31	64.6	11	33.3	46	48.9
F	0	00.0	25	62.5	4	30.8	29	51.8
In-patient[a]	112		150		124		386	
M	28	36.8	41	46.6	29	34.5	98	39.5
F	10	27.8	33	53.2	13	32.5	56	40.6
Total	128		285		449		862	

Compared to viral acute respiratory infection by Gender in 1992: $p < 0.0001$;

[a] – In-patient wards and ICUs, M (male) and F (female)

The prevalence of viral infections showed a similar distribution for males and females (Table 1), for out-patient, emergency-ward, and hospitalized patients. The analysis of the years 1991 and 1992 showed an increase in the positive rate for both sexes in 1991 ($p < 0.0001$).

Table 2, referring to prevalence of viral infections by age and viral types found at the various health care levels, shows a higher frequency of RSV in children under one year of age at all three levels. The percentage of positive NPS samples did not vary significantly in the various age brackets ($p > 0.05$ for all the comparisons between different brackets), but the etiologic agent displayed some significant differences. RSV infections were more prevalent in patients under six months than in those 12-23 months ($p = 0.0162$) and 36-47 months of age ($p = 0.0359$). In hospitalized children aged 12-23 months and out-patient children in the one to three year age bracket, the proportion of adenovirus infection was similar to that of RSV. Influenza A virus displayed increased positivity with age in out-patient children.

Figure 1 refers to the annual prevalence of viral infections in the out-patient, emergency, and in-patient facilities, with the types of virus detected. In all cases there was a higher positive rate in the year 1991 and a higher prevalence of RSV as compared to the other viruses, except for hospitalized patients in 1992, in whom adenovirus was more prevalent. For both hospitalized patients and those in other health case facilities, there was an increase in the detection of adenovirus in 1992. In emergency-ward patients, viral prevalence was higher in 1991 ($p=0.0006$). RSV was the most frequent pathogen in the three years studied, with the highest prevalence in 1991 ($p < 0.0001$), followed by parainfluenza type 3 virus (3.4%) in 1991 and adenovirus (8.7%) in 1992 ($p = 0.047$ – Fisher's exact test). In hospitalized children, there was also a higher number of infected patients in 1991 ($p = 0.0099$). RSV was the most prevalent pathogen in 1991 ($p < 0.0001$) and adenovirus was the most prevalent in 1992 ($p < 0.0001$).

Comparing viral prevalence in two out-patient facilities (Table 3), we note a higher prevalence in the hospital out-patients facility as compared to the outlying public health clinic ($p = 0.0034$). Annual viral prevalence in the hospital out-patient facility was statistically similar in 1991 and 1992 ($p = 0.081$, with Yates Correction).

The number of children in which one of the viruses studied was detected in the period from 1990 to 1992, by months, is shown in Figure 2.

Another interesting aspect was observed in comparing temporal series of mean monthly temperatures, number of deaths, and detection of RSV and adenovirus in children under one year of age with ARI (Figure 3). The analysis showed a significant negative correlation between the average monthly

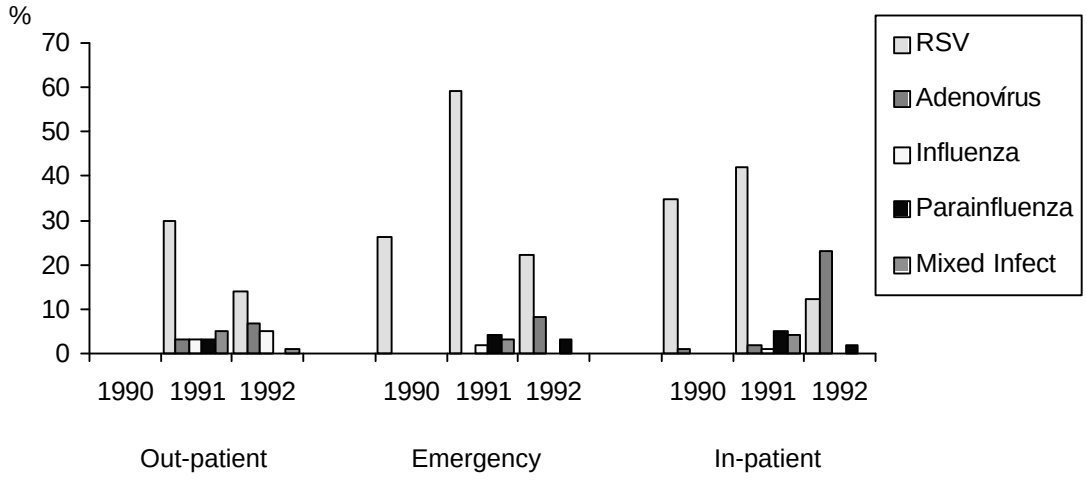

Figure 1-Prevalence of viral ARI by level of medical care, 1990 – 1992, Porto Alegre, Brazil.

Table 2 - Prevalence of viral acute respiratory infections by age (n = 862), Porto Alegre, Brazil.

Age (months)	0-6		7-11		12-23		24-35		36-47		48-59		≥ 60	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Out-Patient														
RSV	12	71	14	67	11	50	8	67	4	36	2	50	-	-
adeno	3	18	3	14	6	27	1	8	4	36	1	25	-	-
FLU A	1	6	4	19	3	14	2	17	2	8	1	25	-	-
FLU B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PF3	-	-	-	-	1	5	-	-	-	-	-	-	-	-
mixed infection	1b	6	-	-	1a	5	1a	8	1b	9	-	-	-	-
Emergency														
RSV	53	86	10	91	1	100	-	-	-	-	-	-	1	100
adeno	4	7	-	-	-	-	-	-	-	-	-	-	-	-
FLU A	1	2	-	-	-	-	-	-	-	-	-	-	-	-
FLU B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PF3	2	3	1	9	-	-	-	-	-	-	-	-	-	-
mixed infection	2ac	3	-	-	-	-	-	-	-	-	-	-	-	-
In-Patient														
RSV	84	78	20	67	4	44	-	-	-	-	-	-	2	29
aAdeno	12	11	10	33	5	56	-	-	-	-	-	-	3	43
FLU A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FLU B	1	1	-	-	-	-	-	-	-	-	-	-	-	-
PF3	8	7	-	-	-	-	-	-	-	-	-	-	1	14
mixed infection	3ac	3	-	-	-	-	-	-	-	-	-	-	1d	14
Nº + samples	187		62		32		12		11		4		8	
Total samples	449		189		104		48		39		15		18	
Positive samples (%)	41.6		32.8		30.8		25		28.2		26.7		44.4	

a - RSV + Adeno (5); b - RSV + FLU B (2); c - RSV + PF3 (2); d - VRS + Adeno + PF3 (1)

Table 3- Prevalence of viral infection in hospital pediatric out-patients and outlying public health clinic (n =326), Porto Alegre, Brazil.

	HCSA *				U.S		
	1991		1992		1992		
	n	%	n	%	n	%	Total (n)
RSV	14	29.8	28	15.2	9	9.5	51
Adenovirus	1	2.1	15	8.2	2	2.1	18
Influenza A	1	2.1	10	5.4	2	2.1	13
Influenza B	0	-	0	-	0	-	0
Parainfluenza 3	1	2.1	0	-	0	-	1
Mixed Infection	2	4.3[ab]	2	1.1[ab]	0	-	4[ab]
Total positive	19		55		13		87
Total samples	47		184		95		326
Positive samples (%)	40.4		29.9		13.7		26.7

* Versus viral infection in outlying public health clinic, p = 0.0034; [a] - RSV + Adeno (2);

[b] - RSV + FLU B (2);

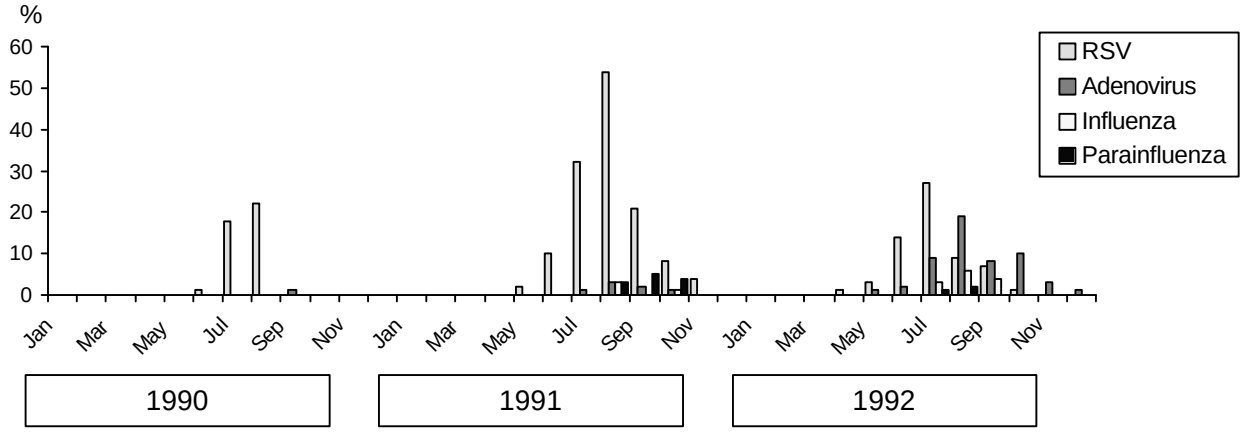

Figure 2 - Seasonality of viral infection in children with ARI, 1991-1992, Porto Alegre, Brazil. he samples were taken from May to September 1990, May to December 1991, and throughout the entire year of 1992.

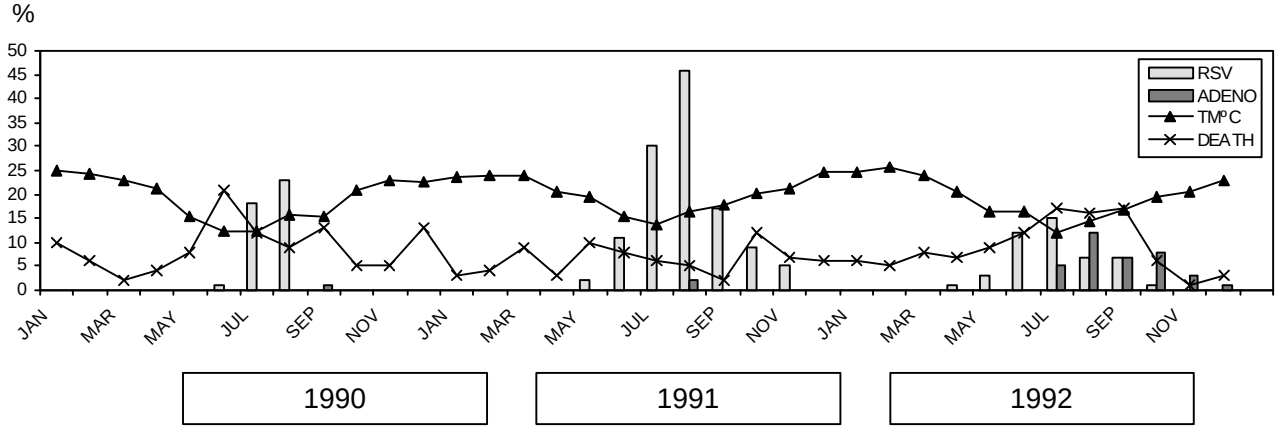

Figure 3 - Mean monthly temperature, mortality, and viral detection in children under one year of age, Porto Alegre, Brazil.

temperature and detection of RSV and adenovirus with r = - 0.599 and p < 0.0001. A similar correlation was observed between the number of deaths per month in children under one year of age with ARI and average monthly temperature, with r = - 0.605 and p < 0.0001. Therefore there was not a significant correlation between the number of deaths and viral detection. Information obtained from the Health's Secretary showed that the year of 1991 was atypical in terms of the number of deaths per month due to ARI in children under one year.

DISCUSSION

Annual variations have been detected in the viral prevalence of ARIs[8 11 23 36]. Frequency of viral detection in ARI depends on many factors, such as patient selection criteria, disease severity, season of the year, and diagnostic method. In the current study we selected ARI patients from different levels of health care in order to encompass mild, moderate, and severe respiratory syndromes existing in the population.

To detect the viral agents we employed indirect immunofluorescence (IIF) due to the speed and facility in diagnosis. We hadn't no laboratory conditions for cell culture viral isolation. In addition, the IFAT have ability to detect more than one type of virus per NPS sample, frequently not detected in cell cultures[10] and allow a rapid characterization of the viral groups and sub-groups circulating in the population, especially for influenza viruses and RSV[30 35]. IIF showed a high level of agreement with viral isolation, although the latter requires greater laboratory support[27].

Studies with RSV[6 20], such as this one, showed a similar viral prevalence for male and female patients, although a higher proportion of boys with lower respiratory tract infections had been hospitalized or treated in the emergency wards compared to girls. At the primary health care level, where there was a greater flow of children with upper respiratory tract infections and a slight predominance of male patients, there was also no significant difference observed between males and females. The significant increase in viral prevalence in both sexes in 1991 was due mainly to greater detection of RSV at the various health care levels, suggesting that this virus circulated more in the population that year.

With regard to age, our data on viral prevalence agreed with those of broader virological surveys in populations of in-hospital and out-patient pediatric patients[11 12 17 19], differing in the 24-35 month age bracket[11 12 19] and that of 36-47 months[11]. In these age brackets, one needs to consider the prevalence of enterovirus, herpes simplex virus, and rhinovirus detected by Hazlett *et al*, the small sample studied by Ong *et al*, and the absence of NPS samples in hospitalized and emergency-ward patients in our study. RSV was the most frequently detected pathogen in the first year of life in all three levels of health care, in agreement with Chanock *et al*., and was associated with upper and lower respiratory tract diseases[36]. The high prevalence of RSV in children up to six months of age was higher than that found in part of the literature, varying from 38% to 65.9%[6 7 8 20], but it was similar to that observed in Argentina[29] and reflects the low antibody response, which tends to increase with age, and the limited protection (or absence there of) provided by maternal antibodies[24].

Our results, similar to another study in Rio de Janeiro[17], showed that the adenovirus was the second most common viral pathogen in children under two years of age, followed by influenza A virus, this prevalence increased gradually with age in these children. Similar adenovirus prevalence data were found in Chile and Argentina[4 13 29]. Variation in the prevalence of influenza A virus was only observed in out-patient children, since at the other health-care levels children in the under-one-year age bracket predominated. Parainfluenza type 3 virus was the third most common viral pathogen in children under six months of age hospitalized for bronchiolitis, with indices ranging from 3% to 7%. This prevalence is similar to that reported in children from tropical Southern India[12], where this virus was the second most frequent viral agent in lower respiratory tract infections. The positive viral test rates for different age brackets vary in the literature, but they agree with regard to the importance of age in prevalence of infections with a viral etiology.

The results for viral infection prevalence in out-patient children were similar to those found in Rio de Janeiro[17], where RSV, adenovirus, and influenza were the three main etiologic agents of ATIs and accounted for 21.8% of the positive cases detected by cell culture and IIF. The low prevalence of viral ARI in out-patient children shows the need for broader virological studies, with techniques capable of detecting other viruses circulating in the population. In emergency ward treatment, which proved to be a better source of data for RSV surveillance, some type of virus was detected in 52.9% of the cases, with 47% of the cases being RSV. This is clearly related to the high incidence of RSV in more severe ARIs in our area. Similar findings were recorded in Montevideo, Uruguay, in 1991 and 1992[9 25]. In hospitalized children, the low prevalence of influenza virus disagrees with findings of some authors[37 41] while agreeing with those of others[3 11 17]. Prevalence of RSV and adenovirus, accounting for 91.4% of the positive virological diagnoses, justifies antigenic and genetic studies of these viruses to help cast light on their role in reinfection and clinical course. Percentages of ARI with the detection of two or more types of virus agreed with 63.1% of the articles reviewed[46].

RSV infection in the area covered by this study, with an annual occurrence of respiratory disease epidemics

in the cold months, displayed a seasonality similar to that of countries with temperature climates[1 16 33], subtropical ones[33], and rainy seasons in tropical countries[11 12]. The epidemics tend to vary as to intensity and severity[39], while this study confirms, as do Navas *et al*., a significant annual variation in the frequency of RSV infection.

A significant positive correlation between frequency of viral detection and the rainy season, mean relative humidity, and mean temperature was observed in children with bronchiolitis[38]. Likewise, the occurrence of RSV, which began with a low frequency in April/May and displayed a maximum peak in July/August, correlated with the minimum mean monthly temperature in Porto Alegre, thus highlighting the relevance of viral respiratory infections in the State. This seasonal pattern is closer to that of respiratory infection in Chile, Argentina, Uruguay[3 26 28], and São Paulo[43] than that of other regions of Brazil[2 17]. We did not investigate a correlation with humidity and rainfall in our study, because Rio Grande do Sul State has a regular pluvial precipitation throughout the year. Adenovirus and parainfluenza type 3 virus, which circulated in the late winter and early spring in 1991, displayed the highest prevalence in the month of October. While, in 1992, adenovirus was detected from May to December, with the highest prevalence also in the month of October. These data are compatible with some annual reports showing the variable circulation of adenovirus[12 38], which may or may not occur throughout the year. Parainfluenza type 3 virus, detected mainly in bronchiolitis, occurred in a similar period in slum-dwellers in Rio de Janeiro[17], although it varied from country to country[12 38]. Influenza A virus was detected more frequently in out-patient children during the winter months. The two influenza B viruses detected in the months of July and August were in hospitalized and out-patient children.

Monitoring respiratory viruses over the course of these three years provided information on RSV infection, since there is little information available on RSV in developing countries[47], and confirms the importance of viral etiology in acute respiratory disease. Prospects for the introduction of an RSV vaccine in Brazil in the coming years and the recent mass immunization for influenza in our Country for individuals over 65 years of age, requiring knowledge of the viral variants circulating in the community to obtain maximum vaccinal efficiency, call for the on-going and in-depth study of respiratory viruses.

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