Clinical and molecular characterization of children and adults with respiratory bocavirus infection in Mexico: a cross-sectional nested study within the ILI002 prospective observational study

Ana Estela Gamiño-Arroyo,[a,k] José Arellano-Galindo,[a,k] Paola Del Carmen Guerra-de-Blas,[b] Ana M. Ortega-Villa,[c] Allyson Mateja,[d] Beatriz Llamosas-Gallardo,[e] Ana A. Ortíz-Hernández,[e] Rafael Valdéz-Vázquez,[f] Alejandra Ramírez-Venegas,[g] Arturo Galindo-Fraga,[h] Ma Lourdes Guerrero,[i] Pilar Ramos-Cervantes,[i] Luis Mendoza-Garcés,[b] Mónica González-Matus,[a] Carmen Marroquín-Rojas,[a] Juan Xicohtencatl-Cortes,[a] Sara A. Ochoa,[a] Ariadna Cruz-Córdova,[a] John H. Powers,[j] Guillermo Miguel Ruiz-Palacios,[i] John Beigel,[c] and Sarbelio Moreno-Espinosa,[a,*] on behalf of the Mexican Emerging Infectious Diseases Clinical Research Network (LaRed)

[a]Hospital Infantil de México Federico Gómez, Instituto Nacional de Salud, Mexico City, Mexico
[b]The Mexican Emerging Infectious Diseases Clinical Research Network (LaRed), Mexico City, Mexico
[c]National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA
[d]Clinical Monitoring Research Program Directorate, Frederick National Laboratory for Cancer Research, Frederick County, MD, USA
[e]Instituto Nacional de Pediatría, Mexico City, Mexico
[f]Hospital General "Dr. Manuel Gea González", Mexico City, Mexico
[g]Instituto Nacional de Enfermedades Respiratorias "Ismael Cosío Villegas", Mexico City, Mexico
[h]Departamento de Epidemiología, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
[i]Departamento de Infectología, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
[j]Clinical Research Directorate, Frederick National Laboratory for Cancer Research, Frederick County, MD, USA

The Lancet Regional Health - Americas 2024;29: 100647

Published Online xxx https://doi.org/10.1016/j.lana.2023.100647

Summary

Background Human Bocaviruses (HBoV) can cause acute respiratory tract infections. High coinfection rates cloud its pathogenicity. This study sought to describe the clinical features of HBoV1 disease in children and adults with Influenza-like illness (ILI), exploring associations between viral load, clinical features, and seasonality.

Methods Patients who tested positive for HBoV1 by polymerase chain reaction, enrolled from April 2010 to March 2014 in the ILI002 prospective observational cohort study were included in this cross-sectional nested study. Participants were included in ILI002 if they presented with signs and/or symptoms suggestive of influenza-like illness. Samples were tested for viral load, and NP1 and VP1/VP2 phylogenetic analyses, except for the samples lacking suitable and viable clinical material for genotyping.

Findings We identified HBoV1 in 157 (2.8%) of participants. Prevalence was 4.5% in children and 1.8% in adults. Single HBoV1 detection occurred in 41.1% and 46.3% of children and adults, respectively. Children commonly experienced fever (83.3%), cough with sputum (74.4%), and shortness of breath (72.2%). In the multivariate analysis of children, significant positive associations were detected between viral loads and age (0.20 [95% CI: 0.07, 0.33]), and the presence of fever (2.64 [95% CI: 1.35, 3.94]), nasal congestion (1.03 [95% CI: 0.07, 1.99]), dry cough (1.32 [95% CI: 0.42, 2.22]), chest congestion (1.57 [95% CI: 0.33, 2.80]), red eyes (1.25 [95% CI: 0.35, 2.14]), cough with sputum (1.79 [95% CI: 0.80, 2.78]), and other signs and symptoms such as chills, dizziness, and diaphoresis (1.73 [95% CI: 0.19, 3.27]). In contrast, significant negative associations were found between viral loads and percent neutrophils on the blood count (–0.04 [95% CI: –0.06, –0.02]), fatigue (–1.60 [95% CI: –2.46, –0.74]) and the presence of other symptoms or signs, including adenopathy and rash (–1.26 [95% CI: –2.31, –0.21]). Adults commonly experienced sore throat (73.1%), fatigue (77.4%), and headache (73.1%). In the multivariate analysis of adults, significant positive associations were detected between viral load and body mass index (0.13 [95% CI: 0.04, 0.21]), and the presence of confusion (1.54 [95% CI: 0.55, 2.53]), and sore throat (1.03 [95% CI: 0.20, 1.85]), and significant negative associations were detected between viral load and chest congestion (–1.16 [95% CI: –2.07, –0.24]). HBoV1 was detected throughout the year irrespective of season, temperature, and humidity.

*Corresponding author. Hospital Infantil de México Federico Gómez, Instituto Nacional de Salud, Doctor Márquez 162, Doctores, Cuauhtémoc, Mexico City 06720, Mexico.
E-mail address: sarbeliomoreno.infecto@gmail.com (S. Moreno-Espinosa).
[k]These authors contributed equally to the work.
Registration: NCT01418287.

Interpretation This study demonstrated the importance of detecting HBoV1 in patients with influenza-like illness either as single infection or co-infection, in both adults and children, and improves the characterization of HBoV1 seasonality, clinical features, and viral load. Phylogenetic analyses show a high conservation.

Funding The Mexican Emerging Infectious Diseases Clinical Research Network (LaRed), CONACYT (Fondo Sectorial SSA/IMSS/ISSSTE, Projects No. 71260 and No. 127088), Fondos federales no. HIM/2015/006, NIAID, NIH through a contract with Westat, Inc. (HHSN2722009000031, HHSN27200002), NCI, NIH (75N91019D00024, 75N91019F00130). Additional information at the end of the manuscript.

Keywords: Human bocavirus (HBoV); Influenza-like illness (ILI); Viral load; Phylogenetic analysis; Seasonality

Research in context

Evidence before this study

We searched in PubMed for studies published from 2005 to March, 2023, using the search terms "('HBoV OR Bocavirus' AND 'respiratory infection OR influenza-like illness') AND ('single infection AND co-infection')" AND ('children OR adults' OR 'Mexican population'). Human Bocavirus is a relatively newly identified virus, isolated for the first time in 2005. Throughout the last decade, the genotype HBoV1 has been detected with increasing frequency, mainly in respiratory samples. It has been associated with both upper and lower respiratory symptoms. Although it shows a relatively high rate of co-infection with other respiratory viruses, HBoV1 has also been identified as a single infection, causing influenza-like illness (ILI). However, contradictory evidence exists about the role of HBoV1 as a causing agent of ILI when presented as a single infection; the role of viral load in the disease is also poorly understood.

Most published data focus on paediatric populations, though some studies have been done on adults infected with HBoV1. Additionally, there is no epidemiological or phylogenetic analysis of this virus done in Mexico that includes a wide range of age groups.

Added value of this study

To our knowledge, this is the one of the largest cohorts to characterize HBoV1 infection in the Mexican population; we aim to assess relationships between viral load, single or co-infection, and clinical manifestations.

Our study has several strengths over previous similar studies. Importantly, it provides a complete characterisation of the clinical and molecular features of an HBoV1-positive cohort of participants and includes both children and adults.

We identified HBoV1 in patients with ILI both as a single infection and co-infection, in both adults and children, highlighting clinical features that include systemic upper and lower respiratory symptoms. We found no association between viral load and severity, in neither single nor co-infection.

Implications of all the available evidence

The surveillance of HBoV1 as a viral agent associated with ILI both as a single and as a co-infection with other pathogens helps to understand the wide spectrum of disease severity in both infection types and may help avoid unnecessary antibiotic prescription. Additional research, including serology or mRNA detection, is needed to establish a direct causal association between HBoV1 and respiratory disease.

Introduction

Respiratory viruses are major causes of morbidity and mortality in patients with influenza-like illness and acute respiratory infections.[1] In 2005, Allander et al., identified human bocavirus (HBoV) in secretions from Swiss children with respiratory infections.[2] Four viral genotypes (HBoV1, 2, 3, and 4) have been identified; HBoV2, 3, and 4 have been detected mainly in human feces, while HBoV1 has been detected mainly in respiratory samples.[3] Most of the studies regarding HBoV1 have focussed on children, finding a variable prevalence worldwide.[4–7] The prevalence of HBoV1 respiratory infections worldwide varies from 1% to 33.3% and it is prevalent in both low- and high-income countries.[8–10] Differences in prevalence could be attributed to the diverse age groups included in various studies. In Mexico, few studies address HBoV. To our knowledge, all of them only evaluate paediatric populations.[11–13]

HBoV1 infection is characterised by influenza-like and lower respiratory tract symptoms (wheezing, respiratory distress).[14,15] Clinical scenarios include acute respiratory infections, co-infection with other viruses, and more severe respiratory disease.[16,17] Viral load has been associated with some symptoms including wheezing and dyspnea.[18] However, other studies found no association between viral load and disease severity.[19] The association between HBoV1 infection, specific clinical symptoms, and disease presentation and course, including co-infections, is incompletely understood.

Most published data focus on paediatric populations and data on wider age ranges are not available. This study is a pioneering effort with one of the largest cohorts of prospective data collected in both children and adult patients presenting with influenza-like illness. This comprehensive investigation provides insights into the clinical and laboratory characterization of HBoV1 infection, spanning several transmission periods. Based on our current awareness, such detailed comparison has not been done within the Mexican adult population, highlighting the unique contribution of this study to the understanding of HBoV1 infections. This effort supports the identification of HBoV1 as an important viral agent that should be studied and searched for in suspected respiratory tract infections and influenza-like illness, both on its own and with other pathogens. Especially during the ILI002 study, bocavirus emerged as a recently identified pathogen in need of comprehensive epidemiological and clinical profiling within the context of Mexico. Furthermore, there was a lack of phylogenetic analyses to assess the distribution of different strains of the virus in the region. Therefore, the objectives of this study were the characterisation of HBoV1 infection in a cohort covering children and adults aged more than 1 month with influenza-like illness, assessing relationships between viral load and clinical manifestations, comparing the disease as a single infection versus as a co-infection with other viral agents, and determining phylogenetic characteristics and seasonality of HBoV1 in Mexico.

Methods

ILI002 is a hospital-based, observational, prospective, longitudinal, multicentre cohort study that enrolled children and adults seeking care for ILI from April 2010 to May 2014.[20] ILI002 was conducted by the Mexican Emerging Infectious Diseases Clinical Research Network (LaRed) in four tertiary-care hospitals in Mexico City and two General Hospitals, one in Mexico City and another in San Luis Potosí, Mexico. During the planning phase of the study, there were no exact estimates available regarding the number of patients exhibiting influenza-like symptoms who progress to severe illness. Considering the observational design of this study and the minimal risk associated with participation, an initial recruitment goal of 5000 participants was established. The participants who sought medical attention at these hospitals were invited to participate and had their demographic data as well as respiratory samples collected at enrolment. The Ethics Committee of each institution approved the study.

Study population and definitions

This is a cross-sectional study nested in the ILI002 cohort. Of the 5662 eligible subjects in the ILI002 cohort, we included 157 study participants seeking medical care for ILI who tested positive for HBoV1. 5505 subjects were deemed ineligible as they did not test positive for HBoV1. Data from all 157 subjects included positive for HBoV1 were included in all subsequent analysis. ILI was defined by at least one respiratory symptom (e.g., cough, sore throat, post-nasal drip) and one of the two following criteria: 1) fever (≥38 °C) on examination, self-reported feverishness in the past 24 h, or participant-reported fever; 2) one or more non-respiratory symptoms (e.g., malaise, headache, myalgia, irritability, etc.). We employed a modified version of the World Health Organization (WHO) definition of ILI, taking into consideration the limitations associated with relying solely on fever as a requirement for diagnosis. In our study, we expanded the criteria beyond fever to include feverishness or systemic symptoms. This decision was based on the fact that a significant proportion of patients having respiratory infections and respiratory symptoms do not exhibit fever as a prominent feature of their illness. We aimed to ensure that our study did not inadvertently exclude a substantial number of participants who might otherwise meet the clinical criteria for ILI in order to capture a more comprehensive representation of individuals experiencing influenza-like symptoms, thus enabling a more accurate assessment of the clinical and epidemiological aspects of ILI within our population of interest.

Study procedures

Written informed consent from adult participants and parents was obtained and assent was obtained for children aged 12–18. Sociodemographic, signs and symptoms data were collected. Physical exams were performed, and nasopharyngeal swabs or nasal aspirates were obtained for PCR for respiratory pathogens. Blood samples were drawn for complete blood counts and chemistry analysis. Information on signs, symptoms, chronic medical conditions, previous treatments, hospitalizations, and death was obtained from medical records and participant report.

DNA extraction

DNA extraction was performed using QIAamp DNA minikit (Qiagen® code 51306) following manufacturer's recommendations.

Laboratory diagnosis

Respiratory samples were tested with RespiFinder 19 (April 2010 to May 2012) or RespiFinder 22 (previously named RespiFinder Plus, June 2012 to March 2014) from PathoFinder BV, Maastricht, the Netherlands. RespiFinder 19 (multiplex RT-PCR test) can identify 15 viruses (coronavirus NL63, OC43, 229E; human metapneumovirus (HMPV); influenza A, AH5N1, B; parainfluenza virus (HPIV) serotypes one through four; respiratory syncytial virus (RSV) A, B; rhinovirus (RV);

adenovirus (HAdV)), and four bacteria. RespiFinder 22 removed influenza H5N1 and added bocavirus (type one), coronavirus HKU1, influenza AH1N1v, and enterovirus. Samples tested with RespiFinder 19 were subsequently tested for HBoV1 using virus-specific primers. Conditions for double nest inhouse PCR using these primers were previously reported.[2] HBoV1 was confirmed by the same inhouse PCR if detected by RespiFinder 22.[2] A participant was categorised as co-infected if the sample at enrolment tested positive for HBoV1 and another virus or bacteria.

Viral load

HBoV1 positive samples were analysed for viral load quantitation using HBoV instrument IV, catalogue MBS598173 (MyBiosource, San Diego Cal.) following manufacturer's recommendations. Fluorescence was read in fluorescence channel FAM for target nucleic acids and HEX/VIC/JOE for internal controls. Viral load was calculated as number of genomes/mL in samples and categorized as: low ($\leq 10^6$ load genomes/mL) and high ($>10^6$ genomes/mL), according to the same cut-off points used by Principi et al., in their study of HBoV in Italy.[21]

HBoV phylogenetic distribution

Phylogenetic distribution was done based on the N terminus of the NP-1 and VP/NC with primers previously reported.[2] The conditions were: for NP-1, buffer with KCl 50 mM, Tris–HCl pH 8.3 10 mM, $MgCl_2$ 3 mM, dNTPs 200 µM, Taq polymerase 5 U/µl (NEB catalog M0273S), primers NP-1 20 pmol and 1 µl of extracted DNA was amplified as previously reported.[22] VP/NC was amplified in the same way but with 1.5 U of taq DNA polymerase and 30 pmol of primer.[23] The products were purified by high pure PCR purification kit from Sigma-Aldrich (Cat. 11732668001) and sequenced in a system of capillaries.

Bioinformatic analysis

Sequences were aligned using software clustal omega (http://www.ebi.ac.uk/Tools/msa/clustalo/). Phylogenetic analyses used the software MEGA 7 (http://www.megasoftware.net). The impact on biological function of the protein was analysed using PROVEAN software (http://provean.jcvi.org/seq_submit.php).

Environmental measures

Humidity and temperature were measured by two weather stations in downtown Mexico City from the Meteorological National Service of Comisión Nacional del Agua México installed at the National School of Biological Science. Station equipment includes sensors of environmental temperature and humidity (Campbell Scientific) and sensors of relative humidity and temperature (Cambell Scientific CS215), automatically measuring daily every ten minutes.

Statistical analysis

Demographics, medical history, and clinical features were compared between participants with HBoV single and co-infection using Fisher's exact tests for categorial variables and Wilcoxon rank-sum tests for continuous variables, analysing children and adults separately. Participants were categorised as co-infected if they tested positive for HBoV1 as defined in the methods section and for another virus or bacteria screened by RespiFinder at enrolment. Categorial variables were summarised by number and frequency. Continuous variables were summarised with mean and standard deviation. Comparisons between clinical presentation and infection status, and between infection status and antibiotic use were performed by Fisher's exact test.

Poisson regression models were used to predict frequencies of bocavirus cases by year and by season (spring, summer, winter, and fall), and by median relative humidity and median temperature per season.

A linear regression model examined univariate relationships of each clinical feature and log10 viral load. Laboratory values CPK, LDH, and creatinine were log10 transformed to reduce variability and skewness. Akaike's Information Criterion (AIC) was used for model selection to explore multivariate relationships between log10 viral load and clinical features where the model with minimum AIC was selected.

Univariate and multivariate logistic regression models with Firth's bias-Reduced penalised-likelihood logistic regression were used to identify any association between the outcome of single versus co-infection with clinical variables and lab parameters.[24,25] We used Firth's bias-reduced penalized likelihood logistic regression during the analysis to address the separation in logistic regression. This analysis was performed using package logistf.[26]

Parameter estimates and 95% confidence intervals were calculated. Statistical analyses were performed in R version 3.6.1 and 4.1.3. $p < 0.05$ was considered statistically significant. Missing data were assumed to be missing at random. Model selection was performed on data with complete observations. We had complete information on signs and symptoms, co-infection status, clinical presentation, and antibiotic use. Any missing data for the variables in the tables is explicitly mentioned in the footer of the tables.

Role of funding source

This research was financially supported by LaRed and NIAID. LaRed & NIAID involvement was restricted solely to the participation of the affiliated authors and did not influence the study design, analysis, writing, or decision to submit the manuscript.

Results

Frequency of infection

Of the 5662 samples that were part of ILI002, 157 (2.8%) were positive for HBoV1. Of the 157 HBoV1-positive

participants, 90 (57.3%) were children ≤18 years old. 67 (42.7%) were adults. Prevalence was 4.5% (90/1988) in children and 1.8% (67/3674) in adults. HBoV1 co-infection occurred in 53/90 children (58.9%) and 36/67 adults (53.7%) (Fig. 1).

Demographic characteristics and medical history

Table 1 presents demographic and medical history data. Among infected children, 37/90 (41.1%) had a single HBoV1 infection, while 53/90 (58.9%) had a co-infection of HBoV1 and another viral agent. In adults, similar proportions of single HBoV1 infection (31/67; 46.3%) and co-infection (36/67; 53.7%) were observed. We detected the presence of two pathogens in 42 children, three pathogens in nine children, and four pathogens in two children. Most adults with co-infection had two pathogens (83.3%); five adults had three pathogens and one adult had five pathogens. The most common co-infections observed in children were with RV (23.3%), followed by influenza (11.1%), HAdV (10.0%), and HMPV (8.9%). In adults, the most common co-infecting agents were RV (22.4%), followed by influenza (16.4%), RSV (9.0%), and HMPV (6.0%). Of the 90 children, 27.8% were outpatients, 10.0% were in the emergency room (ER) for less than 24 h, 34.4% were hospitalized, and 27.8% were in intensive care units (ICU). Of the 67 adults, 53.7% were outpatients, 7.5% were in the ER, 38.8% were hospitalised, and none were detected in intensive care units. In children, 90% (81/90) of cases occurred in those < five years old. In adults, 56/67 (83.6%) cases occurred in participants 18–60 years old. 11/67 (16.4%) cases were in those ≥60 years old. Of the 90 children, 16 (17.8%) had passive smoke exposure. Thirty-three (49.3%) adults were current or former smokers.

Common comorbidities in children were congenital syndromes (21.6% and 20.8% with single and co-infection, respectively). In adults, common comorbidities included asthma (19.4% HBoV1 only, 5.6% co-infections) and cardiovascular disorders (12.9% HBoV1 only, 16.7% co-infections). Other comorbidities included cognitive disfunction, immunodeficiencies, and diabetes mellitus (Supplementary Table S1). Common medications in children and adults were antipyretics and antibiotics. Significantly more children with HBoV1 single infections received antibiotics compared with co-infected children (73% and 49.1%, respectively; p = 0.030). When comparing infection status and antibiotic use for both children and adults combined, we found no significant association (p = 0.104). Other medications included systemic steroids, antihistamines, and cough medicine (Supplementary Table S1). There were no other significant differences in demographics, medical history, or medication use between participants with single and co-infections.

There were 43/75 (57.3%) patients who were outpatient who had a coinfection, compared to 46/82 (56.1%) inpatient who had a coinfection. We found no statistically significant differences between these groups (p = 1.00). We found no statistically significant differences (p = 1.00) between clinical presentation and infection status.

Signs and symptoms

Table 2 presents frequencies of symptoms by age group. Common symptoms in children included fever (83.8% HBoV1 single infection, 83% co-infection), cough with sputum (75.7% HBoV1 single infection, 73.6% co-infection) and shortness of breath (73% HBoV1 single infection, 71.7% co-infection). Significantly more

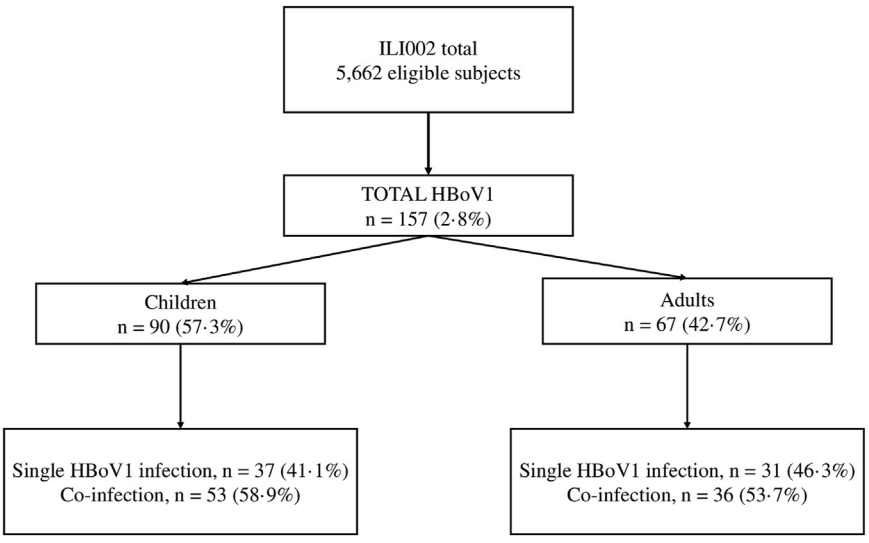

Fig. 1: **Study cohort accrual/Study flow chart.**

	Children				Adults			
	Overall (n = 90)	HBoV1 only (n = 37)	HBoV1 Co-infection (n = 53)	p-value	Overall (n = 67)	HBoV1 only (n = 31)	HBoV1 Co-infection (n = 36)	p-value
	n (%)	n (%)	n (%)		n (%)	n (%)	n (%)	
Status								
Emergency room	9 (10.0)	4 (10.8)	5 (9.4)	0.68	5 (7.5)	3 (9.7)	2 (5.6)	0.69
Hospitalised	31 (34.4)	15 (40.5)	16 (30.2)		26 (38.8)	13 (41.9)	13 (36.1)	
ICU	25 (27.8)	8 (21.6)	17 (32.1)		0 (0.0)	0 (0.0)	0 (0.0)	
Outpatient	25 (27.8)	10 (27.0)	15 (28.3)		36 (53.7)	15 (48.4)	21 (58.3)	
Age								
Median (IQR)	19.20 (10.61, 34.11)	19.76 (10.75, 33.01)	18.97 (10.16, 34.49)	0.51	37.00 (26.50, 54.50)	39.00 (25.50, 54.50)	36.00 (29.25, 54.25)	0.85
Categorical age								
0–1 month	0 (0.0)	0 (0.0)	0 (0.0)	0.48	0 (0.0)	0 (0.0)	0 (0.0)	1.00
>1 month–≤12 months	30 (33.3)	12 (32.4)	18 (34.0)		0 (0.0)	0 (0.0)	0 (0.0)	
>12 months–≤24 months	23 (25.6)	8 (21.6)	15 (28.3)		0 (0.0)	0 (0.0)	0 (0.0)	
>24 months–≤5 years	28 (31.1)	11 (29.7)	17 (32.1)		0 (0.0)	0 (0.0)	0 (0.0)	
6–≤11 years	5 (5.6)	4 (10.8)	1 (1.9)		0 (0.0)	0 (0.0)	0 (0.0)	
12–≤17 years	4 (4.4)	2 (5.4)	2 (3.8)		0 (0.0)	0 (0.0)	0 (0.0)	
18–<60 years	0 (0.0)	0 (0.0)	0 (0.0)		56 (83.6)	26 (83.9)	30 (83.3)	
≥60 years	0 (0.0)	0 (0.0)	0 (0.0)		11 (16.4)	5 (16.1)	6 (16.7)	
Sex								
Male	49 (54.4)	21 (56.8)	28 (52.8)	0.83	49 (54.4)	21 (56.8)	28 (52.8)	0.61
Race								
Mixed	87 (96.7)	35 (94.6)	52 (98.1)	0.57	64 (95.5)	30 (96.8)	34 (94.4)	1.00
White	3 (3.3)	2 (5.4)	1 (1.9)		3 (4.5)	1 (3.2)	2 (5.6)	
Smoking history								
Never smoked	74 (82.2)	31 (83.8)	43 (81.1)	0.79	31 (46.3)	13 (41.9)	18 (50.0)	0.11
Passive smoker	16 (17.8)	6 (16.2)	10 (18.9)		3 (4.5)	0 (0.0)	3 (8.3)	
Current smoker	0 (0.0)	0 (0.0)	0 (0.0)		11 (16.4)	4 (12.9)	7 (19.4)	
Former smoker	0 (0.0)	0 (0.0)	0 (0.0)		22 (32.8)	14 (45.2)	8 (22.2)	
Comorbidities								
Congenital syndromes	19 (21.1)	8 (21.6)	11 (20.8)	1.00	0 (0.0)	0 (0.0)	0 (0.0)	NA
Asthma	4 (4.4)	3 (8.1)	1 (1.9)	0.302	8 (11.9)	6 (19.4)	2 (5.6)	0.13
Cardiovascular disorders	5 (5.6)	2 (5.4)	3 (5.7)	1.00	10 (14.9)	4 (12.9)	6 (16.7)	0.74
Vaccination								
Flu vaccine–Yes	38 (42.2)	18 (48.6)	20 (37.7)	0.39	17 (25.4)	11 (35.5)	6 (16.7)	0.10
Pneumonia vaccine–Yes	75 (83.3)	31 (83.8)	44 (83.0)	1.00	10 (14.9)	3 (9.7)	7 (19.4)	0.32
Current medications								
Antipyretics	49 (54.4)	21 (56.8)	28 (52.8)	0.83	25 (37.3)	10 (32.3)	15 (41.7)	0.46
Antibiotics	53 (58.9)	27 (73.0)	26 (49.1)	0.03	39 (58.2)	18 (58.1)	21 (58.3)	1.00
Co-infections[a]								
Influenza	10 (11.1)	0 (0.0)	10 (18.9)	N/A	11 (16.4)	0 (0.0)	11 (30.6)	N/A
Coronavirus	4 (4.4)	0 (0.0)	4 (7.5)	N/A	1 (1.5)	0 (0.0)	1 (2.8)	N/A
HPIV	8 (8.9)	0 (0.0)	8 (15.1)	N/A	3 (4.5)	0 (0.0)	3 (8.3)	N/A
RSV	5 (5.6)	0 (0.0)	5 (9.4)	N/A	6 (9.0)	0 (0.0)	6 (16.7)	N/A
RV	21 (23.3)	0 (0.0)	21 (39.6)	N/A	15 (22.4)	0 (0.0)	15 (41.7)	N/A
HAdV	9 (10.0)	0 (0.0)	9 (17.0)	N/A	1 (1.5)	0 (0.0)	1 (2.8)	N/A
HMPV	8 (8.9)	0 (0.0)	8 (15.1)	N/A	4 (6.0)	0 (0.0)	4 (11.1)	N/A
Bordetella pertussis	0 (0.0)	0 (0.0)	0 (0.0)	N/A	1 (1.5)	0 (0.0)	1 (2.8)	N/A
Mycoplasma pneumoniae	1 (1.1)	0 (0.0)	1 (1.9)	N/A	0 (0.0)	0 (0.0)	0 (0.0)	N/A

Comorbidities and current medications shown are the most common in either children or adults. p-values from Fisher's Exact Test for categorical variables and Wilcoxon test for continuous variables. HBoV1 = human bocavirus; ICU = intensive care unit; SD = standard deviation; HPIV = human parainfluenza virus; RSV = respiratory syncytial virus; RV = rhinovirus; HAdV = human adenovirus; HMPV = metapneumovirus. Sex was self-reported. [a]The statistical comparisons between single and co-infections for the list of co-infections are not applicable.

Table 1: **Demographics and medical history by age group and infection status.**

	Children				Adults			
	Overall (n = 90)	HBoV1 only (n = 37)	HBoV1 co-infection (n = 53)	p-value[a]	Overall (n = 67)	HBoV1 only (n = 31)	HBoV1 co-infection (n = 36)	p-value[a]
	n (%)	n (%)	n (%)		n (%)	n (%)	n (%)	
Symptoms								
Fever	75 (83.3)	31 (83.8)	44 (83.0)	1.00	42 (62.7)	19 (61.3)	23 (63.9)	1.00
Dry cough	23 (25.6)	10 (27.0)	13 (24.5)	0.81	26 (38.8)	8 (25.8)	18 (50.0)	0.049
Cough with sputum	67 (74.4)	28 (75.7)	39 (73.6)	1.00	41 (61.2)	23 (74.2)	18 (50.0)	0.049
Sore throat	15 (16.7)	7 (18.9)	8 (15.1)	0.78	49 (73.1)	22 (71.0)	27 (75.0)	0.79
Fatigue	26 (28.9)	9 (24.3)	17 (32.1)	0.48	51 (77.6)	24 (77.4)	28 (77.8)	1.00
Headache	10 (11.1)	5 (13.5)	5 (9.4)	0.74	49 (73.1)	23 (74.2)	26 (72.2)	1.00
Muscle ache	8 (8.9)	4 (10.8)	4 (7.5)	0.71	37 (55.2)	17 (54.8)	20 (55.6)	1.00
Red eyes	21 (23.3)	7 (18.9)	14 (26.4)	0.46	19 (28.4)	7 (22.6)	12 (33.3)	0.42
Watery eyes	20 (22.2)	4 (10.8)	16 (30.2)	0.039	28 (41.8)	9 (29.0)	19 (52.8)	0.08
Sneezing	25 (27.8)	7 (18.9)	18 (34.0)	0.15	26 (38.8)	11 (35.5)	15 (41.7)	0.63
Shortness of breath	65 (72.2)	27 (73.0)	38 (71.7)	1.00	20 (29.9)	9 (29.0)	11 (30.6)	1.00
Runny nose	29 (54.4)	16 (43.2)	33 (62.3)	0.088	44 (65.7)	21 (67.7)	23 (63.9)	0.80
Nasal congestion	32 (35.6)	9 (24.3)	23 (43.4)	0.076	39 (58.2)	18 (58.1)	21 (58.3)	1.00
Chest congestion	9 (10.0)	4 (10.8)	5 (9.4)	1.00	14 (20.9)	6 (19.4)	8 (22.2)	1.00
Nasal flattening	24 (26.7)	8 (21.6)	16 (30.2)	0.47	2 (3.0)	0 (0.0)	2 (5.6)	0.50
Nausea	20 (22.2)	4 (10.8)	16 (30.2)	0.039	14 (20.9)	9 (29.0)	5 (13.9)	0.15
Diarrhoea	13 (14.4)	5 (13.5)	8 (15.1)	1.00	6 (9.0)	4 (12.9)	2 (5.6)	0.40
Confusion	0 (0.0)	0 (0.0)	0 (0.0)	–	10 (14.9)	5 (16.1)	5 (13.9)	1.00
Malaise	53 (58.9)	25 (67.6)	28 (52.8)	0.20	44 (65.7)	21 (67.7)	23 (63.9)	0.80
Irritability	44 (48.9)	16 (43.2)	28 (52.8)	0.40	0 (0.0)	0 (0.0)	0 (0.0)	–
Dyspnoea	9 (10.0)	5 (13.5)	4 (7.5)	0.48	27 (40.3)	15 (48.4)	12 (33.3)	0.23
Other	9 (10.0)	6 (16.2)	3 (5.7)	0.15	30 (44.8)	13 (41.9)	17 (47.2)	0.81
Physical exam								
Wheezing	30 (33.3)	15 (40.5)	15 (28.3)	0.26	16 (23.9)	7 (22.6)	9 (25.0)	1.00
Rales	59 (65.6)	21 (56.8)	38 (71.7)	0.18	18 (26.9)	8 (25.8)	10 (27.8)	1.00
Thoraco-abdominal	8 (8.9)	4 (10.8)	4 (7.5)	0.71	1 (1.5)	1 (3.2)	0 (0.0)	0.46
Hyperaemic pharynx	20 (22.2)	9 (24.3)	11 (20.8)	0.80	20 (29.9)	12 (38.7)	8 (22.2)	0.18
Other	27 (30.0)	13 (35.1)	14 (26.4)	0.48	25 (37.3)	10 (32.3)	15 (41.7)	0.46

HBoV1 = human bocavirus. [a]The p-values are not adjusted for multiple comparisons.

Table 2: **Descriptive symptoms by age group and infection status.**

co-infected children had watery eyes and nausea compared to children with single HBoV1 infection (30.2% and 10.8%, respectively, for both symptoms; p = 0.04). When correcting for multiple comparisons the results are no longer statistically significant. Common symptoms in adults were sore throat (71% HBoV1 single infection, 75% co-infection), fatigue (77.4% HBoV1 single infection, 77.8% co-infection), and headache (74.2% HBoV1 single infection, 72.2% co-infection). When evaluating these comparisons at the four clinical presentations (outpatient, emergency room, hospitalization, and ICU) we found no significant differences between single and co-infection after correcting for multiple comparisons (Supplementary Table S2).

The only significant difference in laboratories was lymphocyte count, which was higher in co-infected children compared to children with single infection (median values 31 and 20 $\times$ 10^9/L, respectively; p = 0.024). When correcting for multiple comparisons this result is no longer statistically significant. Death occurred in one child with HBoV1 co-infection. Five adults died, two of them with HBoV1 co-infection (Supplementary Table S3).

Univariate models did not find any significant clinical feature as predictive of a higher probability for having a single HBoV1 infection versus co-infection. However, in a multivariate model, both the presence of wheezing (OR = 0.214; 95% CI: 0.054, 0.763) and younger age (OR = 0.825; 95% CI: 0.666, 0.977) resulted in higher odds of having a single HBoV1 infection in children. In adults, the presence of rales (OR: 6.713, 95% CI: 1.145, 60.202), respiratory distress symptoms (dyspnoea, nasal flattening, thoracoabdominal dissociation, shortness of breath) (OR = 6.256; 95% CI: 1.258, 41.113), and red and/or watery eyes (OR = 4.260; 95% CI: 1.047, 20.481) were associated with higher odds of

having a co-infection with other viral agents, while an increase in white blood cell (WBC) count levels (within normal ranges) (OR = 0.839; 95% CI: 0.702, 0.969) and the presence of chest congestion and/or cough with sputum (OR = 0.084; 95% CI: 0.012, 0.399) were associated with higher odds of single HBoV1 infection. In all participants (both children and adults), the multivariate model showed that the presence of chest congestion and/or cough with sputum (OR = 0.307; 95% CI: 0.104, 0.810), being an adult (OR = 0.371; 95% CI: 0.151, 0.863), and an increase in WBC counts (OR = 0.920; 95% CI: 0.856, 0.979), were associated with higher odds of having a single HBoV1 infection compared with a co-infection (Supplementary Figure S1).

In univariate analyses, we found significant associations between higher antibiotic use and patients presenting with fever (p = 0.027), shortness of breath (p = 0.00034), nasal flattening (p = 0.049), dyspnoea (p = 0.033), and rales (p < 0.0001), and between lower antibiotic use and patients presenting with red eyes (p = 0.0088), sneezing (p = 0.0031), nasal congestion (p = 0.0021), nausea (p = 0.030), and hyperaemic pharynx (p = 0.0088) (Supplementary Table S4).

Viral load

In children, geometric mean viral loads were 677,972.3 genomes/mL with HBoV1 single infection and 739,309.4 genomes/mL with co-infection. In adults, geometric mean viral loads were 423,389.2 genomes/mL in single infection and 336,840.7 genomes/mL with co-infections. Viral load was categorised as: low ($\leq 10^6$ *load* genomes/mL) and high ($>10^6$ genomes/mL). There were no statistically significant differences in viral load categories between single infections and co-infections. High viral loads occurred in 46.7% (n = 42) of children and 32.8% (n = 22) of adults (Supplementary Table S5).

Results of univariate models associating laboratory and clinical features to log10 viral load are shown in Supplementary Table S6. We did not discover any changes in log10 viral load that were associated with any characteristic in adults. Nasal congestion and log10 creatinine were shown to be significantly associated in children (p = 0.029 and p = 0.019, respectively). The outcomes of multivariate models associating these features to log10 viral load are shown in Table 3. In children, we identified significant association between higher log10 viral loads and increases in age (beta = 0.20 [0.07, 0.33], p = 0.0046), the presence of fever (beta = 2.64 [1.35, 3.94], p = 0.00019), nasal congestion (beta = 1.03 [0.07, 1.99], p = 0.037), dry cough (beta = 1.32 [0.42, 2.22], p = 0.0053), chest congestion (beta = 1.57 [0.33, 2.80], p = 0.014), red eyes (beta = 1.25 [0.35, 2.14], p = 0.0075), cough with sputum (beta 1.79 [0.80, 2.78], p = 0.00079), and symptoms captured as "other" (i.e., chills, dizziness, diaphoresis; beta = 1.73 [0.19, 3.27], p = 0.029). We found significant associations between lower log10 viral loads and the presence of fatigue (beta = −1.6 [−2.46, −0.74], p = 0.000591), muscle aches (beta = −1.13 [−2.25, −0.01], p = 0.049), increases in percent neutrophils (beta = −0.04 [−0.06, −0.02], p < 0.00014), and the presence of other physical signs and symptoms (i.e., adenopathy, hypoventilation, rash, hyperemic nasal mucosa, postnasal drip; beta = −1.26 [−2.31, −0.21], p = 0.020). In adults, we found significant associations between higher log10 viral loads and increases in BMI (beta = 0.13 [0.04, 0.21], p = 0.0035), and the presence of confusion (beta = 1.54 [0.55, 2.53], p = 0.0031) and sore throat (beta = 1.03 [0.20, 1.85], p = 0.016). We identified a significant association between lower log10 viral loads and chest congestion (beta = −1.16 [−2.07, −0.24], p = 0.014).

Bocavirus case frequency and seasonality

Years 2011 and 2013 had significantly higher bocavirus cases compared to 2010 (p < 0.0001, p = 0.0002, respectively). Cases were isolated only from Mexico City with no bocavirus cases in San Luis Potosí. In 2010, fewer HBoV1 cases were observed in children than adults. In 2014 more cases were observed in children than adults (Fig. 2). The Poisson model estimated the number of bocavirus cases in 2011 as 3.294 (95% CI = [1.914, 5.667]) times higher than in 2010. Similarly, HBoV1 cases in 2013 were estimated as 2.883 (95% CI = [1.661, 5.007]) times higher than in 2010. Seasonality did not significantly affect the bocavirus case frequency. Neither median relative humidity nor median temperature significantly predicted bocavirus case frequency.

Phylogenetic analysis

We performed phylogenetic analyses with NP1 (Fig. 3A) and VP1 (Fig. 3B). Partial sequences submitted to the gene bank database are registered as MH545717.1 to MH545765.1 for NP1 and MK910868 to MK910905 for VP1.

Phylogenetic analyses for VP1 were distributed between three groups (Fig. 3B). Mexican strains had close identity with strains previously identified in Argentina, Brazil, Egypt, China, Saudi Arabia, Japan, and Taiwan. Identity analysis performed on NP1 showed identity between 99.3% and 99.6% and for VP1 HBoV1 proteins showed identity between 99.3% and 99.6% (Supplementary Tables S7 and S8). The phylogenetic distribution (Fig. 3) confirmed close relationship, despite VP1 being distributed in three branches compared to NP1 in two branches. Polymorphisms found on VP1 were neutral changes without effect on protein structure (Supplementary Table S9).

Discussion

This is a sub-study of ILI002 which was a prospective, multicentre, observational cohort study. All screened samples collected in ILI002 were re-tested in the final

	Estimate [95% CI]	p-value
Children		
Signs and Symptoms		
Age	0.20 [0.07, 0.33]	0.0046
Fever (≥38 °C)	2.64 [1.35, 3.94]	0.00019
Nasal congestion	1.03 [0.07, 1.99]	0.037
Fatigue	−1.60 [−2.46, −0.74]	0.00058
Dry cough	1.32 [0.42, 2.22]	0.0053
Chest congestion	1.57 [0.33, 2.80]	0.014
Hyperaemic pharynx	0.80 [−0.16, 1.77]	0.100
Red eyes	1.25 [0.35, 2.14]	0.0075
Cough with sputum	1.79 [0.80, 2.78]	0.00079
Other symptoms	1.73 [0.19, 3.27]	0.029
Other findings in physical examination	−1.26 [−2.31, −0.21]	0.020
Muscle aches	−1.13 [−2.25, −0.01]	0.049
Rales	1.07 [−0.04, 2.17]	0.057
Nausea	−0.87 [−2.00, 0.27]	0.13
Sneezing	0.46 [−0.34, 1.27]	0.25
Irritability	0.72 [−0.20, 1.64]	0.12
Lab values		
Log10 Creatinine, mg/dl	−1.78 [−3.98, 0.42]	0.11
Log10 LDH, U/L	1.21 [−0.90, 3.33]	0.25
Neutrophils, %	−0.04 [−0.06, −0.02]	0.00014
Adults		
Signs and symptoms		
BMI	0.13 [0.04, 0.21]	0.0035
Confusion	1.54 [0.55, 2.53]	0.0031
Chest congestion	−1.16 [−2.07, −0.24]	0.014
Sore throat	1.03 [0.20, 1.85]	0.016
Diarrhoea	1.28 [−0.17, 2.73]	0.081
Watery eyes	0.61 [−0.14, 1.36]	0.11
Dry cough	−0.68 [−1.44, 0.07]	0.073
Lab values		
Lymphocytes, %	−0.03 [−0.06, 0.01]	0.13
Platelet, 10^6/L (cell/mm^3)	−0.005 [−0.012, 0.001]	0.053

95% CI = 95% confidence interval; LDH = lactate dehydrogenase; BMI = body mass index.

Table 3: **Estimates and 95% confidence intervals of the multivariate models relating clinical features and viral load in children and adults.**

two study years to detect bocavirus. HBoV1 was discovered relatively recently, and clinicians may not suspect HBoV1 as causal in respiratory infections. The impact of positive tests on patients' clinical course and outcomes remains unclear. This study allowed recognition and description of more participants with HBoV1 single and co-infection.

This study identified HBoV1 in 2.8% of the ILI002 participants. Of the 157 with a positive HBoV1 test, 57.3% were children, and 42.7% were adults.

The global prevalence of HBoV1 respiratory infections ranges from 1 to 33.3%.[3,9] Variations may be due to diverse study age groups. Madi et al., analysed samples from participants >1 month old (children and adults), and found HBoV1 in 1.9% of participants with respiratory tract infections, with peak incidence in children < one year old.[27] Ljubin-Sternack et al., found HBoV1 in 7.6% of Croatian children with acute respiratory infections, with 17.8% as single infections, and 82.2% as co-infection with other viruses.[5] In the Middle East and North African region, a systematic review conducted by Abdelqader et al., described a pooled frequency of 3.9% of HBoV1 infection among adults and children.[28]

In Mexico, few studies address HBoV. To our knowledge, all of them evaluate paediatric populations.[11–13] Like other studies, we showed HBoV1 infections are more prevalent in children < five years old.[3,5] Wong-Chew et al., reported lower prevalence (0.4%) in Mexican children < five years old with co-infection rates of 22.1%.[29] Our study not only showed the HBoV1 prevalence in children, but also a 1.8%

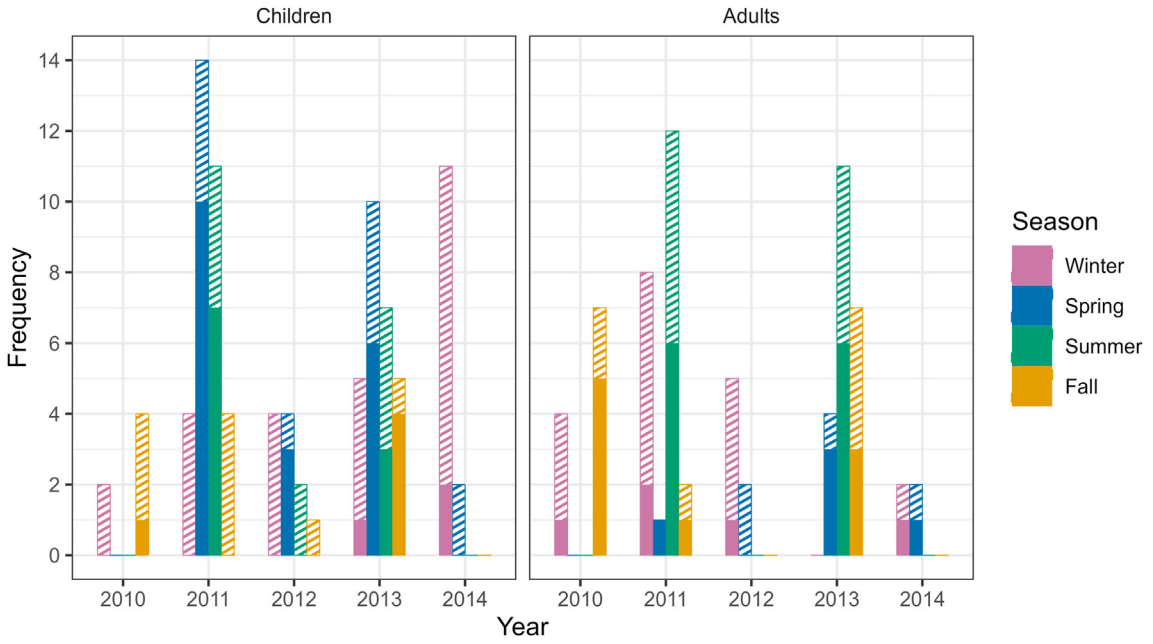

***Fig. 2:* HBoV1 single infection and co-infection in children and adults by season and year.** Legend: Solid bars represent HBoV1 single infection and stripped bars represent co-infection.

prevalence in adults >18 years old in this Mexican population.

The most common symptoms we identified are those related to influenza-like illnesses. The average symptomatic presentations differed between children and adults. The most common symptoms in children were fever and cough. Clinical descriptions of adults in existing literature are less frequent. Common symptoms in adults in this study were sore throat, fatigue, headache, fever, and cough.

HBoV1 has been associated with severe symptoms. A recent study also demonstrated that HBoV1 single infection can cause severe symptoms among children and adults.[29] This study showed dyspnoea in 67.5% of participants (72% of children, 61.2% of adults). Wheezing occurred in 66.7% and rales in 45% of patients who needed hospitalization. Allander et al., investigated HBoV prevalence in children with acute wheezing, finding it in 19% of participants with high viral loads, mainly with single infection.[30] In agreement with Allander et al., we also found lower respiratory tract symptoms, like wheezing as a symptom in children associated with single infection. In adults and overall, congestion and/or cough with sputum were associated with single infection.

Severe HBoV1 presentations could lead to death. In this cohort, death occurred in one child with co-infection, and five adults (two with co-infection). The causal relationship between infection and death is unknown. Most deaths occurred in participants with chronic comorbidities; however, one adult (48 years old) without known comorbidities also died.

Bocavirus circulates throughout all seasons. Some studies have reported greater frequency during winter and spring.[3,5] We found HBoV1-associated respiratory disease in children and adults, irrespective of season, temperature, and humidity. Chen et al., found HBoV infections occur throughout the year, but found a positive correlation with temperature and humidity.[31]

We found an association between fever and increased viral loads in children. This may reflect more intense inflammatory immune responses to viral infection.[32] In children, we found significant associations between higher viral loads and fever, dry cough, cough with sputum, chest congestion, and hyperaemic pharynx. In adults, we found significant relationships between higher viral load and body mass index, confusion, and sore throat. Zhao et al., found associations between viral load and lower respiratory tract infection in children < five years old.[33] In adults, we found a statistically significant association between chest congestion and lower viral loads. The discordant results between adults and children in terms of viral load and chest congestion could be explained by differences in anatomical sites. The amount of virus in the nasopharynx might not reflect the amount of virus in the lung. Lastly, because symptoms are due to host response, viral measures may not accurately reflect symptomatology. It was described that identification of HBoV1 in nasopharyngeal secretions may not be

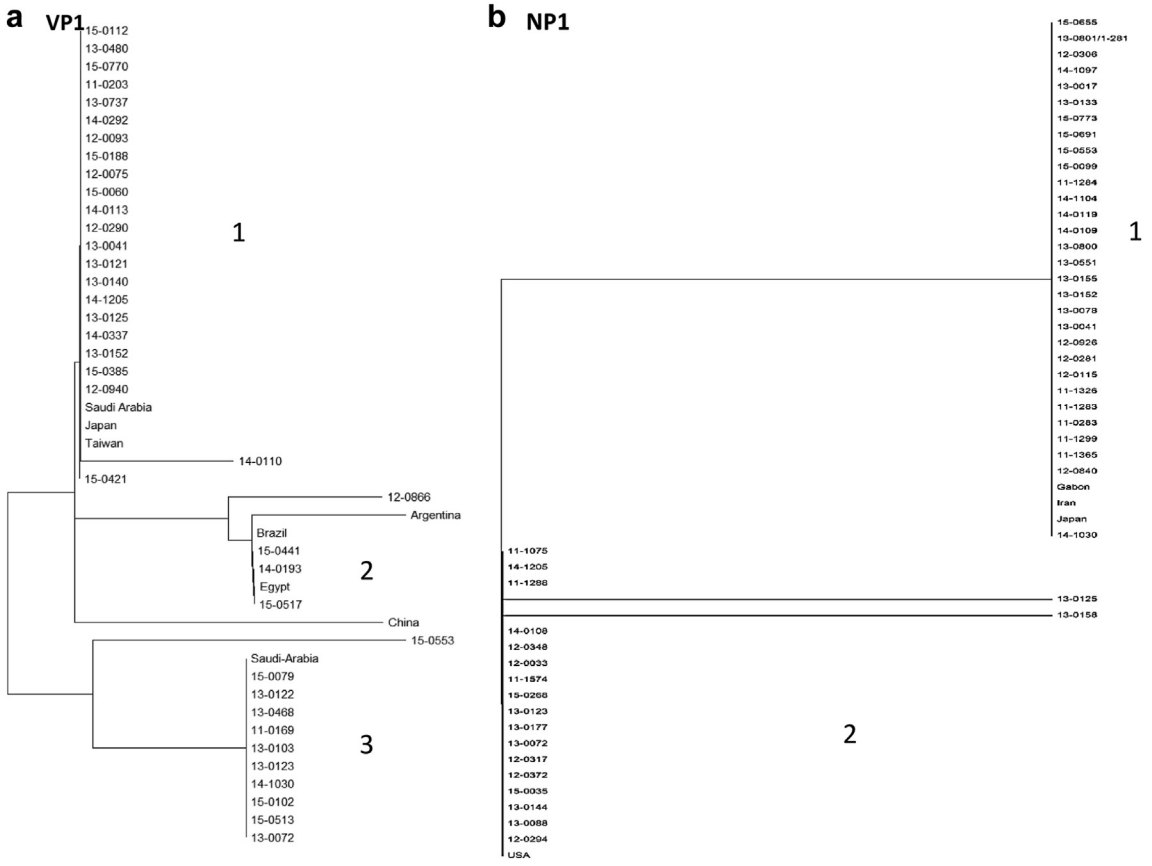

Fig. 3: **Phylogenetic distribution based on NP1, VP, and the non-coding region.** Legend: a) non-coding region VP/NC; b) N terminal NP1. The phylogenetic distribution confirmed close relationship, although VP1 was distributed in three branches and NPI in two branches.

associated with symptomatic infection,[34] and higher viral loads may be more commonly associated with symptoms.[20] However, in this study, despite having low vial loads ($\leq 10^6$ genomes/mL), 22.2% of children and 26.9% of adults with single infection were seeking care for symptoms compatible with influenza-like illnesses. Additionally, there were no statistically significant differences in viral load categories between single infections and co-infections.

Consistent with previous reports,[27] we found high rates of co-infection with other pathogens in HBoV1-positive participants. A study in Seoul reported co-infection rates of 54.5%.[15] The high rates of co-infection for this specific virus may be due to its long persistence, having been reported to last up to six months in the respiratory tract mucosa.[29] This long-lasting persistence and viral shedding increase the possibility of co-infection with more agents. HBoV1 could be associated with the disease by itself, as shown by cases of single infection, or with worse disease with co-infection. In Mexico, HBoV1 has been reported with HAdV co-infection in children with pneumonia.[12] However, our analyses do not show differences in clinical presentation between single and co-infections. Our results support the hypothesis that HBoV1, although not among the most common respiratory pathogens, is still prevalent as a respiratory viral agent that can be associated with severe disease and hospitalization.[13,35]

A Kuwait study illustrated predominance of HBoV genotypes in patients with respiratory diseases with minimal genetic variability.[27] Our analysis of identity performed on NP1 and VP1 proteins of HBoV showed an identity above 97%. Observed changes did not affect final protein structures and all were point mutations. The variability found in analysed regions did not affect clinical course or viral fitness. Further studies are necessary using the complete sequence of the virus to define the role of these and other mutations on the positive selection of the viral diversity and other evolutionary processes.

A limitation of this study is that, given the observational design, microbial diagnostic evaluation decisions were made by attending physicians. The high rate of fever may be due in part to the inclusion of fever in the diagnostic criteria for influenza-like illnesses. The

detection of HBoV1 and co-infecting agents was done by Real Time PCR, but no isolation of the pathogens through culture was performed. This limits the possibility of inferring etiological causality of the viral agents. Also, although viral load was calculated using an HBoV Real Time PCR Kit, using their respective controls, some variation could be found due to previously reported variation in quantitation of housekeeping genes between serial samples. The seasonality analysis was limited by data availability, and there could be other environmental factors not captured in this study related to the frequency of bocavirus infection. It should also be recognised that as a cross-sectional study nested in a cohort, the representativeness of our sample may be limited.

Although we had comparable recruitment of male and female patients (see Table 1), we did not specifically investigate sex-associated differences in our study. Sex differences on influenza-like illnesses have not been thoroughly established, and often contingent upon the pathogen causing influenza-like illness. Further studies may be necessary to delve into this aspect.

In summary, our study is the first to describe the clinical and laboratory characterization of HBoV1 infections in Mexican adults as well as children. Our data were collected in a prospective manner, and we have a complete characterization of clinical and molecular features of our cohort. Further studies in other specific regions can help explore the generalizability of our results. Our study shows the importance of detecting HBoV1 as a cause of respiratory infections among the many other possible etiological agents. The most common medications in participants were antipyretics and antibiotics, highlighting antibiotic overuse in viral diseases and the importance of viral diagnosis in influenza-like illness, to rule out bacterial infection and avoiding avoid unnecessary prescribing. Although antibiotic use was observed more frequently in those with more severe symptoms (respiratory distress), timely diagnostic is highly important, as early use of antibiotics as initial treatment of respiratory diseases, even in those caused by viral agents, is still very common worldwide.

Our study demonstrated the importance of detecting HBoV1 in patients with influenza-like illness due to its association with a wide variety of clinical scenarios ranging from acute respiratory infection both as a single infection and as a co-infection with other viruses, to a more severe respiratory disease. This study allowed us to better characterise the seasonality, laboratory, clinical, and molecular features of bocavirus infection.

Contributors

Conceptualization, AEGA, JAG, GMRP, JB, AGF, and SME; methodology, JAG, CMR, JXC, SAO, ACC, and PRC; formal analysis, PCGB, AMOV, AM, and AEGA; investigation, AEGA, JAG, BLG, AAOH, RVV, ARV, AGF, MLG, LMG, and MGM; resources, GMRP, JB, SME, and JHP; data curation, AEGA, JAG, AMA, and AM; writing—original draft preparation, AEGA, PCGB, AOV, AM, and LMG; writing—review and editing, JHP. GMRP, SME, AC, and MLG; supervision, GMRP, JB, and SM; project administration, AGF; SME, JAG, JB, and GMRP.; funding acquisition, GMRP, JB, SM, and AGF. All authors have read and agreed to the published version of the manuscript.

Declaration of interests

Dr. John Powers discloses consulting fees received for clinical trial design from the following companies: Adaptive Phage, Arrevus, Atheln, Bavaria, Nordic, Cellularity, Eicos, Evofem, Eyecheck, Gilead, GSK, Mustang, OPKO, Otsuka, Resolve, Romark, SpineBioPPharma, UTIlity, and Vir. The remaining authors report no conflicts of interest.

Acknowledgements

The authors thank Ana Vilardell-Dávila, for her assistance in editing this manuscript, and Drs. Caeul Lim and Renee Ridzon for their contributions. We would also like to acknowledge the following individuals, who played vital roles in this research: Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán: Bricia Roa-Martinez, Itzel Cruz-Gaona, Diana Aguilar-Cruz; Instituto Nacional de Enfermedades Respiratorias: Paulina Paulin-Prado, Nora Bautista; Hospital General Dr. Gea González: Patricia Rodríguez, Javier Reyes-Mar, José Alfonso Maya; Instituto Nacional de Pediatría: Diana Andrade-Platas. Central Laboratory at the Department of Infectious Diseases at INCMNSZ: Pilar Ramos-Cervantes, Violeta Ibarra-González, Julia Martínez-López, Luis A. García-Andrade, Fernando Ledesma-Barrientos; National Institute of Allergy and Infectious Diseases (NIAID): Mary Smolskis, John Beigel; Clifford Lane. LaRed Director: Justino Regalado Pineda; LaRed Coordinating Center: Abelardo Montenegro Liendo.

Role of Funding Source: This work was supported by The Mexican Emerging Infectious Diseases Clinical Research Network (LaRed). LaRed is funded by the Mexico Ministry of Health and the U.S. National Institute of Allergy and Infectious Diseases. This project has been funded in part by: CONACYT (Fondo Sectorial SSA/IMSS/ISSSTE, Projects No. 71260 and No. 127088), Fondos federales no. HIM/2015/006, and the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) through a contract with Westat, Inc., Contract No. HHSN2722009000031, Task Order Number: HHSN27200002. This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. 75N91019D00024, Task Order No. 75N91019F00130. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. None of the funding sources had any involvement in the study design, data collection, analysis, interpretation of the data, writing of the report, or the decision to submit the paper for publication.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lana.2023.100647.

References

1 Le YH, Nguyen KC, Coleman KK, et al. Virus detections among patients with severe acute respiratory illness, Northern Vietnam. *PLoS One*. 2020;15(5):e0233117.
2 Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc Natl Acad Sci U S A*. 2005;102(36):12891–12896.
3 Guido M, Tumolo MR, Verri T, et al. Human bocavirus: current knowledge and future challenges. *World J Gastroenterol*. 2016;22(39):8684–8697.
4 Qiu J, Söderlund-Venermo M, Young NS. Human parvoviruses. *Clin Microbiol Rev*. 2017;30(1):43–113.

5 Ljubin-Sternak S, Slović A, Mijać M, et al. Prevalence and molecular characterization of human bocavirus detected in Croatian children with respiratory infection. *Viruses*. 2021;13(9):1728.
6 Oldhoff E, Bennet R, Eriksson M, et al. Human bocavirus 1 epidemiology in children in relation to virus load and codetection. *Acta Paediatr*. 2023;112:1049–1055.
7 Jalving HT, Heimdal I, Valand J, et al. The burden of human bocavirus 1 in hospitalized children with respiratory tract infections. *J Pediatric Infect Dis Soc*. 2023;12(5):282–289.
8 Wang W, Guan R, Liu Z, et al. Epidemiologic and clinical characteristics of human bocavirus infection in children hospitalized for acute respiratory tract infection in Qingdao, China. *Front Microbiol*. 2022;13:935688.
9 Christensen A, Kesti O, Elenius V, et al. Human bocaviruses and paediatric infections. *Lancet Child Adolesc Health*. 2019;3(6):418–426.
10 Joseph OO, Adeniji JA, Faneye AO. Human bocavirus infection among children with respiratory tract infection in Ibadan, Nigeria. *Access Microbiol*. 2022;4:356.
11 Bedolla-Barajas M, Montero H, Morales-Romero J, et al. Prevalence of respiratory viruses in wheezing children not older than 24 months of age. *Gac Med Mex*. 2017;153(3):329–334.
12 Wong-Chew RM, García-León ML, Noyola DE, et al. Respiratory viruses detected in Mexican children younger than 5 years old with community-acquired pneumonia: a national multicenter study. *Int J Infect Dis*. 2017;62:32–38.
13 Uribe-Gutiérrez G, Hernández-Santos H, Manjarrez-Zavala ME, et al. Prevalence and genotypes of the adenovirus infection as well detection of co-infection with bocavirus in Mexican immunosuppressed and non-immunosuppressed children with pneumonia. *Clin Lab*. 2014;60(8):1277–1285.
14 Liu WK, Chen DH, Liu Q, et al. Detection of human bocavirus from children and adults with acute respiratory tract illness in Guangzhou, southern China. *BMC Infect Dis*. 2011;11:345.
15 Lu QB, Wo Y, Wang HY, et al. Epidemic and molecular evolution of human bocavirus in hospitalized children with acute respiratory tract infection. *Eur J Clin Microbiol Infect Dis*. 2015;34(1):75–81.
16 Tabatabai J, Fakhiri J, Meyburg J, et al. Severe human bocavirus 1 respiratory tract infection in an immunodeficient child with fatal outcome. *Pediatr Infect Dis J*. 2019;38(9):e219–e222.
17 Choi SH, Huh JW, Hong SB, et al. Severe human bocavirus-associated pneumonia in adults at a referral hospital, Seoul, South Korea. *Emerg Infect Dis*. 2021;27(1):226–228.
18 Zhou L, Zheng S, Xiao Q, et al. Single detection of human bocavirus 1 with a high viral load in severe respiratory tract infections in previously healthy children. *BMC Infect Dis*. 2014;14:424.
19 Ding XF, Zhang B, Zhong LL, Xie LY, Xiao NG. [Relationship between viral load of human bocavirus and clinical characteristics in children with acute lower respiratory tract infection]. *Zhong Guo Dang Dai Er Ke Za Zhi*. 2017;19(3):327–330.
20 Galindo-Fraga A, Ortiz-Hernández AA, Ramírez-Venegas A, et al. Clinical characteristics and outcomes of influenza and other influenza-like illnesses in Mexico City. *Int J Infect Dis*. 2013;17(7):e510–e517.
21 Principi N, Piralla A, Zampiero A, et al. Bocavirus infection in otherwise healthy children with respiratory disease. *PLoS One*. 2015;10(8):e0135640.
22 Abdel-Moneim AS, Kamel MM, Hamed DH, et al. A novel primer set for improved direct gene sequencing of human bocavirus genotype-1 from clinical samples. *J Virol Methods*. 2016;228:108–113.
23 Abdel-Moneim AS, Mahfouz ME, Zytouni DM. Detection of human bocavirus in Saudi healthy blood donors. *PLoS One*. 2018;13(2):e0193594.
24 Firth D. Bias reduction of maximum likelihood estimates. *Biometrika*. 1993;8:27–38.
25 Heinze G, Schemper M. A solution to the problem of separation in logistic regression. *Stat Med*. 2002;21:2409–2419.
26 Heinze G, Ploner M. *Technical report 2/2004: a SAS-macro, S-PLUS library and R package to perform logistic regression without convergence problems*. Viena, Austria: Section of Clinical Biometrics, Department of Medcal Computer Sciences, Medical University of Viena; 2004.
27 Madi NM, Al-Adwani A. Human bocavirus (HBoV) in Kuwait: molecular epidemiology and clinical outcome of the virus among patients with respiratory diseases. *J Med Microbiol*. 2020;69(7):1005–1012.
28 Abdelqader R, Hasan H, Alanagreh L. Epidemiology of human bocavirus in the Middle East and North Africa: systematic review. *Pathogens*. 2021;10(11):1456.
29 Bagasi AA, Howson-Wells HC, Clark G, et al. Human bocavirus infection and respiratory tract disease identified in a UK patient cohort. *J Clin Virol*. 2020;129:104453.
30 Allander T, Jartti T, Gupta S, et al. Human bocavirus and acute wheezing in children. *Clin Infect Dis*. 2007;44(7):904–910.
31 Chen ZR, Mize M, Wang YQ, et al. Clinical and epidemiological profiles of lower respiratory tract infection in hospitalized children due to human bocavirus in a subtropical area of China. *J Med Virol*. 2014;86(12):2154–2162.
32 Evans SS, Repasky EA, Fisher DT. Fever and the thermal regulation of immunity: the immune system feels the heat. *Nat Rev Immunol*. 2015;15(6):335–349.
33 Zhao B, Yu X, Wang C, et al. High human bocavirus viral load is associated with disease severity in children under five years of age. *PLoS One*. 2013;8(4):e62318.
34 Schildgen O, Müller A, Allander T, et al. Human bocavirus: passenger or pathogen in acute respiratory tract infections? *Clin Microbiol Rev*. 2008;21(2):291–304.
35 Lee HN, Koo HJ, Kim SH, Choi SH, Sung H, Do KH. Human bocavirus infection in adults: clinical features and radiological findings. *Korean J Radiol*. 2019;20(7):1226–1235.