INTRODUCTION

Human bocavirus (HBoV) is a parvovirus with a possible aetiological role in respiratory disease that is currently under investigation. We detected HBoV1 in children and adults hospitalized with acute disease of the lower respiratory tract. HBoV genome was detected by PCR in nasopharyngeal swabs collected from 75 patients aged 0–89 years during 2010. HBoV was found in 17/75 (22.7%) patients, 64.7% of them infants younger than 1 year old and 29.4% adults older than 30 years [the bimodal age distribution among HBoV-positive (HBoV+) patients was statistically significant, P<0.001]. Of all HBoV+ cases, 35.3% were co-infected; all co-infections occurred in children (≤13 years old) and 83.3% of them were HBoV-respiratory syncytial virus (RSV) co-infections. Among infants younger than 1 year, 50% HBoV+ specimens were co-infected, all of them with RSV. The rate of co-infection in infants was significantly higher compared to the frequency of co-infection in the whole cohort (P=0.003). The results suggest that HBoV1 is involved in acute respiratory disease. Interplay between HBoV1 and RSV cannot be discarded as a cause of elevated percentages of co-detections in infants.

METHODS

Patients and clinical specimens. The procedures were evaluated and approved by the Ethics Committee of the Hospital Nacional de Clinicas, Universidad Nacional de Córdoba. Seventy-five clinical specimens (nasopharyngeal swabs) from patients aged 0–89 years hospitalized with a diagnosis of bronchiolitis or pneumonia were tested retrospectively. The samples were collected in the hospital room by qualified personnel and sent properly packaged to the Laboratory of Respiratory Viruses at the Institute of Virology (Laboratorio de Virus Respiratorios, Instituto de Virología) within 24 h of collection. The study involved all available samples derived for viral diagnosis of respiratory viruses [respiratory syncytial virus (RSV), parainfluenza (PIV) 1/2/3, influenza A/B (Flu) and adenovirus (AV)] during 2010.

Abbreviations: ARI, acute respiratory infection; AV, adenovirus; Flu, influenza A/B; HBoV, human bocavirus; PI, parainfluenza; RSV, respiratory syncytial virus.
**Nucleic acid extraction.** Nucleic acids were extracted using guanidine buffer and silica (Boom et al., 1990). Briefly, 100 μl nasopharyngeal swab suspension (in viral transport medium) was incubated with 200 μl lysis buffer (0.1 M Tris pH 6.4, 37 mM EDTA pH 8.0, 0.22 g Triton X-100 ml⁻¹, 1.2 g guanidine isothiocyanate ml⁻¹) and 10 μl silica for 10 min at room temperature. After spinning down, the pellet was washed twice with 70% ethanol and once with 100% acetone, dried at 56 °C for 15 min and resuspended in 25 μl TE buffer (10:1, pH 8.0), followed by incubation for 15 min at 56 °C. The silica was removed by centrifugation at 16,000 g for 2.5 min and 2 μl supernatant was used as template in the PCR.

**Detection of HBoV.** PCRs were performed with 2 μl template and 0.2 mM dNTPs, 0.4 μM forward and reverse primers mix, 2.5 mM MgCl₂, and 0.02 U Platinum Taq DNA polymerase (Invitrogen) μl⁻¹. The primers used amplified the region of HBoV1 DNA from nucleotide 2354 to 2684 (Allander et al., 2005), resulting in a fragment of 354 bp. An amplification cycle of 94 °C, 30 s; 48 °C, 30 s; 72 °C, 1 min was carried out for 35 rounds, preceded by 2 min at 94 °C and followed by a final extension at 72 °C for 10 min. Appropriate negative and positive controls were included. The PCR products were visualized on 8.5% polyacrylamide gels stained with silver solution (0.11 M AgNO₃).

**Analysis of data.** The following epidemiological aspects associated with HBoV-positive (HBoV⁺) samples were considered: prevalence by age; seasonality; and rate of co-infection with other respiratory viruses, for which we used the diagnostic determinations of RSV, PIV, Flu and AV performed by direct immunofluorescence assay (at the Laboratory of Respiratory Viruses, Institute of Virology). Statistically significant differences were identified by the χ² test (two-tailed) with a significance level of 0.05.

**RESULTS AND DISCUSSION**

The mean age (±SD) of all patients included in the study was 14.1 ± 22.2 years (median 1 year). Of the 75 samples, 56 (75%) were from children younger than 15 years and 65 (86.7%) were collected during fall and winter. The prevalence of HBoV in the study population was 17/75 (22.7%). HBoV was detected in patients in the range 15 days to 64 years of age (Fig. 1), but 11/17 (64.7%) were younger than 12 months and 5/17 (29.4%) were adults older than 30 years. The bimodal age distribution among HBoV⁺ patients was statistically significant (P < 0.001). The mean age (±SD) of the 17 HBoV⁺ patients was 14.5 ± 22.2 months (median 0.58 years). Eight out of 33 (24.4%) infants younger than 6 months, 11/38 (28.9%) infants younger than 1 year, and 5/15 (33.3%) adults older than 30 years were HBoV⁺ (Fig. 1). The difference in prevalence between infants younger than 1 year and adults older than 30 years was not statistically significant (P = 0.352). HBoV was detected nearly throughout the year, although most HBoV⁺ cases occurred during the months corresponding to late fall and winter (Fig. 2). Of 17 HBoV⁺ cases, 6 (35.3%) were co-infected with another respiratory virus. All co-infections detected were single co-infections. Five out of 6 (83.3%) were HBoV-RSV co-infections (all of them occurred in infants younger than 1 year) and one was an HBoV-Flu B co-infection, which occurred in a 13-year-old child. The difference between the rate of co-infection in all HBoV⁺ patients (35.3%) and HBoV⁺ patients younger than 1 year (5 out of 10 patients, 50%) was statistically significant (P = 0.003). No co-infections were observed among HBoV⁺ adult patients. HBoV was the second most frequent respiratory virus detected after RSV (20/75, 26.7%), followed by Flu B (6/75, 8%) and PIV 3 (2/75, 2.6%). HBoV was the sole virus detected in 11/75 (14.7%) patients, and the proportion at which it was found in mono-infections was 11/75 (14.7%). The median age among these patients was 1 year (25th percentile 1.5 months; 75th percentile 39.5 years).

This study shows a high prevalence of HBoV1 (22.7%) in 0–89-year-old patients hospitalized with lower ARI in Córdoba, Argentina, during 2010. The virus was detected across the entire age range studied (Fig. 1). Previous studies detected HBoV mainly in children less than 5 years old and mostly in infants less than 2 years old (Choi et al., 2006; Kaplan et al., 2006; Kesebir et al., 2006; Kleines et al., 2007; Brieu et al., 2008; Canducci et al., 2008; Cilla et al., 2008). However, a recent report (Guido et al., 2011) and the present work confirm that HBoV1 is also a frequent virus in adults with respiratory disease. On the other hand, since initially HBoV was detected in patients 5–6 months of age and older (Ma et al., 2006; Allander et al., 2007), and more than 90% of infants younger than 3 months had specific antibodies, some authors proposed that maternal antibodies could prevent neonatal infection by HBoV (Endo et al., 2007). Yet here HBoV was detected among children 0–0.5 years old at a high frequency (24.4%, Fig. 1), indicating a very early incidence of HBoV1 infection. This study and others (Weissbrich et al., 2006; Chow et al., 2008; Al-Rousan et al., 2011) detected HBoV1 as early as few days after birth; thus HBoV1 infection during the first month of life may not be infrequent. HBoV⁺ cases occurred throughout the year but peaked during late fall and winter (Fig. 2), which is consistent with analogous findings by other authors (Allander et al., 2005; Kesebir et al., 2006; Weissbrich et al., 2006; Chow et al., 2008; Cilla et al., 2008; Martin et al., 2010). The higher frequency of detection during the cold months might partially explain the high rate of co-infection with other respiratory viruses that circulate with a similar pattern, in particular RSV. The
present work allows only an estimation of the rate of co-infection with other major respiratory viruses in lower ARI and relative frequency of detection, since we used the results of the diagnosis of RSV, PIV, Flu and AV by immunofluorescence assay. Due to the low sensitivity of this technique compared to PCR, the rate of co-infection in our sample is probably higher. In addition, rhinovirus, associated with the development of lower respiratory disease especially in adults (Pierangeli et al., 2011), was not diagnosed in the patients included in this study. However, considering the high frequency of HBoV1 detection in infants younger than 1 year and during the fall–winter period, plus the circulation pattern of RSV (Leung et al., 2005), a high rate of HBoV1-RSV co-infection is most likely. Elevated percentages of RSV-HBoV1 co-infections have been observed by others and RSV is commonly cited as the most frequent respiratory virus in co-infection with HBoV1 (Kaplan et al., 2006; Weissbrich et al., 2006; Gerna et al., 2007; Cilla et al., 2008; Pilger et al., 2011). Interestingly, we did not find co-infections in HBoV+ samples from adults. Thus, an interaction between HBoV1 and RSV being at least partly responsible for the high rate of co-infections between these two viruses cannot be discarded. Taking into account the low rate of HBoV1 detection among children above 2 years old, and in teenagers and young adults, HBoV1 appears to be a respiratory virus with increased infection rate or pathogenic potential in the extremes of life.

One of the limitations of this and other studies is the lack of a control group (individuals without respiratory symptoms). Thus, several scenarios are still possible for HBoV1 (a mild respiratory agent, a respiratory pathogen with significant impact on health in children and adults, or an agent whose rate of infection and pathogenicity are facilitated by other respiratory agents). Nevertheless, the results presented suggest that HBoV1 is involved in respiratory disease.

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REFERENCES


