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Human respiratory syncytial virus and metapneumovirus in patients with acute respiratory infection in Colombia, 2000–2011

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ABSTRACT

Objective. *To describe the epidemiology of respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) in Colombia from 2000–2011, including seasonal trends.*
Methods. *Nasopharyngeal aspirates and/or throat swabs from 14 870 patients with acute respiratory infections (ARI) were studied. Two subgroups were analyzed using molecular biology techniques. The first consisted of 264 RSV indirect fluorescence assay (IFA)-positive samples, the second of 264 RSV IFA-negative samples. RSV and hMPV were detected using reverse transcription polymerase chain reaction (RT-PCR).*
Results. *2 799 samples (18.8%) contained a respiratory virus. RSV was detected by IFA in 1 333 samples (8.9%). RSV was detected by RT-PCR in 192 samples from the RSV IFA-positive subgroup and in 25 samples from the RSV IFA-negative subgroup. hMPV was detected in eight samples from the RSV IFA-positive subgroup and in 11 samples from the RSV IFA-negative subgroup. Among the RSV infections, subtype A was dominant in two-year intervals, subtype B was dominant in one-year intervals. 85.3% of RSV and 74% of hMPV infections occurred in children younger than 5 years old. RSV and hMPV infections were associated with rainy seasons. Co-infection with RSV A and RSV B was detected in two patients. Five cases of co-infection with RSV and hMPV were detected.*
Conclusions. *This report is the first to examine the epidemiology of ARIs in Colombia, with an emphasis on RSV and hMPV. The samples studied here were obtained over a 12-year period and represent all age groups and both genders.*

Key words Respiratory syncytial viruses; metapneumovirus; polymerase chain reaction; respiratory tract infections; diagnostic techniques, respiratory system; Colombia.

Respiratory infections are the major cause of morbidity and mortality in children worldwide and an important cause of death in the elderly (1). Viruses are the most common pathogens affecting the human respiratory tract (2). Respiratory syncytial virus (RSV), seasonal influenza viruses and human metapneumovirus (hMPV) are among the most common viruses that cause respiratory infections in humans. RSV is the major viral cause of severe lower respiratory tract infection in children, and the major cause of death in children younger than one year old (3). It is also the major cause of bronchiolitis and pneumonia during the first few months of an infant's life (4). A large proportion of children have been infected with RSV by the time they reach two years of age, and half of all children under 2 years of age have experienced at least two RSV infections (5).

hMPV was identified in 2001 as a paramyxovirus associated with upper and lower respiratory tract infections (6). This globally-distributed pathogen contains a non-segmented, negative-sense

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ribonucleic acid (RNA) with a genomic organization similar to that of RSV.

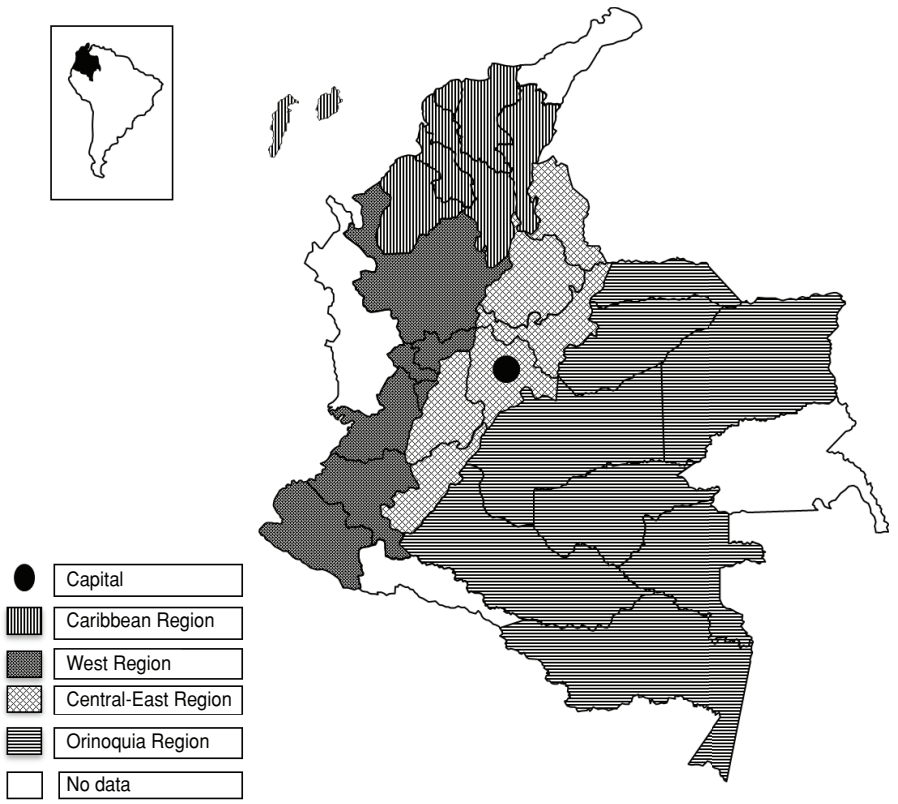
hMPV and RSV infect similar human subpopulations and produce similar symptoms, although symptoms from the former are of lower severity than those from the latter (7, 8). Humans can become simultaneously infected with both viruses because of their overlapping seasonality—indeed, several studies have observed co-infection rates up to 10% (9). Children co-infected with RSV and hMPV have a 10-fold increased risk of suffering more severe symptoms and requiring mechanical ventilation compared with those infected with RSV alone (10). To date, only a few RSV and hMPV case studies have been reported in Colombia; consequently, there are no consistent data on local transmission and circulation patterns of these viruses (11, 12). The aim of this study was to describe seasonal and demographic patterns as well as annual and monthly dynamics of RSV and hMPV in Colombia from 2000 to 2011.

MATERIALS AND METHODS

Sample collection

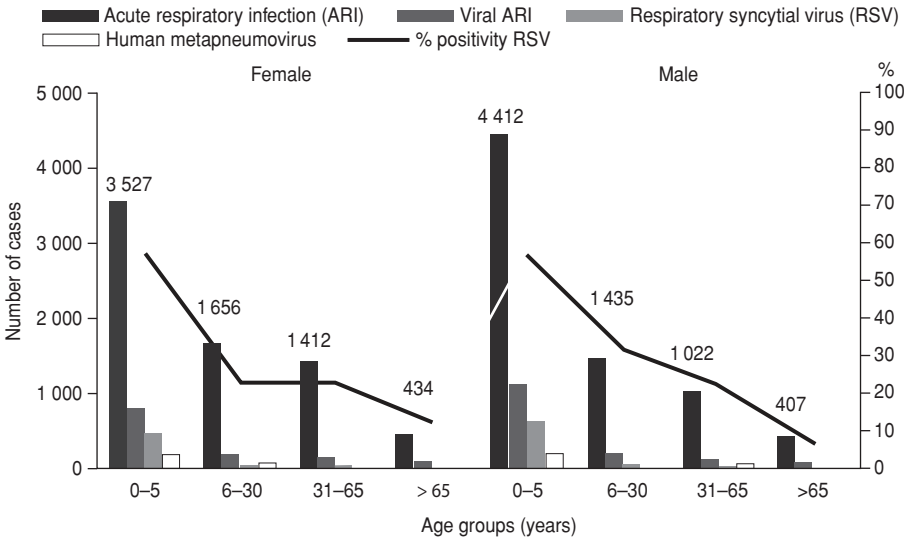
This descriptive and retrospective study was based on the processing of nasopharyngeal aspirates and/or throat swabs from 14 870 patients with acute respiratory infection (ARI), displaying respiratory symptoms such as cough, sore throat, rhinorrhea and general feeling of discomfort (13). Swabs were collected in local health centers and hospitals in 28 departments and the capital city of Colombia from 2000 to 2011 (Figure 1). Samples were placed into 2 mL of viral transport medium—i.e. brain heart infusion medium (BHI) containing bovine serum albumin fraction V, 10 µg/mL gentamicin, and 250 µg/mL amphotericin B—and sent to the national reference laboratory, part of the respiratory infection surveillance system framework. 7 272 samples (48.9%) were collected from females and 7 598 samples (51.1%) from males. In total, 7 939 samples (53.4%) were obtained from children younger than five years of age (3 527 from girls and 4 412 from boys); 3 091 samples (20.8%) were obtained from children and adults between 6 and 30 years of age (1 656 females and 1 435 males); 2 434 samples (16.4%) were obtained from adults between 31 and 65 years of age (1 412 females and 1 022 males); 841 samples (5.6%) were obtained from adults older than 65 years of age (434 females and 407 males), and 565 samples (3.8%) were obtained from patients of unknown age (Figure 2). From 1997 to 2007, respiratory viruses in Colombia were monitored mainly

FIGURE 1. Map of Colombia showing geographical regions in which throat swab samples were collected[a]



[a] Regions colored in white were not included in this study. Colombia's location within South America is shown at upper left.

FIGURE 2. Infection by age and gender, 2000–2011[a]



[a] The number of cases is shown in bars using the scale at the left, and the percentage positive are shown in lines using the scale at the right. Percentage positive of RSV = number of RSV cases x 100/number of viral ARI cases.

in children younger than 5 years and patients older than 65 years. From 2007 onwards, monitoring was expanded to all age groups.

Virus identification

Influenza A, influenza B, parainfluenzas 1, 2, and 3, adenovirus and RSV were detected in a first screening by fluorescent microscopy using a commercial fluorescent polyclonal antibody conjugate (Respiratory Panel Viral Screening and Identification IFA Kit. Light Diagnostics™; cat. # 3105) (Millipore Corporation. Livingston, United Kingdom) (i.e., indirect fluorescence assay, IFA) according to the manufacturer's instructions. The samples were stored at –70°C and thawed at 4°C before reverse transcription polymerase chain reaction (RT-PCR). Nucleic acid quality was tested by quantitative RT-PCR (qRT-PCR) amplification of the human RNAase P gene.

Study subsample

To investigate hMPV and RSV subtype A and B positivity using RT-PCR, a required study subsample size of 528 (Table 1) was calculated using standardized statistical methods, assuming the following parameters: estimated prevalence of hMPV infection in the RSV-negative population with ARI = 10% (14), estimated prevalence of RSV A or B subtype infection in the population with ARI = 7.4%, significance level = 90%, and p = 0.05. The study subsample was selected by convenience from among the 14 870 samples described above, and consisted of two groups: one of 264 RSV IFA-positive samples and a second of 264 RSV IFA-negative samples. The 528 selected samples were collected from patients aged 18 months to 86 years (47.5% female and 52.5% male) in the Colombian provinces with higher RSV frequencies: Bogotá D.C. (capital region, 241 samples), Huila, Cundinamarca and Tolima (central eastern region, 56 samples), Atlántico (Caribbean region, 23 samples), Caldas and Valle del Cauca (western region, 156 samples), and Guaviare (Orinoquia region, 52 samples). These provinces accounted for 94.8% of all the RSV infections in Colombia from 2000 to 2011. The distribution of these samples by age, geographical region, and gender is shown in Table 1.

Nucleic acid extraction and multiplex nested RT-PCR

To detect hMPV and RSV A or B subtypes in the study subsample, nucleic acids from respiratory secretions were extracted using the QIAamp Viral RNA Mini Kit (cat. # 52906) (Qiagen. Hilden, Germany) according to the manufacturer's instructions. A one-step conventional RT-PCR amplification reaction was performed followed by nested conventional PCR amplifications according to previously standardized methodologies (15–17). Briefly, the samples were subjected to an initial cycle of 48°C for 45 min and 95°C for 15 min. The PCR cycling conditions were 45 cycles of 95°C for 30 sec, 53°C for 2 min and 72°C for 30 sec, and a final incubation of 72°C for 5 min. For the nested multiplex PCR, 2 µL of the first reaction was added to 48 µL of a PCR mixture for a final volume of 50 µL. The mixture was heated to 94°C for 2 min. The cycling conditions were as follows: 35 cycles of 94°C for 30 sec, 53°C for 1 min, 72°C for 30 sec, and a final incubation of 72°C for 5 min. The positive controls were RSV 737-base pair (bp) genome fragments cloned into pGEM bacterial plasmid vectors and hMPV 739-bp genome fragments cloned into pCR4 bacterial plasmid vectors. To avoid cross-contamination, safety cabinets located in separate laboratories were used for manipulating respiratory specimens, preparing reagents, processing samples, and performing PCR amplifications. The nested PCR products were analyzed using electrophoresis on 2% low melting point agarose gel (cat. # A9539-1006) (Sigma-Aldrich. Munich, Germany) containing 5 mg/ml of ethidium bromide (cat. # 15585011) (Invitrogen. Grand Island, New York, USA) in 0.5 x Tris-borate EDTA buffer (TBE 10X; cat. # 1001-605) (QBiogene MP Biomedicals. Santa Ana, California, USA). The sizes of the PCR amplicons were 737 bp and 790 bp for generic RSV and hMPV, respectively, and 376 bp for RSV subtype A, 615 bp for RSV subtype B and 487 bp for hMPV after the nested PCR. The properties of the oligonucleotide primers and amplicons are shown in Table 2.

Determination of ARI, viral ARI, RSV and hMPV seasonal and geo-demographic dynamics

Rainfall levels across Colombia from 2000 to 2011 were acquired from the Colombian National Institute of Hydrology, Meteorology and Environmental Studies (IDEAM) for those departments and years for which samples were obtained. The correlation of rainfall with incidence of ARI and viral ARI was measured for the overall sample. Demographic variables such as patient age range and gender, as well as geographical and meteorological variables such as the place of origin of samples and rainfall levels, were analyzed and correlated with the incidence of RSV and hMPV infections in the study subsample.

TABLE 1. Geographical and demographic distribution of the study subsample. Number of samples per age group, geographical region and gender

Age group (Years)	Capital Region		Central-East		Caribbean		West		Orinoquia		Total
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	
0–5	98	77	15	12	6	12	48	47	18	10	343
6–30	19	8	9	3	0	1	14	13	4	5	76
31–65	9	9	6	6	0	0	9	9	6	7	61
> 65	2	3	0	0	0	0	0	4	0	2	11
Unknown	7	9	2	3	1	3	4	8	0	0	37
Total	135	106	32	24	7	16	75	81	28	24	528

TABLE 2. Primers used for respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) gene amplification in the multiplex conventional nested reverse transcription polymerase chain reaction (RT-PCR)

Primer	Nucleotide sequence (5´–3')	Virus	Gene	Gene position	Melting temp (°C)	G+C content (%)	Amplicon size (bp)
SINGLE STEP COMBINED RT-PCR							
RSVAB1F	ATGGAGYTGCYRATCCWCARRRCAARTGCWAT	RSV	F	1–31	62	45	737
RSVAB1R	AGGTGTWGTTACACCTGCATTRACACTRAATTC	RSV	F	705–737	60	39	
HMNV1F	AGTCCTAYCTAGTAGACAC	hMPV	M	2181–2200	41	42	790
HMNV1R	TTGTYCCTTGRTGRCTCCA	hMPV	M	2863–2881	41	42	
NESTED MULTIPLEX-PCR							
RSVA2R	AAATTCCCTGGTAATCTCTAGTAGTCTGT	RSV	F	682–710	57	38	376 (RSV-A)
RSVA2F	AAGGTTTATGAATTATACACTCAACAAT	RSV	F	335–362	55	25	
RSVB2R	GGAGATGCGACAGCTCTGTTGATTTACT	RSV	F	617–644	57	46	615 (RSV-B)
RSVB2F	ATCTTCCTAACTCTTGCTRTTAATGCATTG	RSV	F	30–59	57	35	
HMNV2R	TCTTGCAKATYYTRCTKATGCT	hMPV	M	687–700	53	35	487
HMNV2F	GCRGCIATGTCTGTACTTCC	hMPV	M	222–241	53	50	

Statistical Analysis

The proportion of viruses detected by geographical region, month, gender and age group were analyzed. The Chi-squared test was used for univariate analysis, and P values of < 0.05 were considered statistically significant. Analysis was performed using Stata 11 software (Stata Corp LP. College Station, Texas, USA).

Approval by an ethics committee

This research was approved by the ethics committee of the Colombian National Institute of Health on October 7, 2010.

RESULTS

Virus identification

2 799 samples (18.8% of the study sample) were viral infections that tested positive for either influenza A, influenza B, parainfluenza 1, 2, or 3, adenovirus, or RSV (henceforth referred to as *viral ARI*). In total, 1 333 samples (8.9% of the study sample) were RSV IFA positive. RSV was detected by RT-PCR in 192 samples (72.7%) of the IFA-positive subgroup and 25 samples (9.47%) in the IFA-negative subgroup. Subtype A RSV accounted for 66% of all RSV cases in the study subsample, whereas subtype B RSV accounted for 34%. hMPV was detected by RT-PCR in a total of 19 samples: eight samples (3%) in the RSV IFA-positive subgroup and 11 samples (4.2%) in the RSV IFA-negative subgroup. In addition, 14 cases involved single infections while five involved RSV/hMPV co-infection.

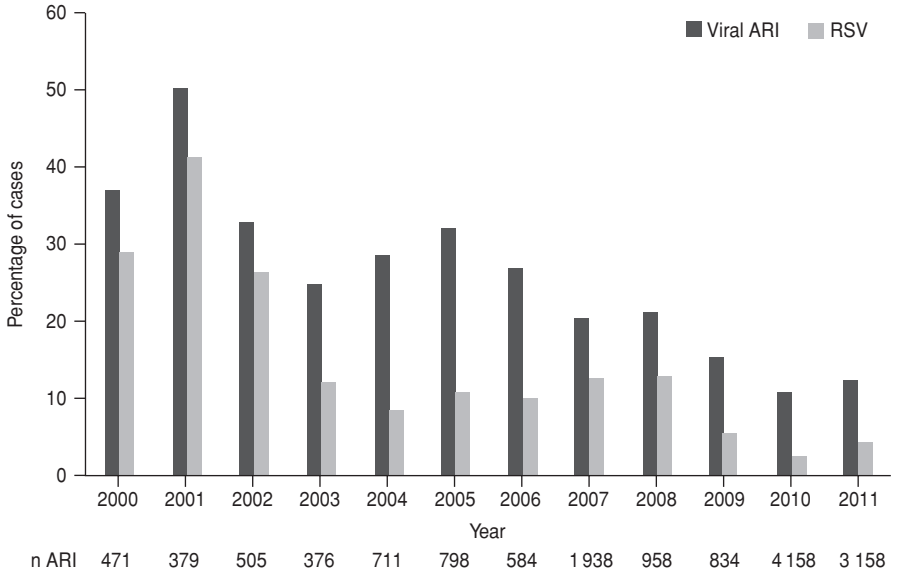
Distribution of RSV by age group

In total, 85.3% of all RSV infections occurred in children younger than 5 years old. In this age group, 58% of RSV infections occurred in males and 42% in females. The proportion of RSV infections among viral ARI by age group was calculated. As expected, the majority (57%) of viral ARI in children younger than 5 years were RSV IFA positive. This proportion decreased with increasing age: 28.1% for people between 6 and 30 years of age, 22.5% between 31 and 65, and just 9.2% in patients over 65**.** The proportion of RSV infections among viral ARI cases by gender and age was also determined. In children younger than 5 years, RSV infections accounted for an identical proportion (57%) of all viral ARIs for males and females. In the 6 to 30-year-old age group, RSV infection rates were 32.6% for males and 23.0% for females; among 31 to 65-year-olds, the equivalent proportions were 22.0% for males and 22.8% for females; and for those over 65, observed rates were 6.5% for males and 12.2% for females. The number of cases of ARI, viral ARI, RSV and the percentage of positive RSV cases by gender and age group are shown in Figure 2.

Seasonal trends for RSV infections

During the 12-year study period, two peaks of respiratory infections occurred annually, the first during the rainy season in April, May, and June, when 29.3%, 37.2%, and 43.8% of ARIs, viral ARIs and RSV infections occurred, respectively, and the second during the secondary rainy season in September, October and November, which saw equivalent rates of 24.8%, 22.0% and 16.3% for ARI, viral ARI and RSV infections. The percentage of positive viral ARI samples among all ARI samples varied widely from 2000–2011. The highest positive percentage was reported in 2001 (50.1%) followed by 2000 (37.1%) and 2002 (32.9%). The lowest positive percentage was reported in 2010 (10.8%), followed by 2011 (12.3%) and 2009 (15.2%) (Figure 3). The percentage of viral ARI samples that was positive for RSV infections also varied widely over the period of observation, with the highest percentage reported in 2001 (82.6%), followed by 2000 (80.7%) and 2002 (78.7%). The lowest percentage was reported in 2010 (23.6%), followed by 2004 (29.6%) and 2005 (34.0%)**.** The percentage of samples that was posi-

FIGURE 3. Annual dynamics of number of acute respiratory infection (ARI) cases (nARI, below), percentage of viral ARI and percentage of respiratory syncytial virus (RSV) infections in Colombia, 2000–2011[a]



[a] Viral ARI and RSV percentages were calculated using nARI annual values as 100%. Viral ARI and RSV were determined by indirect fluorescence assay (IFA).

tive for RSV among reported ARIs also displayed strong variation: the highest percentage was reported in 2001 (41.4%), followed by 2000 (29.1%), and 2002 (26.5%). The lowest percentage was reported in 2010 (2.5%), followed by 2011 (4.5%), and 2009 (5.4%) (Figure 3).

Seasonality and distribution by age group of hMPV infection and hMPV + RSV co-infection

hMPV was detected in 19 samples. These samples were obtained in 2000 (1), 2001 (1 co-infection with RSV), 2002 (1, plus 1 co-infection), 2003 (1 co-infection), 2005 (3, plus 1 co-infection), 2006 (2), 2007 (3, plus 1 co-infection), 2009 (2), and 2010 (2). The majority of hMPV cases (14 cases) were reported during the rainy seasons (March–June and October–December). The percentage of hMPV infections was calculated by age and gender. Of all hMPV infections, 74% occurred in children younger than 5 years of age, with 64% of infections in this age group occurring in female patients. The percentage of infections decreased with increasing age, with just 26% of infections occurring in people between 6 and 30 years of age. No hMPV infection was detected in patients older than 31 years of age. Of hMPV infections, 68% (13 cases) occurred in females, and 32% (6 cases) in males. However, only two co-infections of RSV subtypes A and B were detected in children younger than five years of age (one female and one male). In addition, 5 RSV co-infections with hMPV were detected in children younger than 5 years (four females and one male). Subtype A RSV was involved in four of these co-infections. In this study, co-infections did not display a different pattern than single infections. Furthermore, this analysis does not determine whether disease severity was associated with co-infections.

DISCUSSION

It is well known that RSV and hMPV primarily infect infants (3, 5). This phenomenon has been related to the low level of immune response in that age group, typical within the first two years in humans. In fact, more than 95% of children under two years have been infected with RSV, and 50% of these children subsequently become re-infected (18). RSV re-infection often occurs throughout life because complete immunity against the virus is never developed (18–20).

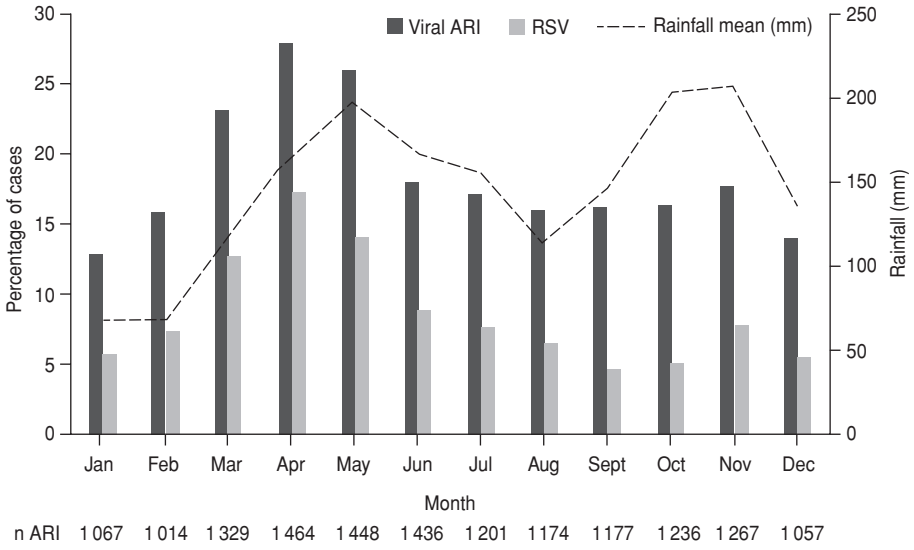
In this study, 85.3% and 74% of RSV and hMPV infections, respectively, occurred in children younger than 5 years old. More RSV infections were observed in boys and more hMPV infections in girls. However, low numbers of cases prevented statistically significant conclusions from being drawn. Similar trends have been reported in previous studies (21). In this study, the frequency of both RSV and hMPV infection decreased with increasing age, as only 5.6% and 0% of RSV and hMPV infections, respectively, occurred in patients more than 31 years old.

Apart from age, other previously-identified risk factors for RSV infection include (20): (i) increased exposure to infectious agents because of day care attendance, birth season, hospitalization, or low socioeconomic status; (ii) decreased body size because of gestational age, malnutrition, or low birth weight; (iii) increased susceptibility because of the absence of breastfeeding or low IgG levels in breast milk; and (iv) factors affecting lung function, such as exposure to smoke and air pollution.

In tropical regions RSV activity varies, from no seasonality to multiple annual peaks. RSV activity has been associated with excessive rainfall, higher environmental temperatures, warm rainy seasons, lower relative humidity and higher magnitude of day-to-day temperature variations (21–25). Regional climate diversity contributes to a corresponding diversity of RSV activity. In South America, RSV infections have been associated with winter in some countries, including Argentina, Brazil, Chile, and Uruguay, and with rainy seasons in other countries, including Colombia, Puerto Rico and Venezuela (26–28). High ambient humidity may increase the lifetime of respiratory viruses outside the host, thereby increasing transmission rates during rainy seasons.

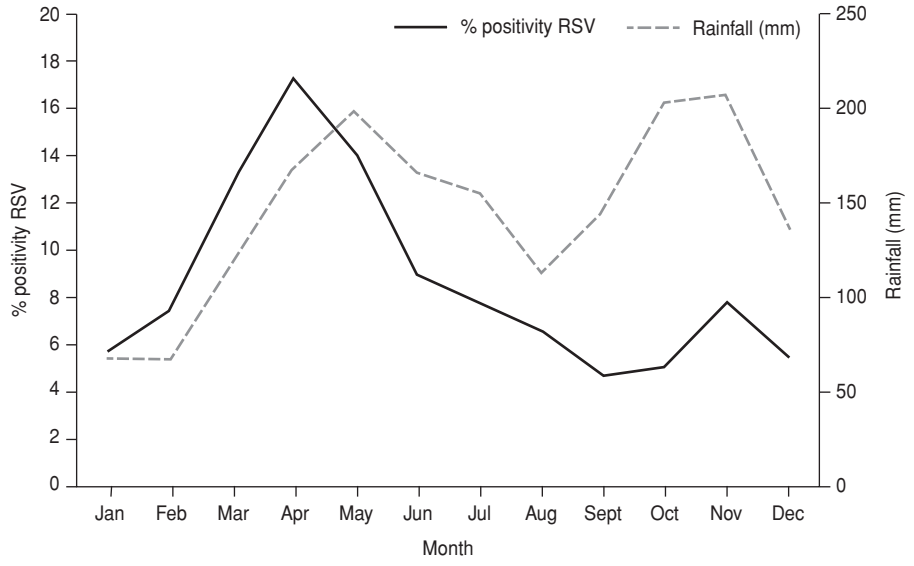
Rainfall is the major relevant climatic variable in Colombia, where temperature varies within a narrow range. This study documented year-round ARI, viral ARI and RSV activity in Colombia from 2000 to 2011. Nevertheless, an association was observed between ARI, viral ARI, and RSV activity and rainfall (Figure 4 and Figure 5). In particular, 54.1%, 59.2% and 60.1% of ARIs, viral ARIs and RSV infections occurred, respectively, during the six rainiest months in Colombia during the period of observation. 10% of ARIs and 48.4% of viral ARIs were caused by

FIGURE 4. Monthly dynamics of number of acute respiratory infection (ARI) cases (nARI, below), percentage of viral ARI, and percentage of respiratory syncytial virus (RSV) infections in Colombia, 2000–2011[a]



[a] Mean rainfall is shown as a dotted line and was measured in millimeters using the scale on the right. Viral ARI and RSV percentages were calculated using nARI monthly values as 100%. Viral ARI and RSV were determined by indirect fluorescence assay (IFA).

FIGURE 5. Monthly dynamics of rainfall mean and % positivity of respiratory syncytial virus (RSV) in Colombia, 2000–2011[a]



[a] Rainfall uses the right-hand scale and was measured in mm. Percent positive of RSV uses the left-hand scale, and was measured as number of RSV cases x 100/number of acute respiratory infection (ARI) cases. Viral ARI and RSV were determined by indirect fluorescence assay (IFA).

RSV during the rainy seasons, whereas the corresponding numbers for dry seasons were only 7.8% of ARIs and 46.5% of viral ARIs. Thus, RSV cases increased slightly among ARI and viral ARI cases during rainy seasons. The association of RSV epidemic periods with the wet seasons validates previous observations in tropical and subtropical countries with seasonal rainfall. Previous studies have shown similar trends in other tropical areas with year-round RSV activity (26, 27, 29–32).

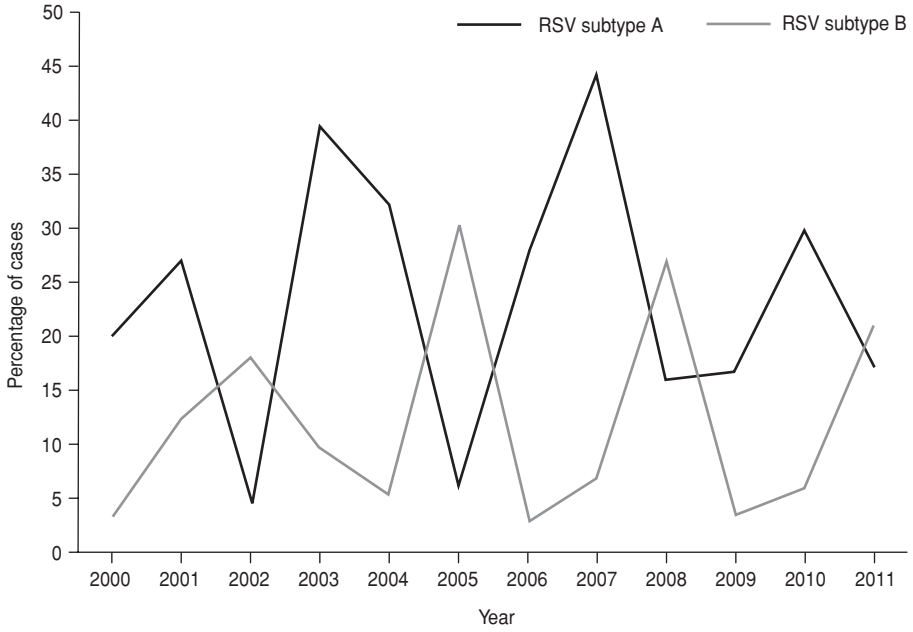
RSV subtype A was dominant among RSV infections and accounted for 66% of all RSV cases in the study subsample, while subtype B accounted for 34% of cases. No demographic variable served to distinguish subtype A from subtype B among the RSV infections, however, subtype A RSV infections were dominant over two-year intervals (i.e., in 2000–2001, 2003–2004, 2006–2007, 2009–2010) and subtype B RSV infections were dominant over one-year intervals (i.e., in 2002, 2005, 2008 and 2011) (Figure 6). Previous studies have revealed similar temporal dynamics for RSV subtypes in Cuba, Uruguay, Hawaii and Argentina where RSV A is dominant and accounts for 62–91% of all RSV infections (26, 31, 33, 34).

During 1997 to 2007, respiratory viruses in Colombia were primarily monitored in children younger than 5 years of age and elderly patients older than 65 years of age. From 2007 onwards, monitoring was increased to cover all age groups. For this reason, the number of ARI samples increased by 2-fold beginning in 2007 (approximately). This is a limitation of this study that may influence the analysis of distribution of RSV and hMPV among age groups. However, because RSV primarily infects children under 5 years old, the number of RSV cases remained stable before and after 2007 (35). In 2009, pandemic H1N1 influenza A emergence resulted in an approximately 4-fold increase in the number of samples collected, primarily from young adults, for ARI monitoring (35). Again, this sudden increase may have influenced the analysis performed on distribution of viruses among the population. However the number of RSV cases remained stable before and after the pandemic, likely because RSV primarily infects children under 5 years old.

An IFA false-positive rate of 27.3% and an IFA false-negative rate of 9.4% were observed when compared to RT-PCR. Diagnostic techniques based on fluorescent microscopy are often dependent upon antigen quality and subjective criteria determined by the technician which are limitations when used for clinical diagnosis.

Because hMPV prevalence is low, the study subsample was not large enough to draw statistically significant conclusions. This became a limitation when

FIGURE 6. Annual dynamics of respiratory syncytial virus (RSV) subtype A and B infections in Colombia, 2000–2011[a]



[a] Nodes show annual percentages of RSV-A and RSV-B as detected by reverse transcription polymerase chain reaction (RT-PCR) among study subsamples. Percentages were calculated as the number of samples RT-PCR positive for either subtype x 100/number of samples (n) tested by RT-PCR in any specific year

investigating the demographic patterns and monthly and annual dynamics of hMPV infections.

For future studies, the authors recommend the selection of a time period during which the surveillance of respiratory viruses is performed uniformly without major deviations in target population groups.

The molecular methods described in this study are suitable for detecting RSV and hMPV. However, the results clearly show the need for extreme care when using and interpreting IFA. Although IFA is a good option for screening and surveillance purposes, a recommendation derived from this study is that IFA should not be used as a confirmatory test for clinical diagnosis.

Future studies on the epidemiology of respiratory viruses should calculate required sample size based on prevalence data for the country or area of study, in order to guarantee that results are statistically significant and conclusions are solid.

Conclusions

Although RSV showed year-round activity and infected all age groups, it clearly increased during rainy seasons and infected mainly children under 5 years old. RSV infections were more frequent in boys than in girls. During the 12-year period, subtype A RSV dominance occurred in periods of two years followed by a year of subtype B RSV dominance.

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Conflict of interests. The authors of this paper declare no conflict of interests in this research.

REFERENCES

1. Hall CB, Weinberg GA, Iwane MK, Blumkin AK, Edwards KM, Staat MA, et al. The burden of respiratory syncytial virus infection in young children. N Engl J Med. 2009;360(6):588–98. Available from: http://www.nejm.org/doi/full/10.1056/NEJMoa0804877
2. Van Woensel JBM, Van Aalderen WMC, Kimpen JLL. Viral lower respiratory tract infection in infants and young children. BMJ. 2003;327(7405):36–40. Available from: http://www.bmj.com/content/327/7405/36
3. Anderson LJ, Parker RA, Strikas RL. Association between respiratory syncytial virus outbreaks and lower respiratory tract deaths of infants and young children. J Infect Dis. 1990;161(4):640–6. Available from: http://jid.oxfordjournals.org/content/161/4/640.full.pdf
4. Brandt CD, Kim W, Arrobio JO, Jeffries BC, Wood SC, Chanock RM, et al. Epidemiology of respiratory syncytial virus infection in Washington, D.C. 3. Composite analysis of eleven consecutive yearly epidemics. Am J Epidemiol. 1973;98(4):355–64. Available from: http://aje.oxfordjournals.org/content/98/4/289
5. Coggins WB, Lefkowitz EJ, Sullender WM. Genetic variability among group A and group B respiratory syncytial viruses in a children's hospital. J Clin Microbiol. 1998;36(12):3552–7. Available from: http://jcm.asm.org/content/36/12/3552.full
6. Van den Hoogen BG, de Jong JC, Groen J, Kuiken T, de Groot R, Fouchier RA, et al. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat Med. 2001;7(6):719–24. Available from: http://www.nature.com/nm/journal/v7/n6/abs/nm0601_719.html
7. Boivin G, Abed Y, Pelletier G, Ruel L, Moisan D, Cote S, et al. Virological features and clinical manifestations associated with human metapneumovirus: a new paramyxovirus responsible for acute respiratory-tract infections in all age groups. J Infect Dis. 2002;186(9):

1330–4. Available from: http://jid.oxfordjournals.org/content/186/9/1330.full
8. Williams JV, Harris PA, Tollefson SJ, Halburnt-Rush LL, Pingsterhaus JM, Edwards KM, et al. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. N Engl J Med. 2004;350(5):443–50. Available from: http://www.nejm.org/doi/full/10.1056/NEJMoa025472
9. Caracciolo S, Minini C, Colombrita D, Rossi D, Miglietti N, Vettore E, et al. Human metapneumovirus infection in young children hospitalized with acute respiratory tract disease: virologic and clinical features. Pediatr Infect Dis J. 2008;27(5):406–12. Available from: http://ovidsp.tx.ovid.com.sp.12.0b/ovidweb.cgi?WebLinkFrameset=
10. Semple MG, Cowell A, Dove W, Greensill J, McNamara PS, Halfhide C, et al. Dual infection of infants by human metapneumovirus and human respiratory syncytial virus is strongly associated with severe bronchiolitis. J Infect Dis 2005;191(3):382–6. Available from: http://jid.oxfordjournals.org/content/191/3/382.long
11. Bedoya VI, Abad V, Trujillo H. Frequency of respiratory syncytial virus in hospitalized infants with lower acute respiratory tract infection in Colombia. Pediatr Infect Dis J. 1996;15(12):1123–4. Available from: http://journals.lww.com/pidj/Citation/1996/12000/Frequency_of_Respiratory_Syncytial_Virus_in.14.aspx
12. Espinal DA, Hurtado IC, Arango AE, García J, Laguna-Torres A, Jaramillo S. Human metapneumovirus in children: first cases in Colombia. (Article in Spanish). Biomédica 2012;32(2):174–8. Available from: http://www.scielo.org.co/scielo.php?pid=S0120-41572012000200003&script=sci_arttext
13. Organización Panamericana de la Salud. Guía operativa para la vigilancia nacional intensificada de infección respiratoria auda grave (IRAG). Washington D.C., USA: Organización Panamericana de la Salud; 2011. Available from: http://www.paho.org/hq./index.php?option=com_docman&task=doc_view&gid=17126&Itemid=
14. Barenfanger J, Mueller T, O'Brien J, Drake C, Lawhorn J. Prevalence of human metapneumovirus in central Illinois in patients thought to have respiratory viral infection. J Clin Microbiol 2008;46(4):1489–90. Available from : http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2292915/
15. Coiras MT, Aguilar JC, Garcia ML, Casas I, Perez-Breña P. Simultaneous Detection of Fourteen Respiratory Viruses in Clinical Specimens by Two Multiplex Reverse Transcription Nested-PCR Assays. J Med Virol. 2004;72(3):484–95. Available from: http://onlinelibrary.wiley.com/doi/10.1002/jmv.20008/abstract;jsessionid=D163291583F1197EF399318655172F7A.f03t04
16. Coiras MT, Perez-Brena P, Garcıa ML, Casas I. Simultaneous detection of influenza A, B, and C viruses, respiratory syncytial virus, and adenoviruses in clinical samples by multiplex reverse transcription nested-PCR assay. J Med Virol. 2003;69(1):132–44. Available from: http://onlinelibrary.wiley.com/doi/10.1002/jmv.10255/abstract
17. Lopez-Huertas MR, Casas I, Acosta-Herrera B, Garcia Ml, Coiras MT, Perez-Breña P. Two RT-PCR based assays to detect human metapneumovirus in nasopharyngeal aspirates. J Virol Methods 2005;129(1):1–7. Available from: http://www.sciencedirect.com/science/article/pii/S0166093405001540
18. McIntosh K, Masters HB, Orr I, Chao RK, Barkin RM. The immunologic response to infection with respiratory syncytial virus in infants. J Infect Dis. 1978;138(1):24–32. Available from: http://jid.oxfordjournals.org/content/138/1/24.long
19. Watt PJ, Zardis M, Lambden PR. Age related IgG subclass response to respiratory syncytial virus fusion protein in infected infants. Clin Exp Immunol. 1986;64(3):503–9. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1542426/
20. Tregoning J, Schwarze J. Respiratory viral infectious in infants: Causes, Clinical Symptoms, Virology, and Immunology. Clin Microbiol Review. 2010;23(1):74–88. Available from: http://cmr.asm.org/content/23/1/74.abstract
21. Moura FEA, Nunes IFS, Silva JB, Siqueira MM. Short report: Respiratory syncytial virus infections in northeastern Brazil: seasonal trends and general aspects. Am J Trop Med Hyg. 2006;74(1):165–7. Available from: http://www.ajtmh.org/content/74/1/165.full.pdf
22. Heirholzer JC, Tannock GA, Heirholzer CM. Subgrouping of respiratory syncytial virus from Australia and Papua New Guinea by biological and antigenic characteristics. Arch Virol. 1994;136(1–2):133–47. Available from: http://link.springer.com/article/10.1007%2FBF01538823
23. Chew FT, Doraisingham S, Ling AE, Kumarasinghe G, Lee BW. Seasonal trends of viral respiratory tract infections in the tropics. Epidemiol Infect. 1998;121(1):121–8. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2809482/
24. Loscertales MP, Roca A, Ventura PJ, Abacassamo F, Dos Santos F, Sitaube M, et al. Epidemiology and clinical presentation of respiratory syncytial virus infection in a rural area of southern Mozambique. Pediatr Infect Dis J. 2002;21(2):148–55. Available from: http://ovidsp.tx.ovid.com.ezproxy/sp-3.12.0b/ovidweb.cgi?WebLinkFrameset=1&S=FIINFPGFOCDDFJANNC
25. Tsai HP, Kuo PH, Liu CC, Wang JR. Respiratory viral infections among inpatients and outpatients in Taiwan from 1997 to 1999. J Clin Microbiol. 2001;39(1):111–8. Available from: http://jcm.asm.org/content/39/1/111.full
26. Frabasile S, Delfraro A, Facal L, Videla C, Galiano M, de Sierra MJ, et al. Antigenic and genetic variability of human respiratory syncytial viruses (group A) isolated in Uruguay and Argentina: 1993-2001. J Med Virol. 2003;71(2):305–12. Available from: http://onlinelibrary.wiley.com/doi/10.1002/jmv.10484/
27. Cane P. Molecular epidemiology of respiratory syncytial virus. Rev Med Virol. 2001;11(2):103–16. Available from: http://onlinelibrary.wiley.com/doi/10.1002/rmv.305/abstract
28. Costa LF, Yokosawa J, Mantese OC, Oliveira TF, Silveira HL, Nepomuceno LL. Respiratory viruses in children younger than five years old with acute respiratory disease from 2001 to 2004 in Uberlandia, MG, Brazil. Mem Inst Oswaldo Cruz. 2006;101(3):301–6. Available from: http://www.scielo.br/pdf/mioc/v101n3/v101n3a14.pdf
29. Straliotto SM, Siqueira MM, Muller RL, Fischer GB, Cunha ML, Nestor SM. Viral etiology of acute respiratory infections among children in Porto Alegre, RS, Brazil. Rev Soc Bras Med Trop. 2002;35(4):283–91. Available from: http://www.scielo.br/pdf/rsbmt/v35n4/a02v35n4.pdf
30. Weber MW, Dackour R, Usen S, Schneider G, Adegbola RA, Cane P, et al. The clinical spectrum of respiratory virus disease in the Gambia. Pediatr Infect Dis J. 1998;17(3):224–30. Available from: http://ovidsp.tx.ovid.com/sp-3.12.0b/ovidweb.cgi?
31. Reese PE, Marchette NJ. Respiratory syncytial virus infection and prevalence of subgroups A and B in Hawaii. J Clin Microbiol 1991;29(11):2614–5. Available from: http://jcm.asm.org/content/29/11/2614.long
32. Checon RE, Siqueira MM, Lugon AK, Portes S, Dietze R. Seasonal pattern of respiratory syncytial virus in a region with tropical climate in southeastern Brazil. Am J Trop Med Hyg. 2002;67(5):490–1. Available from: http://www.ajtmh.org/content/67/5/490.long
33. Ramirez OV, Savón C, Goyenechea A, Valdivia A, González G, Palermo L, et al. Different circulation patterns of subgroups A and B of human respiratory syncytial virus in some provinces of Cuba. (Article in Spanish). Rev Cubana Med Trop. 2004;56(2):94–102. Available from: http://scielo.sld.cu/scielo.php?pid=S037507602004000200003&script=sci_arttext
34. Galiano M, Palomo C, Videla C, Arbiza J, Melero J, Carballal G. Genetic and antigenic variability of human respiratory syncytial virus (Groups A and B) isolated on seven consecutive Seasons in Argentina (1995 to 2001). J Clin Microbiol. 2005;43(5):2266–73. Available from: http://jcm.asm.org/cgi/pmidlookup?view=long&pmid=15872254
35. Instituto Nacional De Salud. Laboratorio de Virología y Dirección de Redes en Salud Pública. Circulación de Influenza y otros virus respiratorios en Colombia 2000–2012. Bogotá D.C., Colombia: Instituto Nacional de Salud; 2012.

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Virus sincitial respiratorio y metapneumovirus humano en pacientes con infección respiratoria aguda en Colombia, 2000–2011

RESUMEN

Objetivo. Describir la epidemiología y tendencias estacionales del virus sincitial respiratorio (VSR) y metapneumovirus humano (MPVh) en Colombia durante el período 2000–2011.

Métodos. Se recolectaron aspirados nasofaríngeos o hisopados faríngeos de 14 870 pacientes con infección respiratoria aguda. Se analizaron dos subgrupos por técnicas de biología molecular. El primero estuvo compuesto por 264 muestras positivas para el VSR por inmunofluorescencia indirecta (IFI), y el segundo estuvo compuesto por 264 muestras negativas para el VSR por IFI. La técnica utilizada para la detección del VSR y el MPVh en ambos subgrupos fue la transcripción inversa asociada a la reacción en cadena de la polimerasa (RT-PCR).

Resultados. 2 799 muestras (18,8%) contenían algún virus respiratorio. Se detectó VSR por IFI en 1 333 muestras (8,9%), e igualmente fue detectado por RT-PCR en 192 muestras en el subgrupo de muestras positivas para el VSR por IFI y en 25 muestras en el subgrupo de muestras negativas para el VSR por IFI. Se detectó MPVh en 8 muestras en el subgrupo de muestras positivas para el VSR por IFI, y en 11 muestras en el subgrupo de muestras negativas para el VSR por IFI. De las infecciones causadas por el VSR, el subtipo A fue dominante en períodos bianuales; en contraste, el subtipo B fue dominante en períodos anuales. El 85,3% de las infecciones por el VSR y 74% de las infecciones por el MPVh ocurrieron en niños menores de 5 años de edad. Las infecciones causadas por el VSR y el MPVh se asociaron con las estaciones de lluvia. Se encontró coinfección con VSR A y VSR B en 2 pacientes, y coinfección con VSR y MPVh en 5 pacientes.

Conclusiones. Esta investigación es la primera en estudiar la epidemiología de las infecciones respiratorias agudas en Colombia, con énfasis en las causadas por el VSR y el MPVh. Las muestras estudiadas fueron recolectadas durante un período de 12 años y representan todos los grupos etarios de ambos sexos.

Palabras clave Virus sincitiales respiratorios, metapneumovirus, reacción en cadena de la polimerasa, infecciones del sistema respiratorio, técnicas de diagnóstico del sistema respiratorio, Colombia.