Frequency of viruses associated with acute respiratory infections in children younger than five years of age at a locality of Mexico City

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A locality in the district of Tlalpan, Mexico City, was selected in order to identify the viral agents in children younger than 5 years of age with acute respiratory infection (ARI). A total of 300 children were randomly selected and were included in this study for a period of 13 months. During this period nasopharyngeal exudates were collected for the isolation of viral agents. Monoclonal fluorescent antibodies were used for viral identification after cell culture. Viral infection was detected in 65% of the specimens. The respiratory syncytial virus (RSV) was the most common virus agent detected. Children required an average of two consultations during the study period. Two high incidence peaks were observed, one during the summer and the other during winter; the most frequent viruses during these seasons were influenza A and RSV, respectively. The largest number of viruses was isolated in the group of children between 1 and 2 years of age and in the group between 4 and 5 years of age. This study demonstrated the presence of ARI and of different viruses in a period of 13 months, as well as the most frequent viruses in children younger than 5 years of age from a community of Mexico City.

Key words: respiratory viruses - acute respiratory infections - respiratory tract viruses - respiratory syncytial virus - influenza virus - adenovirus parainfluenza virus - viral infection - Mexico

Acute respiratory infections (ARIs) are the main cause of morbidity and mortality in children younger than 5 years of age (OPS/OMS 1992, Monto & Lehmann 1998, Demers et al. 2000, Garbino et al. 2004). Deaths due to ARIs are estimated at 4 million in average per year in the world (Beltran et al. 2000, Garbino et al. 2004), most of them in underdeveloped countries of Latin America. Mexico, and Central America (OPS/OMS 1992, Gutierrez et al. 1999, Secretaria de Salud 2000, Straliotto et al. 2002) present the highest rates of morbidity and mortality due to ARIs.

In Mexico, mortality due to pneumonia was the third cause of death in 1999 in the population younger than 1 year old with a rate of 112.6/100,000 infants born alive and a rate of 8.1/100,000 pre-school children (Secretaria de Salud 2001a). Pneumonia-caused mean mortality rates in South America were 59/100,000 inhabitants varying from 20 in Chile to 111 in Bolivia; rates in Central America were 57 /100,000 inhabitants, varying from 18 in Costa Rica to 96 in Honduras; and mean rates in the Caribbean were 58 varying from 15 in Cuba to 96 in Haiti (Secretaria de Salud 1999). In Mexico, the mortality rate was 48/100,000 infants born alive (Secretaria de Salud 1999, 2000). ARIs are still the most frequent cause of disease in Mexican children (Secretaria de Salud 1999, 2001b) in spite of advances achieved in preventive and control measures such as vaccination campaigns and family health education. ARIs are the main reason for consultation in pediatrics services in primary and secondary health care units (Gutierrez et al. 1999, Guiscafre et al. 2001) representing the second most important cause of death in children younger than 5 years of age (Secretaria de Salud 2000, Guiscafre et al. 2001). The number of deaths due to ARI decreased significantly between 1990 and 1996. However, in 1996, ARI was still the second cause of death in children younger than 5 years of age (Secretaria de Salud 2001a,b) and 87% of the deaths were due to pneumonia (Secretaría de Salud 2000). In 2001, mortality due to ARIs was again the first cause of death in children younger than 5 years (Secretaría de Salud 2001), which could be the result of a greater ability for the identification of the causes and for recording the cases, as well as a greater use of medical services by the population; however, ARI etiologic agents are not investigated or are not reported, therefore, the predominant agents and viruses responsible for these are unknown. On the other hand, most ARI cases are treated with antibiotics without considering the probable viral etiology. Consequently, this study was designed to identify the ARI-associated viral agents and the involvement of some risk factors in children younger than 5 years old at a locality in the Tlalpan district which is located in the southwest of Mexico City.

MATERIALS AND METHODS

Study site - The Tlalpan district is located in the southwest of Mexico City, it comprises 20.6% of the territorial land of the Federal District and has various primary health care centers. For the study, the locality of San Nicolas Norte was selected and in it, the National Institute of Respiratory Diseases (INER, by its initials in Spanish) established a clinic for the attention of ARIs at health center T10. The locality is 2400 m above sea level and the total

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Received 28 June 2005
Accepted 5 January 2006

population in 1997 was 16,146 inhabitants; 13% were children younger than 5 years of age (Secretaria de Salud 2000, 2001a,b). The predominant climatic conditions are cold in autumn and winter, temperate in spring and a rainy season from May to September; temperature varies between 0 and 30ºC.

Type of study and selection of population - A prospective study from January 1997 to February 1998 was undertaken. The present cohort included 300 children and was assembled after a previous mapping by means of three procedures: (1) the community was divided into four areas; (2) each area was assigned 25% of the city blocks; (3) each area included 75 dwellings. A child younger than 5 years of age was selected from each family. The probable presence of ARI was considered when children presented two or more of the following signs and symptoms: sore throat, dry or productive cough, aphonia, and fever. The following were considered as acute respiratory infections: nasopharyngitis, sinusitis, pharyngitis, tonsillitis, laryngitis, tracheitis, bronchitis, bronchiolitis, pneumonia, and other respiratory tract infections of multiple or unspecified location. Informed consent for participation and follow-up of the children in the study was obtained from their parents.

Type of samples - Nasopharyngeal exudates were collected to isolate infectious agents. Samples were divided into two parts, one for virus isolation, for which the sample was placed in tubes with transport medium (Leibovitz medium enriched with 0.5% bovine albumin, 300 Ul/ml penicillin, and 300 µg/ml streptomycin) (In Vitro, Mexico), and the other part was used for bacteria isolation, inoculating it directly onto blood agar and chocolate agar media.

Virus isolation and identification - The nasopharyngeal exudates or lavage samples were inoculated in Vero, HeLa, MDCK, and HEp-2 cells previously grown in 8-well chambers and processed as described else were (Manjarrez et al. 2003). Viral identification was made by indirect immunofluorescence, using monoclonal antibodies for the following viruses: RSV, influenza A (IA), influenza B (IB), adenovirus (AD), and parainfluenza 1, 2, and 3 (PI) (Dako, California, US).

Statistical analysis - A χ^2 test was used to compare differences in viral type and frequencies between different age groups of children, seasons, and risk factors.

RESULTS

The study included 300 children, 10 of which dropped out of the study before it was completed. All the remaining 290 children were in infant and pre-school age (0 to 4 years-11 months old), with a mean of 2 years-11 months (SD 1 year-6 months), 47% were girls and 53% were boys (Table I). Table II shows background and characteristics of the study population. The most frequent symptoms and signs were dry (29.4%) or productive (17.6%) cough with presence of mucopurulent sputum (40.5%), accompanied with transparent (33.6%) or green (11.6%) rhinorrhea, adenomegaly (37%), pharyngeal hyperemia (34%), and tonsillar hypertrophy, sneezing (24.6%) and frontal headache (6.2%). Standardized procedures were performed for bacterial isolation, and infection was recorded in 38 samples (13%). The most frequently identified bacterium was *Haemophilus* sp. Virus infection was detected in 252 children (87%), as sole agent in 188 (65%) and mixed infections with bacteria in 64 children (22%) (Fig. 1). RSV was identified most frequently with 96 isolates (33%) (Fig. 2).

TABLE I

Sex and age in children with acute respiratory infection

Age (years)	Female	Male	Total	Virus isolates
>1	17	15	32	40
1-2	48	67	115	51
2-3	31	27	58	45
3-4	21	22	43	57
4-5	18	24	42	59
Total	135	155	290	252

TABLE II

Living environment

Sex	%
Male	53
Female	47
Average age	2 years 11 months (SD 1.6)
Average weight	3.138 kg (SD 0.424)
Breast feeding index	82
Vaccination scheme completed	80%
Favorable nutrition	97
Smoking at home	39
Asbestos ceiling	24.8
Crowded	63.3
Family income	minimum wage 75% < minimum wage 4% > minimum wage 21%

Viral frequency was analyzed in relation to age and 5 groups were formed: (1) children younger than 1 year of age (n = 32); (2) children between 1 year and 1 year and 11 months of age (n = 115); (3) children between 2 years and 2 years and 11 months of age (n = 58); (4) children between 3 years and 3 years and 11 months of age (n = 43); and (5) children between 4 years and 5 years of age (n = 42). Results are shown in Table I and Fig. 3. RSV and IA predominated in the five groups. Two children younger than 1 year of age had pneumonia, the etiological agent was RSV. In some cases there was more than one virus isolated as some children presented more than one infection process during the study period. In a few cases we isolated more than one virus.

Records were maintained throughout all the year in order to determine if seasons have an influence in the frequency of ARIs manifestation and the type of virus. An average of two applications for medical attention per child was recorded during the surveillance period totaling 543 ARI events, which were not evenly distributed: two peaks were detected, one in the summer, with the higher frequency of manifestations (40%) (Fig. 4a), and another in winter. Fig. 4b depicts the predominant viruses

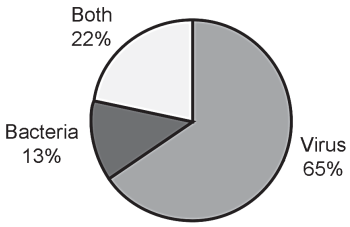

Fig. 1: identified viruses and bacteria in children with acute respiratory infection.

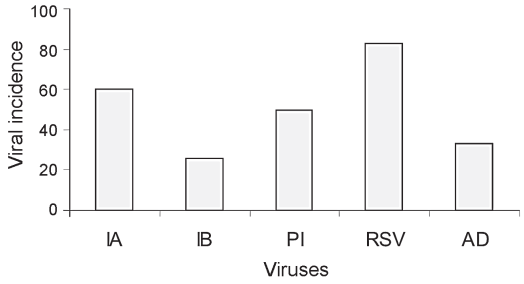

Fig. 2: isolated and identified viruses in children with acute respiratory infection. IA: influenza virus; IB: influenza B virus; PI: parainfluenza virus; RSV: respiratory syncytial virus.

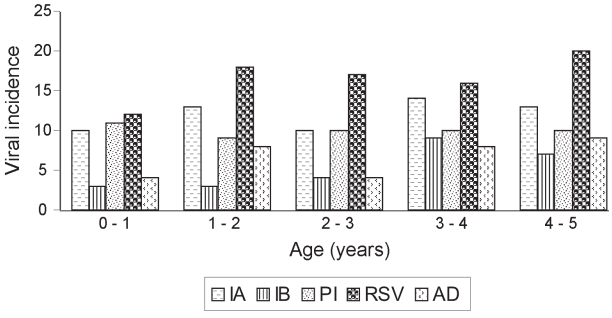

Fig. 3: identified viruses by age group IA: influenza virus; IB: influenza B virus; PI: parainfluenza virus; RSV: respiratory syncytial virus.

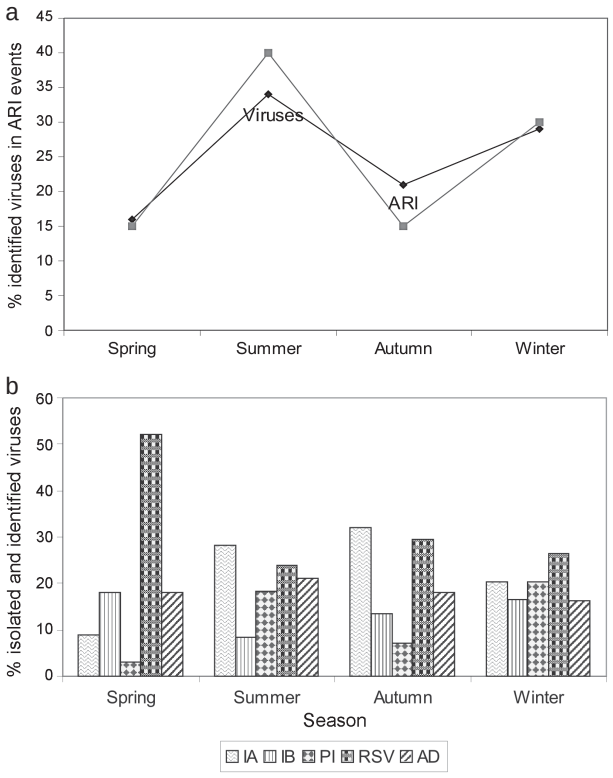

Fig 4: acute respiratory infection (ARI) events and viruses by year season: a) acute respiratory infection distribution by year season; b) viruses frequency by year season; IA: influenza virus; IB: influenza B virus; PI: parainfluenza virus; RSV: respiratory syncytial virus.

during each season. All viruses were detected through the year; AD and IB viruses were detected with a relatively constant frequency through the 4 seasons; PI was detected with a higher frequency during summer and winter; IA was detected in higher frequency during summer and autumn. RSV was the most predominant virus detected in the study in all the seasons and peaked in the spring (approximately 50% of the isolates in that season.

DISCUSSION

Viruses were isolated and identified in 65% of the children included in the study as sole infectious agents and in 22% as co-infection with bacteria. These results reveal that viral infection predominated in ARIs, which agrees with reports by other authors (Sutmoller et al. 1995, Sharma et al. 1999, Gatchalian et al. 1999, Greenwood 1999, West 2002, Manjarrez et al. 2003, Noyola et al. 2004) who mention that between 30 and 90% of ARIs are caused by viruses. In relation to age, we expected to find a higher frequency of both total viruses and RSV in group 1 (children younger than 1 year of age) however, it is known that the frequency of this virus is higher in younger children, in which it is devastating; however, the lowest percentage of total viruses (16%) was detected in this group. RSV showed the highest percentage (24%) in group 5 (children of 4 to 5 years of age). We presume that this is because these children attend school and are in daily contact with other children and, even if they have antibodies, these may not be enough to protect them against re-infections from different strains of the virus. IA was present in all groups. We expected to achieve the largest number of positive isolations in children younger than 1 year of age, but no significant differences were observed between the 5 groups.

We observed that the largest number of cases and virus isolations was present in the summer and winter. The rainy season in this zone of Mexico is in the summer; thus, humidity and temperature increase and perhaps, this combination of heat and humidity influences the amount of viral infections, while in winter, lower temperatures favor respiratory tract infections.

RSV was present in the highest percentage (around 50%, $P < 0.001$) in relation to other viruses. Several authors have reported that this virus predominates in winter; however, we found that the virus peaked in the spring.

IA was also frequent (around 30%) with a peak in the summer and autumn; the lowest number of isolates was

attained in spring (6.4%). IB was less frequent (10%) with a non-significant peak in the winter and spring, and constant in the other seasons. PI was detected with a low frequency (20%) but with two significant peaks, one in summer and another in winter. Finally, AD was less frequent (13%) with a peak in the summer.

Records revealed maternal or paternal positive smoking history in 38% of the families, not specifying intensity, but no significant relationship was found between this and the presence or absence of viral respiratory infections ($P = 0.53$), nor the presence or absence of bacteria ($P > 0.05$). Another important socio-economic factor associated with ARIs was family income, which was minimum wage in 75% of the families, 21% earned more than the minimum wage, and only 4% earned less than this.

We found that 63% of the families included in this study lived in crowded conditions, which is a predisposing factor to ARIs; 24.8% of these families had asbestos ceilings in their houses.

The record revealed that 87% of the mothers and 88% of the fathers had elementary education, and 56% had technical education or high school.

We consider that breast feeding (82%), complete Sabin vaccination series, DPT, measles, and BCG vaccinations (80%), as well as a favorable nutrition (97%) of children are factors that influence the low incidence of viral infections in children younger than 1 year of age, and the absence of severe complications in children of all the ages.

In Mexico, as in the world, ARIs have a high incidence in children due to environmental factors, but the difference is the higher mortality in underdeveloped countries in comparison with developed countries. Fortunately, no complications worsening the disease evolution were found in the present study, probably because of the favorable individual conditions of the children, the follow-up carried out by the health staff and the involvement of parents in the child's attention.

This study shows ARIs manifestation during 1 year and 1 month, the different viruses involved, as well as those more frequent in each age group. The obtained information may be important in the future for the development of prophylactic and control measures.

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