

Acute Human Bocavirus 1 Infection in Children Hospitalized for Acute Bronchiolitis

A 2-Year Prospective Study

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Background: The objective of this prospective study was to assess the proportion and clinical consequences of human bocavirus 1 (HBoV1) replication in children hospitalized for acute bronchiolitis (AB) with HBoV1 DNA in the nasopharynx (NP).

Methods: For this purpose, we detected HBoV1 DNA and mRNA (evidence of viral replication and viable virus) in NP in cases and healthy control children. This research allowed us to distinguish active HBoV1 infections from inactive ones.

Results: HBoV1 DNA was detected in the NP of 37 of 307 patients with AB (12.1%) and 9 of 150 children in a healthy control group (6%) with a high codetection rate with other respiratory viruses in AB patients, 28 of 37 (75.7%). Only 9 of 37 HBoV1 DNA-positive NP swabs (24.3%) with high DNA load were also HBoV1 mRNA positive, moreover, HBoV1 DNA was also detected in the plasma of these patients.

Conclusions: Based on the results of our study, we can conclude that children with AB acute HBoV1 infection has a high HBoV1 DNA load in NP samples together with detected HBoV1 mRNA and detected HBoV1 DNA in plasma.

Key Words: HBoV1, HBoV1 mRNA, acute bronchiolitis, virus replication, respiratory samples

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Human bocavirus 1 (HBoV1) was first described in 2005. This small nonenveloped icosahedral virus with a DNA genome of 5.2 kb belongs to the *Parvoviridae* family. HBoV1 is recognized as an important cause of bronchiolitis in small children. In addition, it also causes upper respiratory tract infections and sometimes severe or even fatal lower respiratory tract infections in children and immunocompromised adults.^{1–4} Therefore, HBoV1 DNA is often detected in nasopharyngeal (NP) swabs of children with upper and lower respiratory tract infections.⁵ However, because HBoV1 is frequently codetected with other respiratory pathogens and its DNA may persist for a month or even longer after acute illness, thus it is often difficult to determine whether it is a real cause of the disease or merely an innocent bystander.^{6–8} The presence of HBoV1 mRNA rather than only viral DNA might help distinguish these 2 conditions.⁹ It is not surprising that it is one of the most frequently detected respiratory viruses in small children with bronchiolitis, because of the high IgG seroprevalence (70%) in schoolchildren.^{5,10–13} Several rather convincing case reports as well as retrospective and prospective studies have suggested the etiologic role of this virus in respiratory infections.^{1–3,6,9,14,15}

This study aimed to determine the frequency of HBoV1 infection in children with acute bronchiolitis (AB) and a healthy control group and to assess its etiological role according to the presence of HBoV1 mRNA to distinguish between acute and inactive infection.

MATERIALS AND METHODS

Study Population and Sample Preparation

This study is part of a large 2-year prospective study on viral respiratory infections in children under 6 years of age in which the clinical part was performed from October 2009 to September 2011. The study protocol was approved by the National Medical Ethics Committee of the Republic of Slovenia (No. 87/08/09) and was registered at the ClinicalTrials.gov registry (reg. NCT00987519). Written informed consent was obtained from the parents of all participants. The principles of the Helsinki Declaration, the Oviedo Convention on Human Rights and Biomedicine and the Slovenian Code of Medical Deontology were strictly followed. In this study, only a subset of children hospitalized for respiratory infection (ie, those with AB and the corresponding healthy control group) were enrolled. AB was defined as the presence of nasal discharge, cough, wheezing and/or crackles on lung auscultation. The control group consisted of children

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without respiratory or gastrointestinal infections who were admitted for elective surgery. NP swabs were collected during anesthesia to minimize discomfort, and their parents were asked to provide consent for participation in the study. These children were included during the same period as the study subjects. Children showing signs or symptoms of infection during the presurgery examination were excluded from the study. Additional specific information regarding the presence of signs and symptoms compatible with gastrointestinal and/or respiratory infections within the past 14 days was also collected.¹⁶

An NP swab and ethylenediaminetetraacetic acid blood sample were obtained on admission from each child with AB. Only NP swabs were obtained from the control group of children.

The study population, procedures for sample preparation, nucleic acid extraction and detection of HBoV1 DNA together with detection of human respiratory syncytial virus, human rhinovirus, human metapneumovirus, human coronaviruses, parainfluenza virus types 1–3 and influenza virus (Flu) types A and B have been described in previous publications;¹⁶ mRNA of HBoV1 was detected using the protocol published by Christensen et al.⁹ HBoV1 DNA was tested in plasma only in children with HBoV1 DNA–positive NP swabs. Cycle threshold value (Ct value) was only used as a rough comparison of viral load within one method.

Statistical Analysis

Numerical data were summarized with means (standard deviations) or medians [interquartile ranges (IQR)], and categorical data with frequencies (percentages). Binomial distribution was used to derive 95% confidence intervals (CI) for proportions. Groups were compared using the χ^2 test for categorical variables and the *t* test for numerical variables; comparisons involved (1) HBoV1 DNA–positive versus HBoV1 DNA–negative patients, (2) HBoV1 mRNA–positive versus HBoV1 mRNA–negative patients among HBoV1 DNA–positive children and (3) HBoV1 mRNA–positive children versus HBoV1 mRNA and DNA–negative children.

The co-occurrence of viruses was presented graphically for the subset of patients with positive HBoV1 DNA.

R programming language (version 4.0.2) was used for all the statistical analyses.¹⁷

RESULTS

Patients and Controls

Of 814 children under 6 years of age diagnosed with AB at the Department of Infectious Diseases of the Ljubljana University Medical Center in a 2-year period, 307 (37.7%) were included in the study. The others were not enrolled because their parents did not consent to their inclusion in the study (the large majority) or because an NP swab was not performed. The healthy control group comprised 150 children admitted for elective surgery.

Children with AB were younger compared with the healthy control group (AB: mean age 12.6 months, IQR: 4.9–19.8; healthy controls: mean age 30.4 months, IQR: 14.8–45.8; $P < 0.001$). The female:male ratio was 1:1.6 (118/307; 38.4% females) among children with AB and 1:6.1 (21/150; 14% females) among controls ($P < 0.001$). The overall characteristics of the children included in the study have been reported previously.¹⁶

Samples

In the study, 307 NP swabs and 283 ethylenediaminetetraacetic acid blood samples from children with AB were obtained, and 150 NP swabs from the healthy control group were acquired.

HBoV1 DNA Detection

HBoV1 DNA was detected in 37 of 307 NP swabs (12.1%, 95% CI: 8%–16%) of patients with AB and 9 of 150 (6%, 95% CI: 3%–11%) children in the control group ($P = 0.064$). In 20 of 37 (54%, 95% CI: 37%–70%) NP swab–positive patients, HBoV1 DNA was also detected in plasma (Fig. 1). AB patients had lower HBoV1 DNA mean Ct values than controls, but the difference was not statistically significant (27.9 vs. 30.6, $P = 0.34$).

Compared with HBoV1 DNA–negative patients, HBoV1 DNA–positive patients were older (17.6 vs. 11.9 months) and there were more males (75.5% vs. 40.4% HBoV1), whereas clinical and laboratory findings in the 2 groups were similar (Table 1).

HBoV1 mRNA Detection

Of 37 HBoV1 DNA–positive NP swabs in children with AB, 9 (24.3%) were also HBoV1 mRNA positive, whereas HBoV1 mRNA was not detected in any of 9 HBoV1 DNA–positive NP samples from the control group ($P = 0.26$). None of the 37 children with HBoV1 DNA–positive results in NP swab had HBoV1 mRNA detected in plasma (Fig. 1).

In children with AB, the mean Ct value of DNA was lower in those with positive HBoV1 mRNA (15.8, range 12.6–17.9) than in those with negative HBoV1 mRNA in NP swabs (30.4, range 18.0–37.2); $P \leq 0.001$. Furthermore, in all 9 children with AB and HBoV1 mRNA detected in NP swabs, HBoV1 DNA was demonstrated in plasma, whereas the corresponding proportion in HBoV1 mRNA–negative NP swabs was 11 of 28, 39.3%; $P = 0.005$, 9/9 vs. 11/28. In addition, the mean Ct value of HBoV1 DNA in plasma was lower in children with positive mRNA in NP swabs (31.6) than in those with negative mRNA swabs (33.3), but the difference was small and not statistically significant.

Comparison of demographic, clinical and laboratory characteristics of the group with HBoV1 mRNA–positive and HBoV1 mRNA–negative NP swab results revealed no significant differences, but the groups were rather small for a reliable statistical assessment (Table 2).

Co-presence of HBoV1 and Other Respiratory Viruses

The viruses observed most frequently with HBoV1 were human respiratory syncytial virus and human rhinovirus (Fig. 2).

In NP swabs of AB patients, only HBoV1 DNA without HBoV mRNA was more often detected together with other viruses compared with the control group (26/28, 92.8% vs. 2/9, 22.2%, $P = 0.009$, 95% CI for the difference: 34%–100%; Fig. 1).

Co-detections with other viruses were less common when both HBoV1 DNA and mRNA were detected compared with the samples with only HBoV1 DNA detected (2/9, 22.2% vs. 26/28, 92.8%, $P < 0.001$). Additionally, detections of other non-HBoV1 viruses were more common in children negative for HBoV1 DNA compared with those positive for HBoV1 DNA, although this difference was not statistically significant (226/270, 83.7% vs. 28/37, 75.7%, $P = 0.33$, 95% CI for difference: –8% to 24%).

DISCUSSION

Respiratory infections in children are very common and are most often caused by respiratory viruses. Virus diagnostics, which are performed only in a minority of patients with respiratory infection, are usually based on the detection of virus nucleic acid in NP swabs. When interpreting the presence of virus nucleic acid in NP smears, it is important to be aware of some limitations. The presence of a virus in the upper respiratory tract does not guarantee

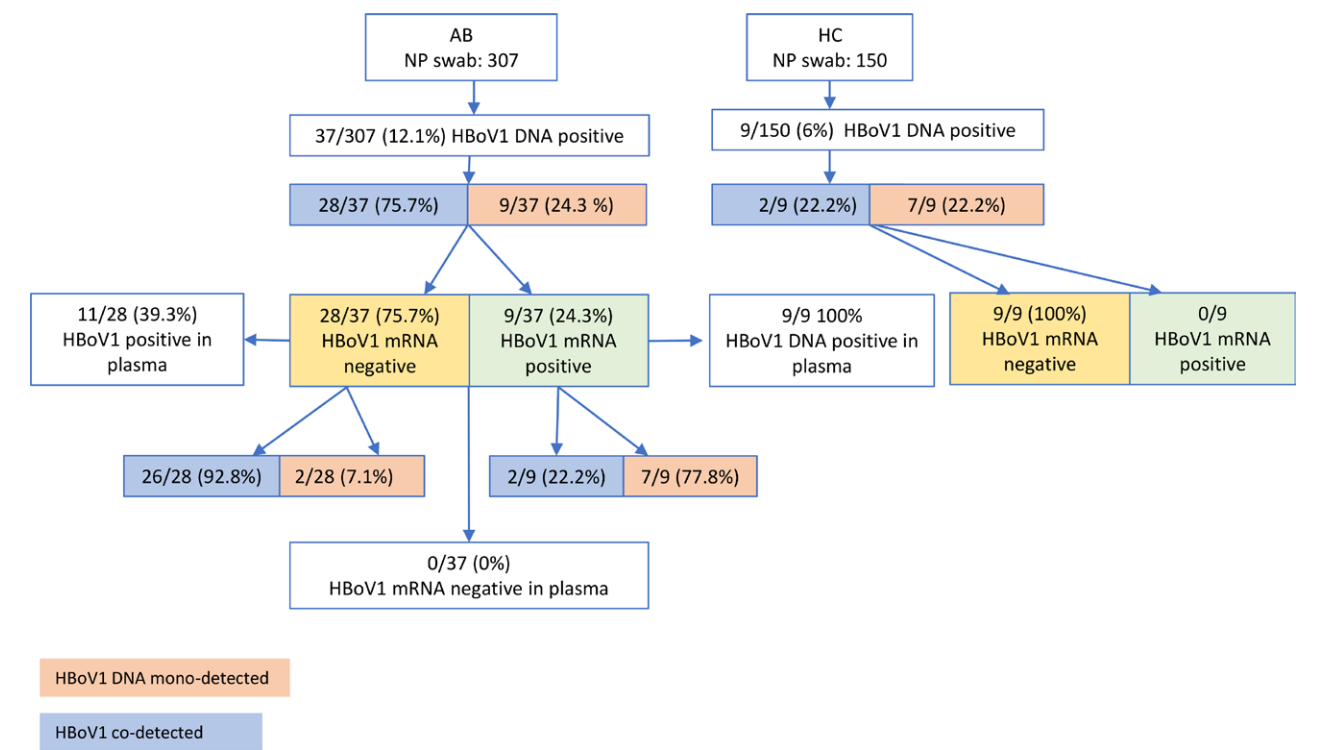


FIGURE 1. Flowchart of HBoV1 DNA and mRNA testing. AB indicates acute bronchiolitis; HBoV1, human bocavirus 1; HC, healthy controls; NP, nasopharyngeal.

TABLE 1. Clinical, Laboratory and Demographic Characteristics of Children With Acute Bronchiolitis: Comparison of Nasopharyngeal Swabs for the HBoV1 DNA–Positive and HBoV1 DNA–Negative Groups

Characteristics	HBoV1 Positive (n = 37)	HBoV1 Negative (n = 270)	P Value
Demographic data			
Mean age, (mo) (SD)	17.6 (6.1)	12 (8.8)	<0.001
Males, number (%)	28 (75.5)	161 (59.6)	0.009
Clinical data			
Hospitalization in days, mean (SD)	3.3 (2.4)	3.3 (2.3)	0.945
Disease duration in days, mean (SD)	9.5 (5.9)	9.7 (5.5)	0.833
Oxygen therapy, number (%)	17 (45.9)	128 (47.4)	1.000
Oxygen therapy in days, mean (SD)	1.7 (2.5)	1.5 (2.1)	0.668
Bronchodilator, number (%)	21 (56.8)	107 (42.1)	0.134
Breathing, inhales/min, mean (SD)	47.3 (11.2)	48.7 (11)	0.460
Birth weight (g), mean (SD)	3374.6 (678.9)	3190.3 (733.9)	0.149
Fever >38 °C, number (%)	21 (56.7)	132 (48.9)	0.483
Otitis, number (%)	10 (27)	65 (24.1)	0.851
Sore throat, number (%)	28 (75.7)	200 (74.1)	1.000
Laboratory findings			
Mean O ₂ saturation (%)	93.7	93.4	0.793
CRP (mg/L), mean (SD)	20.8 (25.3)	22.5 (26.5)	0.711
BSS (points), mean (SD)	5.8 (2.2)	5.8 (2.5)	0.869
WANG (points), mean (SD)	3.6 (1.6)	3.6 (1.9)	0.945

BSS indicates bronchiolitis severity score (includes cough, sputum production, rales/rhonchi, chest pain when coughing and dyspnea); CRP, C-reactive protein; HBoV1, human bocavirus 1; SD, standard deviation; WANG, Wang respiratory score (includes general appearance, respiratory rate, presence of wheezing and retractions).

that the same virus is also present in the lower respiratory tract of a patient who has a lower respiratory tract infection. It also does not make it possible to distinguish between viral infection and the simple presence of a virus. Furthermore, in DNA viruses, such as HBoV1, it does not indicate whether a live virus or only viral DNA is present. On the other hand, the absence of a virus nucleic acid as well as viral mRNA may be due to poorly collected NP smears and the absence of human cells.

The biggest advantage of this research is inclusion of control group of healthy children, which allows us to understand the etiology of viruses and their importance in acute infections. The proportion of NP swab HBoV1 DNA–positive patients is following previous findings, but the corresponding reports for healthy children are scarce.⁶ Nevertheless, in this study, the difference in proportions comparing patients with AB and healthy children was not statistically significant ($P = 0.064$). A significant challenge in

TABLE 2. Clinical, Laboratory and Demographic Characteristics of mRNA HBoV1–Positive and mRNA HBoV1–Negative Children With Acute Bronchiolitis

Characteristics	HBoV1 DNA Positive			HBoV1 DNA Negative	
	Group A	Group B	A vs. B	Group C	A vs. C
	HBoV1 mRNA Positive (n = 9)	HBoV1 mRNA Negative (n = 28)	P Value	HBoV1 mRNA Negative (n = 298)	P Value
Demographic data					
Mean age (mo) (SD)	14.2 (4.5)	18.7 (6.2)	0.053	12.6 (8.8)	0.583
Females, n (%)	0 (0.0)	9 (32.1)	0.131	118 (39.6)	0.040
Clinical data					
Hospitalization (d), mean (SD)	3 (1.7)	3.5 (2.6)	0.625	3.3 (2.3)	0.664
Disease duration (d), mean (SD)	9 (5.3)	9.7 (6.2)	0.779	9.7 (5.6)	0.712
Oxygen therapy, number (%)	5 (55.6)	12 (42.9)	0.779	140 (47.0)	0.866
Oxygen therapy (d), mean (SD)	1 (1.1)	1.9 (2.7)	0.334	1.58 (2.2)	0.428
Bronchodilator, number (%)	6 (66.7)	15 (53.6)	0.762	122 (43.3)	0.293
Breathing, inhales/min, mean (SD)	50.6 (10.7)	46.3 (11.4)	0.327	48.5 (11.1)	0.587
Birth weight (g), mean (SD)	3665 (627.3)	3281.2 (678.8)	0.142	3198.8 (728.3)	0.059
Fever >38 °C, number (%)	2 (22.2)	19 (67.9)	0.044	151 (50.8)	0.176
Otitis, number (%)	3 (33.3)	7 (25)	0.954	72 (24.2)	0.812
Sore throat, number (%)	8 (88.9)	20 (71.4)	0.538	220 (74.1)	0.538
Laboratory findings					
Mean O ₂ saturation (%) (SD)	92.8 (2.4)	93.9 (2.9)	0.280	93.4 (6.9)	0.784
CRP (mg/L), mean (SD)	7.2 (7.7)	24.6 (27.3)	0.087	22.7 (26.6)	0.102
BSS (points), mean (SD)	6.1 (1.8)	5.7 (2.4)	0.682	5.8 (2.5)	0.679
WANG (points), mean (SD)	3.9 (1.7)	3.5 (1.5)	0.508	3.6 (1.9)	0.600

BSS indicates bronchiolitis severity score (includes cough, sputum production, rales/rhonchi, chest pain when coughing and dyspnea); CRP, C-reactive protein; HBoV1, human bocavirus 1; SD, standard deviation; WANG, Wang respiratory score (includes general appearance, respiratory rate, presence of wheezing and retractions).

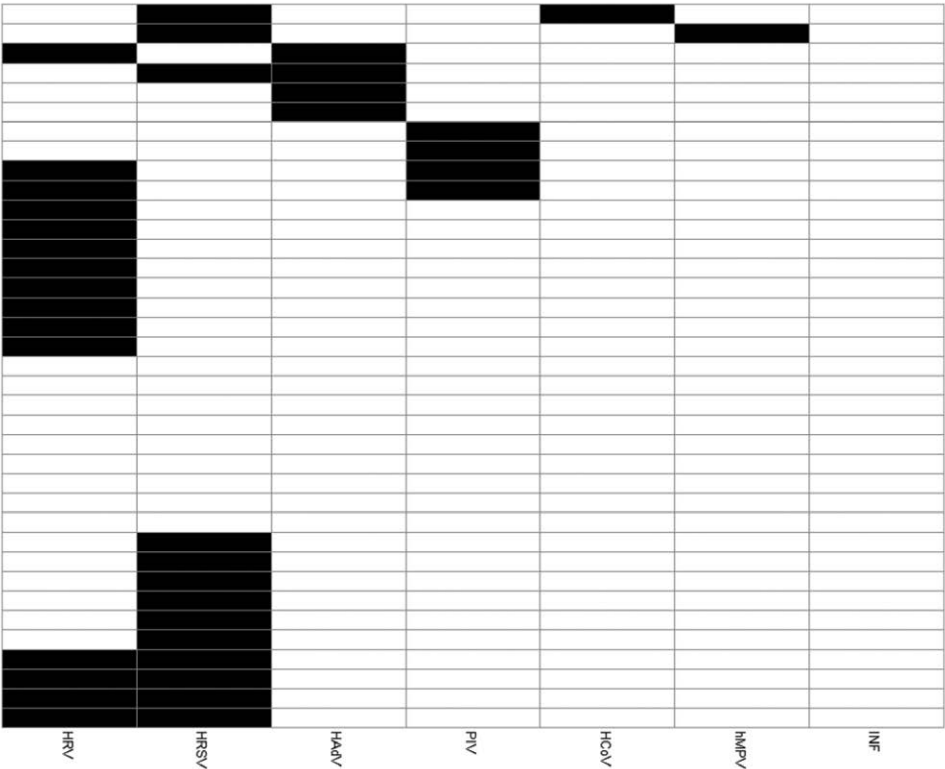


FIGURE 2. Co-occurrence of respiratory viruses in NP swabs of HBoV1-positive patients with acute bronchiolitis. Positive samples are marked in black. The y-axis represents the number of samples. HAdV indicates human mast adenovirus; HBoV1, human bocavirus 1; HCoV, human coronavirus; HMPV, human metapneumovirus; HRSV, human respiratory syncytial virus; HRV, human rhinovirus; INF, influenza virus; NP, nasopharyngeal; PIV, parainfluenza virus.

clinical research is obtaining a control group that closely matches the cases in terms of demographics, particularly age and sex. The control group in this study consists of children who are generally older than those in the case group, as bronchiolitis typically affects children up to the age of 3 years. In contrast, the children undergoing certain elective procedures, who serve as controls, tend to be older on average. Furthermore, a comparison of HBoV1 DNA–positive and HBoV1 DNA–negative patients with AB revealed statistically significant differences only for age and sex but not for several other clinical and laboratory parameters. Moreover, the very high co-detection of other respiratory viruses in AB patients, 28 of 37 (75.7%), further complicated the situation. All these findings are more in favor of the idea that HBoV1 is an innocent bystander than a true pathogen. We tried to distinguish these 2 conditions with a demonstration of actual HBoV1 replication as indicated by the presence of viral mRNA.

In this study, 9 of 37 AB patients with HBoV1 DNA in NP swabs also had HBoV1 mRNA in swabs, suggesting that only 24.3% of HBoV1 DNA–positive cases had an actual infection, and that in children with AB 9 of 307 patients (2.9%) and not 37 of 307 (12.1%) were acutely infected with HBoV1. Several other findings of this study additionally support this outcome, including the absence of virus mRNA in all (9/9) healthy HBoV1 DNA–positive children, and the presence of a lower co-detection rate with other respiratory viruses in mRNA-positive than in mRNA-negative AB patients (2/9, 22.2% vs. 26/28, 92%; $P < 0.001$). Furthermore, a lower mean HBoV1 DNA Ct value in NP swabs of children with HBoV1 mRNA than in those with no mRNA detected (25.7 vs. 32.2; $P = 0.0123$) as well as a higher detection rate of HBoV1 DNA in the plasma of mRNA-positive patients in comparison to mRNA-negative patients (9/9, 100% vs. 11/28, 39.3%; $P = 0.005$) also point in the same direction. The latter findings are in agreement with some published case reports showing that actual HBoV1 infection presents with a high viral DNA load in NP swabs and the presence of HBoV1 DNA in plasma (“viremia”).^{2,9,15,18,19} In this study, we did not detect HBoV1 mRNA in plasma in any HBoV1 DNA plasma–positive patient. This is in line with the knowledge about HBoV1 tropism; that is, the viable virus can be detected in the respiratory tract but does not replicate in blood and, consequently, HBoV1 mRNA could not be detected in plasma.⁷ It is well known that HBoV DNAs have been detected in the tonsils, intestine and heart, but these samples are difficult to access for diagnosis.²⁰ Additionally, the prolonged presence of HBoV DNA in respiratory tract samples often with low viral loads, complicates distinguishing between acute and past infections, especially in immunocompromised children as well as immunocompetent children where the duration of HBoV DNA shedding is unclear. To address this issue, high viral loads, HBoV DNA detection in plasma, serological evidence of primary HBoV infection and HBoV mRNA detection in respiratory samples, as shown in this study, are useful indicators.⁷

Despite the relatively large number of children with AB, the number of those found to have HBoV1 DNA in NP swabs was much smaller (37), and less than a quarter (<3%) of them (9) also had HBoV1 mRNA in NP swabs, showing actual HBoV1 replication. The validity of this finding was additionally supported by the absence of NP virus mRNA in all healthy HBoV1 DNA–positive children and with several distinctions comparing mRNA-positive and mRNA-negative patients. Namely, mRNA-positive AB patients had a significantly lower co-detection rate of other respiratory viruses, had a lower mean HBoV1 DNA Ct value in NP swabs, and in all cases were associated with the presence of HBoV1 DNA in plasma.

A crucial limitation in clinical research is the number of patients that can be included. The number of included patients was too small for a reliable assessment of the statistical correlations of some parameters. To improve the statistical power for certain parameters, particularly given the small number of mRNA-positive samples, we would need to double or even triple the number of children with AB included in the study. The small number of mRNA-positive children did not allow reliable statistical comparisons between mRNA-positive and mRNA-negative children, which is a major limitation of this study.

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