Acute Respiratory Viral Infections in Ambulatory Children of Urban Northeast Brazil

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The morbidity of acute respiratory infections in young children and the role of respiratory viruses were evaluated in a 29-month household-based study in an impoverished urban population in Fortaleza, Brazil; subjects were 175 children <5 years of age in 63 families. Home visits were conducted three times weekly during which staff recorded the presence of respiratory and systemic symptoms and collected upper respiratory tract samples for viral isolation. A large and sustained burden of respiratory illness was observed, and respiratory viruses were isolated in 35% of the samples collected. Of the isolates, 45.6% were rhinoviruses, 16% parainfluenza viruses, 15.8% enteroviruses, 9.9% adenoviruses, 7.0% herpes simplex viruses, and 5.7% influenza viruses. The results indicate that poor children in northeast Brazil have a high prevalence of respiratory illness and that rhinovirus is the most frequent respiratory virus.

Acute respiratory infections (ARI) are major causes of morbidity and mortality in developing countries, where ~4 million ARI-related deaths are estimated to occur each year among young children [1]. In these countries, ~50% of all deaths occur in children <5 years of age, and one-fourth to one-third of those deaths are due to ARI [2]. The incidence, severity, and case-fatality ratio of lower respiratory tract infections, specially pneumonia, are higher in developing countries [2–4]. In contrast, the overall incidence of ARI among children has been reported to be similar in developed and developing nations [2]. However, the number of outpatient or community-based studies providing reliable ARI attack rates in developing countries is limited [5–10].

On the basis of studies in developed countries, respiratory viruses are regarded as the predominant cause of ARI, either as principal pathogens or by predisposing to secondary bacterial infection [2]. Several household-based studies have been conducted on the epidemiology of ARI in developing nations [5–17], and some have assessed the importance of respiratory virus infections. The present household-based study was carried out over a 29-month period (April 1984–August 1986) to assess the morbidity of respiratory illnesses in young ambulatory children in an impoverished urban population of northeast Brazil and to determine the contributory role of respiratory viruses in the etiology of these illnesses.

Methods

Study area and population. Fortaleza, capital of the state of Ceará, is a city in northeast Brazil with nearly 2 million inhabitants. It is located ~4° south of the equator at sea level. The climate has a constant high relative humidity (usually >70% throughout the year) and narrow temperature range. During this study, the mean monthly minimal and maximal daily temperatures were 22.3°C (range, 21.1–24.0) and 31.3°C (range, 30.2–33.4), respectively. Rainfall is the major climatic variable. The rainy season usually occurs each year between January and May. Relative humidity remained >69% throughout the study, reaching 85%–90% during the rainy season.

The Gonçalves Dias district of Fortaleza is an urban slum area located close to the University Hospital of the Federal University of Ceará. The population of Gonçalves Dias, ~1500 inhabitants, is affected by high illiteracy rates, high infant mortality, low vaccination coverage, poor sanitation, and crowded living conditions. The sampling unit in the current study was the family, defined as the group of people living in the same household. For initial enrollment in the study, the mother had to have two or more children <5 years of age or to have one child <5 years of age and to be pregnant. When a family was excluded from the

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This study was approved by the University of Ceará and University of Virginia committees responsible for human investigation. Informed consent was obtained from parents or guardians.

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study, either when their children became >5 years or when they moved from the study area, a replacement was sought in an attempt to maintain the total number of children being followed at the original levels. To replace families, if a mother with two children could not be located, a mother with a single child was enrolled. All eligible children within that age range were enrolled from participating families and followed until 5 years of age or until the end of the study period. During the 29 months of study, 175 children <5 years of age were involved.

Surveillance, symptom recording, and analysis. House-to-house surveillance was conducted three times per week over 29 months by eight health-care workers under supervision of a registered nurse. The results of the first 4 months of study, when the health-care workers were completing training and families were being enrolled, were not considered for analysis. During the thrice-weekly visits, health-care workers recorded the occurrence of selected respiratory, systemic, and gastrointestinal symptoms for each day of the week for each child under surveillance. Respiratory symptoms were sneezing, rhinorrhea, sore throat, hoarseness, cough, dyspnea, conjunctivitis, and earache. Systemic symptoms were irritability, malaise, feverishness, and apathy. Gastrointestinal symptoms were diarrhea, vomiting, anorexia, and abdominal pain. All symptoms were recorded in a yes or no fashion, based on either the mother's judgement or the direct observations by the health-care workers. A special outpatient clinic was established close to the area, to which ill children could be referred regardless of the cause. We intended to use the symptom-recording method to define respiratory illnesses as was done in earlier longitudinal studies in the United States [18, 19]. However, because of the continuous presence of respiratory symptoms in a large proportion of the children, it was not possible to determine when a new illness had started or terminated. No solution to the problem of identifying discrete illnesses was found and, therefore, the clinical information from this study is presented entirely on the basis of presence of symptoms.

Sampling. Nasal aspirates and throat swabs were collected for viral studies when a new respiratory illness was judged to have occurred by either the mother or the health-care worker without use of a preestablished definition. We originally intended to define illness onsets using previous criteria [18, 19], but this was not possible because the continuous presence of symptoms prevented recognition of discrete periods of illness. The samples were collected in the homes by members of the study team, and all new recognized illnesses were sampled.

For the nasal aspirates a rubber bulb was used [20]. A 3-ml aliquot of PBS, pH 7.2, was squeezed into and aspirated from each nostril and immediately emptied into screw-capped centrifuge tubes containing fourfold concentrated viral transport medium (VTM: 8 g/100 ml beef heart infusion broth; Difco, Detroit), antibiotics (vancomycin, 80 μg/ml; gentamicin, 400 μg/ml; amphotericin B, 4 μg/ml), and 4% bovine serum albumin. For the throat, cotton-tipped applicators were swabbed over the tonsils and posterior pharynx, with the help of a tongue depressor. The swabs were broken off into the screw-capped tubes of VTM. Specimens were transported on ice to the laboratory.

Viral cultures. Samples for viral isolation were transported on ice to the diagnostic laboratory established at the Federal University of Ceará Hospital where they were processed no more than 4 h after collection. Specimen tubes were vortexed and the swabs discarded. After centrifugation at 380 *g* for 10 min, the supernatant was used for inoculation of cell culture, 0.2 ml inoculated into two tubes of each continuous cell line (HEp-2, LLC-MK_2, and MDCK), and the rest was frozen at −70°C. After stationary incubation for 1 h at 36°C, monolayers were washed free of specimen with Hanks' balanced salt solution and then fed with appropriate maintenance medium. MEM with 2% fetal bovine serum was used for HEp-2 and LLC-MK_2 and serum-free MEM with 2 μg/ml bovine pancreatic crystalline trypsin (Cooper Biomedical, Malvern, PA) for MDCK. Tubes were incubated in roller drums at 33°C (one LLC-MK_2 and both HEp-2 tubes) and 36°C (one LLC-MK_2 and both MDCK tubes). Tubes were examined for cytopathic effects every other day for 2 weeks. One of the HEp-2 tubes was blind-passaged at day 7. Hemadsorption with guinea pig red blood cells was done on day 7 in one tube of LLC-MK_2 and both tubes of MDCK and again on day 14 in the second tube of LLC-MK_2.

To increase the isolation rates of viruses for which the cell cultures used in Fortaleza were not sensitive, the frozen specimens were transported on dry ice to the University of Virginia, Charlottesville, where they were inoculated onto monolayers of WI-38 and/or FT (fetal tonsil), PRMK (primary rhesus monkey kidney), and HEp-2 cells. Isolates were identified by standard techniques using immunofluorescence (influenza, herpes simplex, and adenoviruses), hemadsorption-inhibition (parainfluenza virus), acid-sensitivity (rhinovirus), and neutralization assays (enterovirus).

Respiratory syncytial virus (RSV) antigen detection. The cell pellet obtained by centrifugation of the specimen was washed by gentle pipette aspiration in 2 ml of PBS, centrifuged, and resuspended in a suitable volume of PBS to obtain as many cells as possible to be used for immunofluorescence. Drops of the cell suspension were placed on slides and permitted to air dry. Slides were fixed in acetone at 4°C for 10 min and stored at −20°C. Indirect immunofluorescence test for RSV antigen was done on the cell pellet from each specimen the same day as the sample collection, following standard procedures, using a bovine polyclonal anti-RSV antibody (Welcome Diagnostics, Research Triangle Park, NC) [21].

Aliquots of frozen supernatants were subsequently tested for the presence of RSV antigen by a time-resolved fluoroimmunoassay (TR-FIA) [22] carried out at the Department of Virology, University of Turku, Finland.

Data analysis. For analysis, 1 respiratory symptom–day was defined as 1 day on which a child presented one respiratory symptom. For example, a child with cough and sneeze on 1 day of the week would be recorded as having 2 respiratory symptom–days in that week. Data from the symptom diaries were entered in an IBM computer using dBASE III, and data analysis was done using SAS software (SAS Institute, Cary, NC).

Results

Participation. Sixty-three families were enrolled in the study. On average, 37 families were under surveillance each month. Except for the first 4 months of study, when enrollments were being conducted, the number of families re-

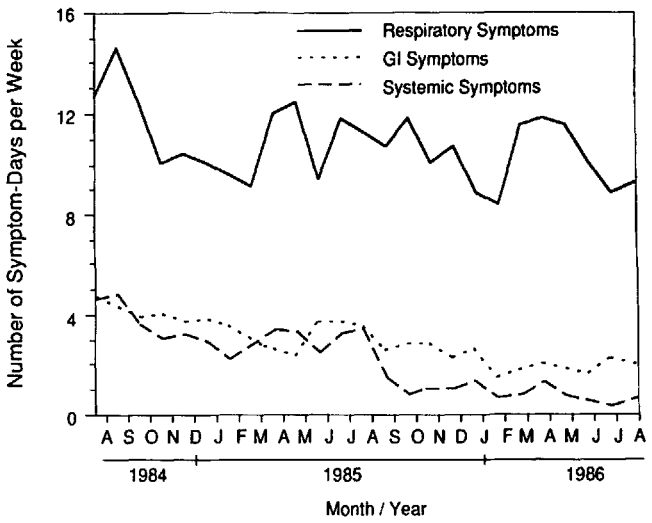

Figure 1. Monthly means of respiratory, gastrointestinal (GI), and systemic symptom-days per week in children <5 years old in Gonçalves Dias, Fortaleza, Brazil (August 1984–August 1986).

mained fairly constant: 32–41 families under surveillance each month. There were 175 children (94 boys, 81 girls) involved in the study, which consisted of 4360 child-weeks of observation. The number of children under surveillance each month averaged 86.5 (range, 75–92). Each family unit had a mean of 2.8 children <5 years old under observation.

Symptom occurrence. Respiratory symptoms were reported frequently throughout the study. In 1984–1985 and 1985–1986, respectively, 60% and 68% of the children had at least one respiratory symptom on ≥75% of the days of the year, 20% and 28% on 50%–75% of the days, 8% and 10% on 25%–50% of the days, and <5% on ≤25% of the days of the year. The children experienced a mean rate of 11.7 (range, 9.6–14.9) respiratory symptom-days per week, which remained relatively constant throughout the year. In comparison, the mean rates of symptom-days per week per child were 2.90 (range, 1.44–5.73) for gastrointestinal symptoms and 2.14 (range, 0.35–4.91) for systemic symptoms. The monthly means of the rates of symptom-days per week are shown in figure 1.

When corrected for the number of symptoms considered in the study (eight respiratory and four each gastrointestinal and systemic), there were means (±SD) of 1.46 ± 0.15 respiratory symptom-days, 0.72 ± 0.23 gastrointestinal symptom-days, and 0.53 ± 0.34 systemic symptom-days per week ($P < .001$, respiratory vs. gastrointestinal or systemic symptoms, two-tailed t test). When any child with one or more respiratory symptoms was considered ill, results indicated that ~80% of the children were ill with respiratory illness on an almost continuous basis. That rate only decreased to 70% when we considered any child with two or more respiratory symptoms as being ill.

The seasonal patterns of three selected respiratory symptoms (rhinorrhea, cough, and sneezing) indicated that each of these symptoms was present at high frequency throughout the year (figure 2). On average, ~60% of the children experienced rhinorrhea or cough and ~45% sneezing. Minor variations in prevalence of respiratory symptoms occurred over the seasons of the year, but the large seasonal variations observed in temperate areas did not. The consistent presence of respiratory symptoms prevented us from defining clearly asymptomatic periods. Consequently, identification of discrete periods of illness and calculation of ARI attack rates were not possible for most of the children in this study or for the whole population. During the period of the study, 16 children had radiographically documented pneumonia. Two of those are known to have died during the acute illness.

Respiratory viral infections. Of 1052 specimens obtained for viral culture, 369 (35%) were positive and yielded a total of 386 isolates (table 1). Viruses recovered comprised 176 rhinoviruses, 62 parainfluenza viruses (34 type 2, 13 type 1, 8 type 3, 7 untyped), 61 enteroviruses (13 echoviruses, 6 coxsackieviruses, 6 each polioviruses types 1 and 2, 2 polioviruses type 3, 28 untyped), 38 adenoviruses, 27 herpes simplex viruses type 1, and 22 influenza viruses (19 type A, 3 type B). RSV was not isolated during this study, nor were its antigens detected by immunofluorescence or TR-FIA.

The frequency distribution of the viruses by age, expressed as percentages of the samples positive in each age group, is shown in figure 3. Rhinoviruses were the most frequent isolates in each age group. Compared with the rhinovirus isolation rates in the older groups (6 months–5 years), rhinoviruses were recovered more frequently (47%) in the group 0–6 months old ($P < .05$, χ^2 test). The frequencies of other viruses did not differ significantly across the different age groups.

To assess the seasonality of specific viral infections, the number of different viral isolates per month was considered in relation to the main seasonal variable, rainfall (figure 4). The overall sampling rate averaged 0.5 samples per child per month without seasonal variation (figure 5). Rhinovirus ac-

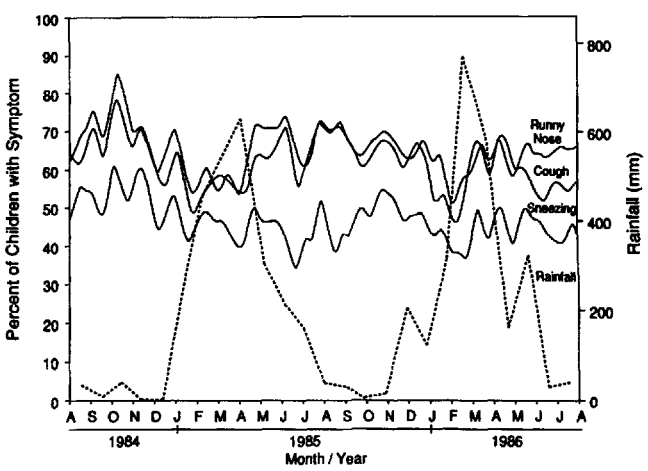

Figure 2. Daily occurrence of selected respiratory symptoms in children <5 years old in Gonçalves Dias, Fortaleza, Brazil (August 1984–August 1986).

Table 1. Recovery of viruses from the upper respiratory tract specimens of children in Gonçalves Dias, Fortaleza, Brazil, 1984–1986.

Virus	Number of isolates			% of specimens yielding virus‡	% of total isolates
	Brazil*	Virginia†	Total		
Rhinovirus	0	176	176	16.7	45.6
Parainfluenza	7	55	62	5.9	16.0
Enterovirus	21	40	61	5.7	15.8
Adenovirus	31	7	38	3.6	9.9
Herpes simplex	10	17	27	2.5	7.0
Influenza	7	15	22	2.1	5.7
Total	76	310	386	36.5	100.0

* Isolates recovered from original samples at the University of Ceara's laboratory only.
† Additional isolates recovered at the University of Virginia's laboratory.
‡ Of 1052 specimens, 369 yielded 386 viral isolates. Two isolates were recovered from 17 specimens. The most common associations were with rhinovirus: adenoviruses in 8 samples, enteroviruses in 2, parainfluenza virus in 1, influenza virus in 1, and herpes simplex virus in 1. Two associations occurred with parainfluenza virus: one with adenovirus and one with enterovirus. Influenza virus was isolated once in association with enterovirus.

tivity occurred throughout the study (figure 4) but varied considerably in intensity from month to month. No obvious seasonal pattern or change in activity related to rainfall was discernible. In contrast, close association was observed between rainfall and peaks of influenza and parainfluenza virus activity (figure 4). One influenza A and one influenza B peak came during the height of rain periods, and another influenza A outbreak developed during a lesser period of rainfall in November–December 1985. Parainfluenza 2 activity peaked sharply during the rain season (February–June) in the second year of study but was negligible during the first year. Enterovirus activity tended to predominate in the drier season (figure 4). Adenovirus and herpes simplex infections occurred on a year-round basis.

Unlike observations in temperate areas where the school term coincides with the respiratory viral disease season, no clear patterns were observed between school terms and occurrence of respiratory viruses in Fortaleza, where there are two school terms during the year.

It was not possible to correlate viral recovery with discrete periods of illness. Therefore, the role of the viruses recovered in this study in causing the symptoms observed cannot be established with the same degree of certainty as in earlier studies [18, 19] in which viral recovery was associated with specific self-limited periods of illness. However, because it has been well established that rhinovirus, influenza virus, and parainfluenza virus are respiratory pathogens that commonly cause the kinds of symptoms reported in this study, it seems reasonable to suggest that respiratory viruses caused at least some of the respiratory symptoms reported.

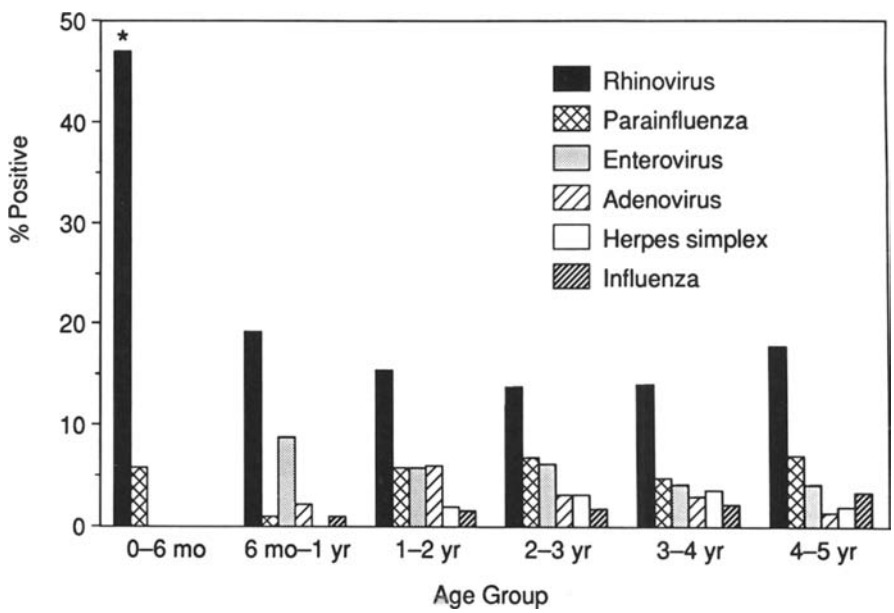

Figure 3. Frequency distribution of virus isolation by age in children <5 years old in Gonçalves Dias, Fortaleza, Brazil (August 1984–August 1986). *$P < .05$.

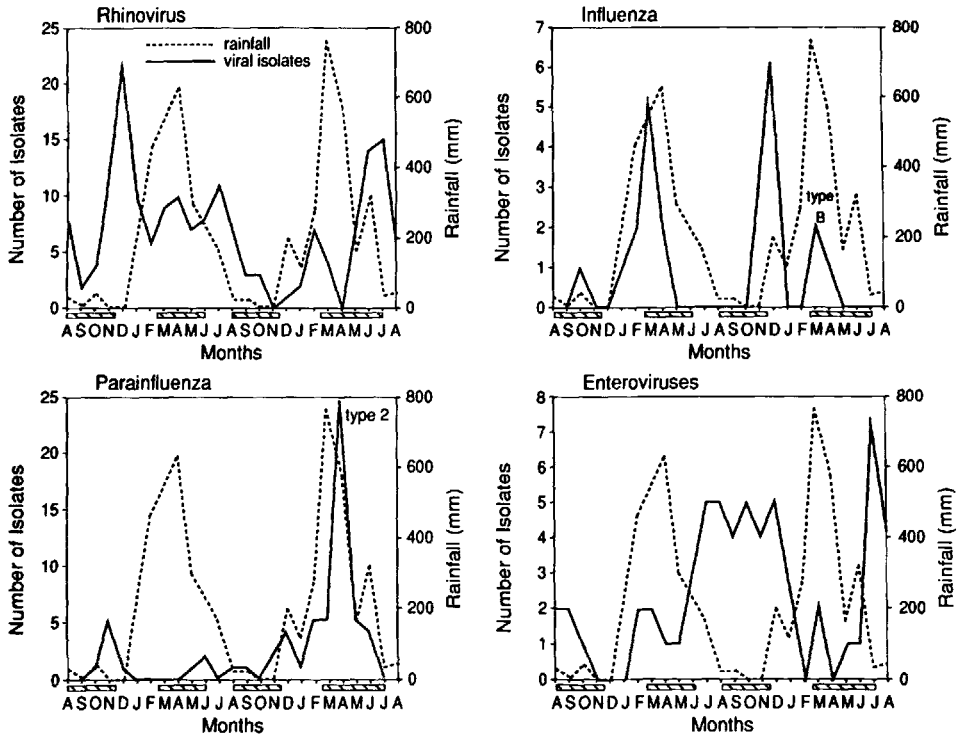

Figure 4. Monthly occurrence of rhinovirus, influenza, parainfluenza, and enterovirus in children <5 years old in Gonçalves Dias, Fortaleza, Brazil (August 1984–August 1986). ▨, school terms in Fortaleza.

Discussion

Community-based research provides essential information about ARI that is not obtainable in any other way [23]. The principal findings of our study were the sustained burden of respiratory symptoms and the associated high frequency of rhinovirus infections. Only a few studies have been undertaken in urban pediatric populations in developing countries, in which longitudinal surveillance of ARI and sampling for viral isolation were simultaneously conducted in the home [10, 17]. In the present study, surveillance was conducted by trained nonmedical staff with similar background in terms of beliefs and disease concepts as the study population. Thus, we believe that the data in this work reflect an accurate picture of ARI as it was perceived by the population.

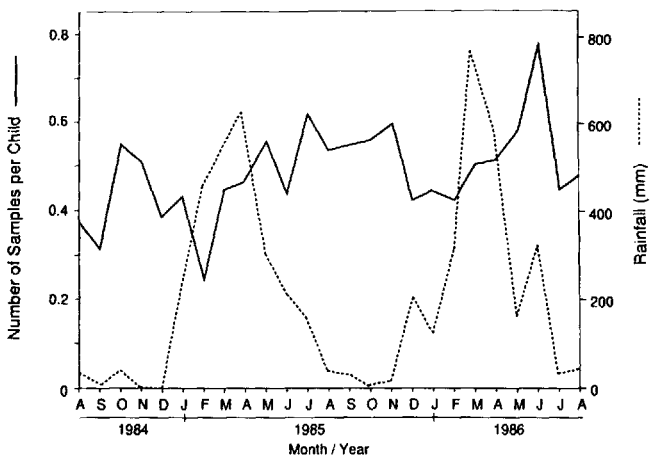

Figure 5. Monthly rates of samples for viral isolation per child < 5 years old in Gonçalves Dias, Fortaleza, Brazil (August 1984–August 1986).

Several community-based studies in developing countries have also documented the frequent occurrence of respiratory illness [5–7, 11, 12]. Mata [6] conducted a 5-year longitudinal house-to-house surveillance of illnesses among 45 children in a rural setting in Guatemala. In that study ARI was the cause of 35% of the recognized illnesses, second only to diarrheal disease. Also supportive of the importance of ARI are the studies by Black et al. [12] in Bangladesh and McAuliffe et al. [7] in a village in northeast Brazil, nearby our study area. Both studies found ARI to be the most frequent illness in young children.

In a household-based surveillance of diseases among children in urban India conducted by Sharma et al. [5], upper ARI represented 26.8% of all illnesses, being the leading cause of morbidity in young children. Similar findings were reported by de Romaña et al. [11] in an impoverished urban area in Lima, Peru, where upper ARI caused 27.8% of all illnesses in a cohort of infants in their first year of life. In the present study, 60%–70% of the children had at least one respiratory symptom on ≥75% of the days of the year. Moreover, the finding of an average of 11.7 respiratory symptom-days per week during the study (figure 1) indicates that a heavy symptom burden was sustained throughout the year.

The most frequently used criterion to measure the incidence of ARI is the attack rate, based on arbitrary definitions

of what constitutes an episode of ARI. In the children of Gonçalves Dias, symptoms of ARI were almost constantly present, usually without any identifiable asymptomatic period. If we defined an episode of illness simply by the presence of one or more respiratory symptoms, we found that ~80% of the children would have been considered ill throughout the study period. Thus it was not possible to define distinct episodes of illness for most children, a finding similar to that observed in urban Peru by de Romaña et al. [11]. In the presence of such a burden of respiratory illnesses, we thought the best description of events was to quantify symptoms rather than impose an artificial definition of an ARI episode. Although we did not investigate bacterial pharyngitis, asthma, sinusitis, or allergic rhinitis that may have contributed to some of the symptoms, it seems reasonable to assume that acute respiratory viral infection is an important problem in this population [3].

The second major finding of our study was the high frequency of documented respiratory viral infections, especially by rhinovirus (table 1, figure 4). Respiratory viruses were isolated in 36.5% of the samples, and 45.6% of the isolates were rhinoviruses. This is similar to the respiratory virus isolation rate of 42% obtained in children in a group day care in the United States [24]. The high rhinovirus isolation rate in the present study was obtained from cultures done at the University of Virginia laboratory on the samples brought frozen from Fortaleza. This highlights the importance of using human diploid fibroblast cell lines, known to be sensitive for rhinovirus. Rhinovirus was the most frequently isolated respiratory virus in some community-based studies in developed countries [25–28], and our study extends this finding to young children with ARI in Brazil.

Most studies in which viral etiology of ARI in children in developing countries was determined were based on sampling conducted in a health-care facility [29–41]. Only a few studies did surveillance and sampling in the home [10, 15–17]. Zijiang et al. [15] conducted ARI surveillance in a semirural population in China and did not report rhinovirus isolation. Kloene et al. [16] extensively surveyed respiratory virus activity in rural West Bengal, India. In that study children were sampled monthly, regardless of the presence of ARI symptoms, and a broad range of viruses was isolated. The overall isolation was 11.5%, and rhinovirus was rarely recovered, presumably because they did not use sensitive cell cultures. Vathanophas et al. [10] tested respiratory tract specimens obtained from a randomly selected sample of Thai children with ARI and obtained a viral isolation rate of 15.5%. Rhinovirus was recovered in only 1.7% of the total tested samples. Hortal et al. [17], in a study conducted in Uruguay, obtained an overall isolation rate of 15.3%. They did not report rhinovirus isolation.

An intriguing finding in our study was the lack of detection of RSV over the 2-year period. Several hospital-based studies in various developing countries have documented RSV infection, sometimes as the most frequent isolate, in children with ARI [29, 32–35]. In a pilot study in the emergency room of the University Hospital in Fortaleza before the surveillance reported here, we isolated RSV from several clinical samples. The variable sensitivity of different cell line strains to RSV and the loss of sensitivity of continuous cell lines over time are well known [42]. However, it would be hard to explain our complete lack of RSV detection on that basis, as our samples were cultured in several cell types in Fortaleza and at the University of Virginia laboratory, and we used two antigen detection tests that also failed to detect RSV infection in the study participants. Although these techniques probably lacked sufficient sensitivity to be categorical about the absence of RSV, the results suggest that RSV was not a frequent pathogen in our outpatient population during the period of study. This contrasts with the frequency of RSV isolation in most studies of young children with respiratory tract illness [3, 10].

Attempts to correlate prevalence of the different viruses with climatic variables gave interesting but inconclusive results. Unfortunately, it was not possible to continue the study beyond 2 years because of lack of support. The outbreak of parainfluenza that occurred during the rainy period in the second study year was due primarily to type 2, in contrast to outbreaks in the United States, which have not been associated with parainfluenza type 2 alone.

In summary, this study has shown that children of low socioeconomic level living in urban ghettos in Brazil, where 25 million of a population of 130 million are estimated to live in slums [43], suffer a large burden of respiratory symptoms; rhinovirus was the most frequent viral pathogen isolated in association with those symptoms. Even though the inability to identify discrete episodes of illness creates a problem in evaluating the exact clinical significance of a particular agent, our results showed rhinovirus to be the major recognized contributor to the respiratory symptoms in this population. The importance of rhinovirus in predisposing to secondary bacterial complications, such as otitis media, sinusitis, pneumonia and their sequelae, requires further study.

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