

# Frequency of human bocavirus (HBoV) infection among children with febrile respiratory symptoms in Argentina, Nicaragua and Peru

Gabriela Salmón-Mulanovich,<sup>a,b</sup> Merly Sovero,<sup>a</sup> V. Alberto Laguna-Torres,<sup>a</sup> Tadeusz J. Kochel,<sup>a</sup> Andres G. Lescano,<sup>a,c</sup> Gloria Chauca,<sup>a</sup> J. Felix Sanchez,<sup>d,e</sup> Francisco Rodriguez,<sup>f</sup> Eduardo Parrales,<sup>g</sup> Victor Ocaña,<sup>h</sup> Melvin Barrantes,<sup>i</sup> David L. Blazes,<sup>j</sup> Joel M. Montgomery<sup>a,k</sup>

<sup>a</sup>Naval Medical Research Center Detachment, Callao, Peru. <sup>b</sup>Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA.

<sup>c</sup>Universidad Peruana Cayetano Heredia, Lima, Peru. <sup>d</sup>Hospital Infantil Manuel de Jesús Rivera, Ministerio de Salud, Nicaragua. <sup>e</sup>Facultad de Medicina de Universidad Nacional Autónoma, Nicaragua. <sup>f</sup>Hospital Departamental Humberto Alvarado de Masaya, Nicaragua. <sup>g</sup>Dirección de Planificación, Ministerio de Salud, Nicaragua. <sup>h</sup>Centro de Salud Chiclayito, Piura, Perú. <sup>i</sup>Hospital Materno-Infantil Dr. Eduardo Oller, San Francisco Solano, Buenos Aires, Argentina. <sup>j</sup>Division of GEIS Operations, Armed Forces Health Surveillance Center, Silver Spring, MD, USA. <sup>k</sup>US Centers for Disease Control and Prevention, Atlanta, GA, USA.

*Correspondence:* Gabriela Salmón-Mulanovich, Naval Medical Research Center Detachment, Av. Venezuela s/n cdra 36, Bellavista, Callao, Peru. E-mail: gsalmom@jhsph.edu

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**Background** Globally, respiratory infections are the primary cause of illness in developing countries, specifically among children; however, an etiological agent for many of these illnesses is rarely identified.

**Objectives** Our study aimed to estimate the frequency of human bocavirus (HBoV) infection among pediatric populations in Argentina, Nicaragua and Peru.

**Methods** We conducted a cross-sectional study using stored samples of an influenza-like illness surveillance program. Irrespective of previous diagnosis, nasopharyngeal or nasal swab specimens were randomly selected and tested using real-time PCR

from three sites during 2007 from patients younger than 6 years old.

**Results** A total of 568 specimens from Argentina (185), Nicaragua (192) and Peru (191) were tested. The prevalence of HBoV was 10·8% (95% CI: 6·3; 15·3) in Argentina, 33·3% in Nicaragua (95% CI: 26·6; 40·1) and 25·1% in Peru (95% CI: 18·9; 31·3).

**Conclusions** These findings demonstrate circulation of HBoV in Argentina, Nicaragua and Peru among children with influenza-like symptoms enrolled in a sentinel surveillance program.

**Keywords** Epidemiology, human bocavirus, respiratory illness.

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## Introduction

Globally, respiratory infections are one of the primary causes of illness in developing countries, specifically among children younger than 5 years old. However, the etiology of these acute respiratory infections diagnosed as pneumonia is determined in only 75% of the cases.<sup>1</sup> Of these identified respiratory pathogens, typically determined with molecular techniques, viruses account for approximately 15% of the infections.<sup>2</sup> Nonetheless, by employing molecular diagnostic techniques in hospital-based studies, one can typically identify up to 90% of the viral agents associated with respiratory tract infections.<sup>3</sup>

Human bocavirus (HBoV) was originally described by Allander *et al.*<sup>4</sup> among children with respiratory tract

infection in Sweden, 2005. Since then, it has been reported in different countries<sup>3,5–7</sup> and in patients of differing ages, although the majority of the infections have been detected in young children.<sup>5,6</sup>

Although our understanding of the epidemiology and genetic characteristics of this novel virus has been enhanced with the increasing information, we are still unable to discern the role of this virus in respiratory infections.<sup>8–10</sup> Longtin *et al.* and Garcia-Garcia *et al.* have also identified the virus among asymptomatic children.<sup>11</sup> The virus has also been identified in fecal samples of Spanish and Brazilian children with diarrheal symptoms.<sup>12</sup> Additionally, it is not uncommon to find the virus in co-infection with other known pathogens. Given these inconclusive and confusing findings, the scientific

community remains divided as to whether HBoV is a true respiratory pathogen.

Since 2006, the US Naval Medical Research Center Detachment (NMRCDD) in Peru has conducted passive influenza-like illness (ILI) surveillance for numerous pathogens in Peru<sup>13</sup> and other Central and South American Countries. The ILI sentinel surveillance program enrolls all patients who approach the participating health services with oral temperature of 38°C or higher and sore throat or cough lasting <5 days. Under the regular ILI surveillance program, the specimens are tested for evidence of influenza A and B, adenovirus, parainfluenza 1–3, herpesvirus and respiratory syncytial virus (RSV) infection.

In this framework, our study aimed to identify the frequency of HBoV infection among a pediatric subset of stored samples of the ILI-enrolled population under surveillance from Argentina, Nicaragua and Peru (1, 2, 3, respectively, see Figure 1).

## Materials and methods

We randomly selected a total of 568 stored nasopharyngeal swabs or nasal washes collected in 2007 from children



Figure 1. Study sites.

younger than 6 years old who suffered fever and cough or sore throat in three study sites from the program. The facilities included in the study were three city hospitals in Buenos Aires, Argentina, in Managua and Masaya, Nicaragua; and one primary healthcare center in Managua, Nicaragua and four in Piura, Peru: 1) Hospital Infantil from Buenos Aires, Argentina; 2) Hospital Departamental Humberto Alvarado from Masaya, Hospital Infantil Manuel Jesus Rivera “La Mascota” and Centro de Salud Villa Venezuela from Managua, Nicaragua; and 3) Centro de Salud Chiclayito, Bellavista, Pachitea and Centro Medico Militar, Piura, Peru (Figure 1).

All samples tested for HBoV were assessed using real-time PCR as described by Lu *et al.*<sup>14</sup> The DNA was extracted and eluted from 200 µl of transport media using QIAmp DNA blood Mini kit (Qiagen, Valencia, CA, USA). The samples were tested for NS1 and NP1 genes (Table 1) by real-time PCR using the TaqMan® Universal PCR Master Mix Reagent kit (Applied Biosystems, Foster City, CA, USA). The PCR was carried out in a volume of 20 µl of master mix and 5 µl of sample. The thermocycling conditions were as follows: 95°C for 3 minutes, followed by 45 cycles of 95°C for 15 seconds and 60°C for 1 minute. Amplification was performed on an ABI 7500 real-time PCR system (Applied Biosystems). Sequencing was conducted on any positive real-time PCR samples.

HBoV infection was determined from stored samples from each site, and 95% confidence intervals were estimated with the binomial exact test. An exploratory analysis was performed to determine the differences in distribution of HBoV infection, by country and within country, by age or gender, using a *t*-test for equal variance and a chi-square test. The presence of co-infections was assessed with cross-tabulations. All analyses were performed with Stata/SE 10.1, StataCorp LP, College Station, TX, USA.

Table 1. Primers and probes used to detect human bocavirus

Primer/probe	Sequence (5'–3')	Gene
NS1-F	TGC AGA CAA CGC YTA GTT GTT T	NS-1
NS1-R	CTG TCC CGC CCA AGA TAC A	NS-1
NS1 P	6FAM-CCA GGA TTG GGT GGA ACC TGC AAA-BHQ	NS-1
NP1-F	AGA GGC TCG GGC TCA TAT CA	NP-1
NP1-R	CAC TTG GTC TGA GGT CTT CGA A	NP-1
NP1 P	VIC-AGG AAC ACC CAA TCA RCC ACC TAT CGT CT-BHQ	NP-1

## Results

### Study population

A total of 568 nasopharyngeal swabs were tested. Of these, 185, 192 and 191 individuals from Argentina, Nicaragua and Peru, respectively, met the inclusion criteria. All individuals were children with a median age of 1 year (range: 0–5 years). All sites had the same median age; however, mean ages were 1.21 years in Nicaragua (SD: 1.40), 1.75 years in Argentina (SD: 1.45) and 1.79 years in Peru (SD: 1.50). Forty-five percent of all participants were girls. A slight predominance in male participants was observed in each of the sites: 52.4% Argentina, 58.8% Nicaragua and 53.4% Peru, although this finding was not statistically significant.

### HBoV prevalence

The prevalence of HBoV for the entire study population was 23.2% (95% CI: 19.7; 26.7) ( $n = 132$ ). The site in Argentina showed a lower prevalence of HBoV infection when compared to Peru or Nicaragua (both  $P < 0.001$ , using Fisher's exact test). The estimated prevalence for each site and binomial exact confidence intervals are presented in Table 2. HBoV was detected in 132/568 (23.2%) specimens, 34% (45/132) of which were co-infected with another respiratory virus.

The  $t$ -test for equal variance to assess the age distribution between children positive or negative for HBoV infection (variance ratio test:  $P = 0.517$ ) showed no age effect ( $P = 0.056$ ), and we obtained the same outcome when examining the age distribution within each of the countries.

Sixty-one percent of the children who tested positive for HBoV were boys, but the gender distribution across sites was not significantly different ( $P = 0.110$ ).

## Discussion

This study, performed in a population of participants in a respiratory surveillance program in three different geographical sites in Latin America, presents evidence of the

circulation of HBoV in Argentina, Nicaragua and Peru. The virus had previously been reported to circulate in Brazilian children.<sup>5,14,15</sup> The prevalence reported in the original study from Allander *et al.* was 3.5% in hospitalized patients in Sweden. Additional studies, mostly among hospitalized children and infants with respiratory infections, have identified prevalence proportions ranging from 1.5% to 19% in different regions.<sup>11,16,17</sup> The different values reported from these studies may be because of sampling techniques, study populations and the sensitivity of the diagnostic assay.<sup>18</sup> The prevalence of infection detected in our study in Argentina is consistent with these findings. However, the prevalence rates that we identified in Nicaragua and Peru are higher than have been previously reported for this virus. The reason why these two sites have higher prevalence rates may be related to the circulation of the virus in these areas caused by appropriate environmental conditions (susceptible population, climate, geography, migration, etc.) that may also favor persistent infection as has been reported in other studies.<sup>19</sup> Despite the difference in prevalence rates among the three sites, the age and gender distribution of our samples are similar and comparable to other studies with a peak in HBoV infection in children 1 year of age and a slight increase in detection among boys. In our study, as in previous studies, HBoV has been identified as a co-infecting agent in a high proportion (ranging from 9.5% to 83.0%) of respiratory illnesses.<sup>4,6,20–23</sup>

The role of HBoV as a pathogen remains unclear.<sup>8,9</sup> In our study, we detected HBoV as the most common viral agent, i.e. 23.2% of the total samples tested. However, we were unable to detect an agent in 49.0% of the samples, and we did not test for some known respiratory viruses, such as human rhinovirus, coronavirus or for bacterial agents. Previous studies have shown that upper respiratory infections among children may be caused by a variety of viral agents and very frequently occur as co-infections<sup>24,25</sup>, and this may be the case for these HBoV-positive samples. Furthermore, additional studies have detected HBoV in fecal samples of patients with both respiratory and gastrointestinal symptoms<sup>12,26,27</sup>, and in Brazil, there was evidence of infection in children with only symptoms of gastrointestinal distress, no respiratory symptoms.<sup>14</sup> This may suggest an alternative transmission route for the virus and its pathogenic role in the gastrointestinal tract. However, a recent study by Garcia-Garcia *et al.*<sup>28</sup> demonstrated that HBoV infection among hospitalized children with respiratory symptoms was more frequent than in healthy ones.

Future studies should include testing for evidence of HBoV within populations from additional surveillance sites from a variety of locations globally, evaluating the severity of HBoV cases, and assessment of non-respiratory patients (healthy individuals) to help better define this virus as a potential pathogen.

**Table 2.** Prevalence of human bocavirus in study population by country

Country	Sample size	Positive samples	Prevalence	95% CI
Argentina	185	20	10.8	6.3; 15.3
Nicaragua	192	64	33.3	26.6; 40.1
Peru	191	48	25.1	18.9; 31.3
Total	568	132		

## Conclusions

These findings demonstrate circulation of HBoV in Argentina, Nicaragua and Peru. The age and gender distribution is similar to what has been reported previously. HBoV was found in 23% of the samples collected from children with respiratory symptoms, which is somewhat higher to what has been reported previously. As in previous studies, HBoV was found mainly as a co-infecting agent in our samples, making it difficult to determine whether it is a pathogenic virus, exacerbates the symptoms of other viral infections<sup>22</sup>, or is merely a persistent non-pathogenic (commensal) virus.<sup>19</sup> Routine testing for HBoV among patients with respiratory symptoms should be included in the testing strategy among any respiratory illness surveillance system, as has been previously suggested.<sup>3</sup>

## Addendum

G Salmon-Mulanovich, JM Montgomery, Blazes DL Blazes, AG Lescano contributed to the concept and design of the study. G Salmon-Mulanovich, M Sovero analyzed and interpreted the data. VA Laguna-Torres, TJ Kochel, G Chacuca, JM Montgomery, Blazes DL Blazes, AG Lescano revised the intellectual content of the manuscript. JF Sanchez, F Rodriguez, E Parrales, V Ocaña, M Barrantes revised and approved the version to be published.

## Disclaimer

The views expressed in this manuscript are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the US Government.

## IRB statement

The Naval Medical Research Center Detachment Institutional Review Board determined the investigation (PJT.NMRCD.028) did not meet the definition of human subject research.

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## Conflict of interest

The authors declare no conflict of interest or significant financial interest TO DISCLOSE related to the entity funding the project or any other institution involved with the investigation.

## Meetings

The majority these findings were presented as a poster at the 25th Annual Clinical Virology Symposium, in Daytona Beach, Florida 19–22nd April, 2009.

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