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RESEARCH ARTICLE

Detection of human bocavirus (HBoV) in children with acute respiratory infection (ARI) during the covid-19 transition period

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ABSTRACT

Acute respiratory infections (ARIs) are the highest cause of death in children in the world. Based on the 2018 Riset Kesehatan Dasar Nasional, ARI cases in Indonesia showed a prevalence of 4.4%, with the highest cases occurring in children. One of the new viruses first identified in 2005 in human nasopharyngeal samples is the human bocavirus (HBoV). HBoV is a single-strand DNA virus belonging to the *Parvoviridae* family. This study aimed to assess the prevalence of HBoV in children presenting with ARI during the transitional period of the Covid-19 era. HBoV detection was conducted using multiplex PCR and reverse hybridization methods on nasopharyngeal and oropharyngeal swab samples collected from symptomatic children. This study reported a prevalence rate of 4.94% for HBoV in 2022 and 5.04% in 2023.

Furthermore, the study identified favorable detection rates for HBoV in children with ARIs as 14.81% in 2022 and 8.45% in 2023. These rates ranked 2nd and 5th highest among other pathogens detected in ARIs. Additionally, there was an increase in positive HBoV samples from 4 samples in 2022 to 6 samples in 2023, which was attributed to the relaxation of nonpharmaceutical Covid-19 interventions by mid-2022. HBoV was identified at a significant rate among children with ARI in Jakarta during the transitional phase of the Covid-19 era (2022-2023). Given its potential to induce severe ARI, HBoV necessitates heightened attention as an etiological agent.

1. Introduction

Acute respiratory infection (ARI) is a common disease in children worldwide. It ranks as the highest cause of under-five deaths, based on the World Health Statistics 2023 report from the World Health Organization (WHO) (El Baroudy *et al.,* 2018; WHO, 2023). ARI cases in Indonesia based on the 2018 National Basic Health Research (Riskesdas) showed a prevalence of 4.4% (Badan Penelitian dan Pengembangan Kesehatan Kementerian RI, 2018). ARIs are generally caused by respiratory pathogens, including bacteria, viruses, and fungi (Bhuyan *et al.,* 2017). Globally, 76-90% of pathogens causing ARI are viruses (Setiawaty *et al.,* 2018; Walker *et al.,* 2022). Viruses that commonly cause ARI in children include respiratory syncytial virus, influenza virus types A and B, adenovirus, parainfluenza virus, human metapneumovirus, human rhinovirus, and human enterovirus (Bhuyan *et al.,* 2017; El Baroudy *et al.,* 2018; Setiawaty *et al.,* 2018). In recent years, besides SARS CoV2 that caused a pandemic in 2020-2022, human bocavirus (HBoV) has become an etiologic agent of ARI with an increasing prevalence in pediatric patients

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(El Baroudy *et al.,* 2018; Trapani *et al.,* 2023).

HBoV has four strains: HBoV1, HBoV2, HBoV3, and HBoV4. HBoV1 strain is most commonly detected in respiratory specimens and associated with ARI, while the other strains are most commonly found in stool specimens and are associated with gastrointestinal disease (Kang *et al.,* 2018; Kobayashi *et al.,* 2019). Previously, HBoV1 infection was known to cause mild, self-limiting respiratory symptoms and was often found to co-infect with other etiologic agents (Liao *et al.,* 2022). However, HBoV1 has been reported in many cases of severe respiratory infections, leading to death in children without co-infection with other respiratory pathogens. The high prevalence of HBoV in pediatric ARI with various clinical manifestations and severe symptoms indicates that HBoV infection in children requires more vigilance. The molecular knowledge and pathogenesis of HBoV in pediatric ARI are not fully understood and are still under active research. Therefore, this study aims to examine the prevalence of HBoV in children with ARI and asses the co-infection rates of HBoV with other respiratory viruses in children presenting with ARI during the Covid-19 transition period. The results of this study will enhance comprehension of the role of HBoV as a primary causative agent in respiratory infections, highlighting its potential to cause severe respiratory complications leading to mortality. Furthermore, the findings may inform public health strategies to control and prevent future HBoV outbreaks in pediatric populations.

2. Materials and Methods

The Clinical Microbiology Laboratory Faculty of Medicine Universitas Indonesia (LMK FK UI) received nasopharyngeal and oropharyngeal swab samples from children exhibiting symptoms of ARIs. Collected between 2022 and 2023 from hospitals in Jakarta, these samples underwent testing utilizing the Hybrispot Respiratory Flow Chip Kit (Vitro Master Diagnostica®, Spain), employing multiplex PCR and reverse hybridization methods with 2720 Thermal Cycler (Applied Biosystems®, California) and Vitro Hybrispot 12 (Vitro Master Diagnostica®, Spain). This approach aimed to identify pathogens responsible for acute respiratory infections. Nasopharyngeal and oropharyngeal swab samples were extracted with QIAamp RNA mini kit (Qiagen, Germany) and RNA extraction with QIAamp RNA mini kit (Qiagen). Hybrispot Respiratory Flow Chip tests simultaneously amplify viral DNA, viral RNA, and bacteria by reverse transcription and multiplex PCR (RT-multiplex PCR), which can amplify several targets in one PCR process. Primers contained in the respiratory RT-multiplex PCR consist of 23 pathogenic species, including bocavirus, influenza virus type A, influenza A H3 virus, influenza A H1N1 virus (2009 pandemic), influenza B virus, respiratory syncytial virus (RSV) type A, RSV type B, rhinovirus, enterovirus, human metapneumovirus, adenovirus, human parainfluenza virus type 1, human parainfluenza virus type 2, human parainfluenza virus type 3, human parainfluenza virus type 4, human coronavirus 229E, human coronavirus KHU-1, human coronavirus NL63, human coronavirus OC43, human coronavirus SARS-CoV-2, *Bordetella pertussis, Bordetella parapertussis,* and *Mycoplasma pneumoniae.* Following the RT-multiplex PCR process, the obtained results underwent reverse hybridization on membranes containing species-specific probes utilizing DNA-flow technology. The use of biological samples for Hybrispot Respiratory Flow Chip testing and data derived from these tests in this article has been approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia, with protocol number 23-10-1596.

3. Results

The analysis of respiratory pathogens detected in oropharyngeal and nasopharyngeal swab samples obtained from children displaying symptoms of acute respiratory infections (ARIs) revealed a prevalence rate of 4.94% for human bocavirus in 2022 and 5.04% in 2023 (Table 1 & Figure 1). In 2022, human bocavirus ranked as the second most prevalent pathogen in respiratory pathogen-positive samples, with a detection rate of 14.81%. The predominant pathogen identified

Table 1. HBoV detection from children with ARI symptoms by Hybrispot Respiratory Flow Chip

Variables Data	Year	
	2022	2023
Total samples	81	119
Number of positive samples for respiratory pathogens	27	71
Number of positive samples for human bocavirus	4	6
Prevalence rate (%)	4,94	5,04
Detection rate (%)	14,81	8,45

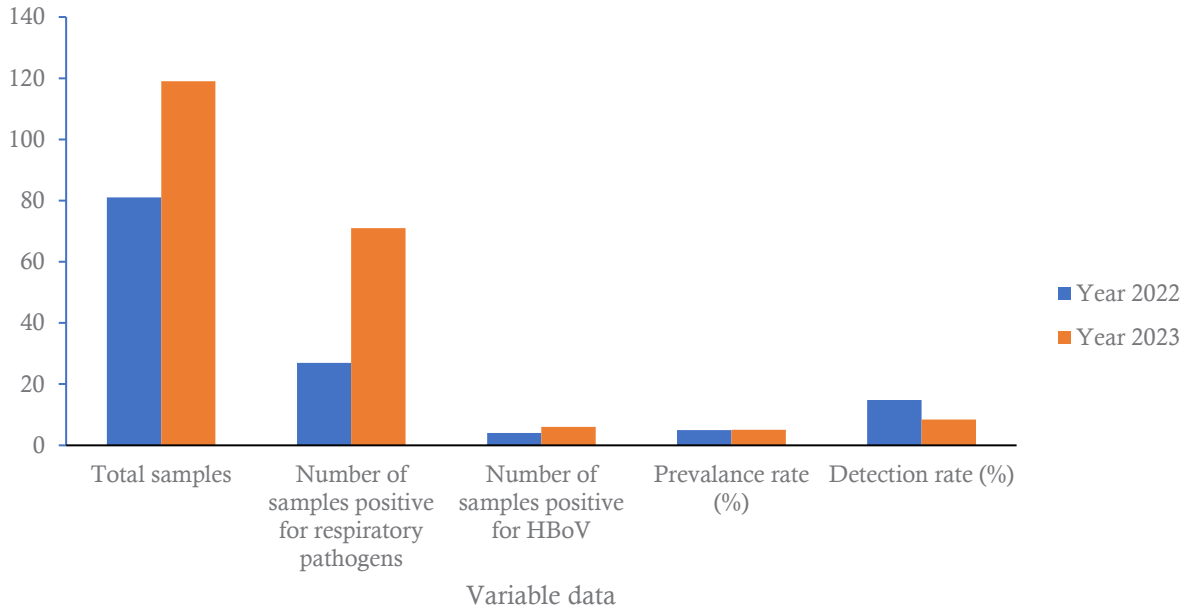

Figure 1. HBoV detection from children with ARI symptoms by Hybrispot Respiratory Flow Chip

Table 2. Basic characteristics of children who are HBoV positive

No	Age (years, months)	Sex	Co-infection	Test Conducted (Month, Year)
1	1,10	male	none	August, 2022
2	4,9	female	rhinovirus	August, 2022
3	4,6	female	none	August, 2022
4	0,3	female	respiratory syncytial virus type A	August, 2022
5	0,11	male	none	March, 2023
6	0,6	female	virus influenza A H1N1 2009	March, 2023
7	1,1	female	none	March, 2023
8	1,4	female	human coronavirus OC43, human parainfluenza virus type 3	September, 2023
9	6,3	male	adenovirus	September, 2023
10	4,6	female	virus influenza A H1N1 2009, *Mycoplasma pneumoniae*	September, 2023

in positive samples in 2022 was human coronavirus SARS-CoV-2, with a detection rate of 40.74%. Conversely, in 2023, the human bocavirus detection rate is 8.45%, positioning it as the fifth most common pathogen detected in respiratory pathogen-positive samples, following rhinovirus (18.30%), adenovirus (18.30%), influenza A H1N1 2009 virus (14.08%), and influenza A H3 virus (9.85%). Additionally, there was an increase in the number of positive HBoV samples from 4 samples in 2022 to 6 samples in 2023 (Table 1 & Figure 1). Notably, 90% of patients testing positive for human bocavirus were under five, while the remaining 10% were between five and seven years old (Table 2). Furthermore, co-infection was observed in 60% of samples testing positive for human bocavirus, indicating simultaneous detection with other respiratory pathogens such as rhinovirus, respiratory syncytial virus type A, influenza A H1N1 2009 virus, human coronavirus OC43, human parainfluenza virus type 3, adenovirus, and *Mycoplasma pneumoniae* (Table 2).

4. Discussion

Human bocavirus (HBoV), initially identified in 2005, belongs to the *Parvoviridae* family, *Parvovirinae* subfamily, under the genus *Bocaparvovirus* (O. Schildgen & Schildgen, 2018). HBoV is a non-enveloped virus with a single-stranded linear negative-sense DNA genome type, measuring approximately 25 nm in size and roughly 4.7-5.7 kb (Christensen *et al.,* 2019). Unlike other viruses in the family *Parvoviridae* which only have two open-reading frames (ORF), the HBoV genome has 3 ORF which expresses a total of 9 proteins via

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alternative splicing and alternative polyadenylation, consisting of 6 non-structural proteins (NS1, NS1-70, NS2, NS3, NS4, NP1) and 3 structural proteins (VP1, VP2, VP3) (Shao *et al.,* 2021).

The worldwide prevalence of HBoV from data collected between 2005 and 2016 was 6.3% for respiratory infections and 5.9% for gastrointestinal infections (Guido *et al.,* 2016). Those data are not significantly different from the HBoV detection results in Jakarta by the LMK FKUI, with a prevalence rate of 4.94% in 2022 and 5.04% in 2023. HBoV has also been documented to have a positive detection rate of 4.46% in 2012 in Indonesia from samples of individuals exhibiting influenza-like symptoms (ILI) (Adam *et al.,* 2017). Furthermore, HBoV has been documented to have a positive detection rate of 17.8% in 2014 in Surabaya, Indonesia, from samples of children experiencing ARI and ranked as the second-highest detected virus in pediatric patients with pneumonia (Setyoningrum *et al.,* 2020). The findings of a study conducted by Wertheim *et al.* (2015) also showed that out of 1122 patients hospitalized in Thailand, Vietnam, and Indonesia with symptoms of ARI, 200 patients were identified to have HBoV (16.4%), ranking second highest among detected viruses in patients with ILI (Wertheim *et al.,* 2015). The data results indicate a high level of HBoV detection over recent years and continue to show high detection rates in 2022 and 2023, with HBoV positive detection rates reaching 14.81% in 2022 and 8.45% in 2023, respectively. These rates rank as the second-highest and the fifth-highest detection rates among other detected respiratory pathogens in children with ARI (Table 1).

Data from LMK FK UI for 2022-2023 indicate an increase in positive HBoV samples in 2023, with 6 samples positive for HBoV compared to 2022, with 4 samples positive for HBoV. This contrasts with the decrease in positive samples of human coronavirus SARS-CoV-2, which decreased from 11 positive samples in 2022 to 3 positive samples in 2023. The reduced detection of human coronavirus SARS-CoV-2 in 2023 in children may be attributed to the formation of herd immunity among individuals aged over 6 years who have been fully vaccinated against Covid-19. The rise in the incidence of positive cases of HBoV in 2023 is likewise reflected in the identification of other respiratory pathogens, including rhinovirus, adenovirus, influenza A H1N1 2009 virus, and influenza A H3 virus, the latter demonstrating a higher detection rate compared to HBoV in 2023. This could be connected to 2022, which is still in the stage of implementing nonpharmaceutical measures to control the transmission of Covid-19. Thus, in 2023, when nonpharmaceutical interventions were no longer in place, there was an increase in cases of respiratory pathogens other than Covid-19 (Ali *et al.,* 2022). This increase will also be reinforced by a decrease in immunity due to reduced exposure to respiratory pathogens during the implementation of nonpharmaceutical interventions until 2022 (Baker *et al.,* 2020).

Data from LMK FK UI for the years 2022-2023 also indicate that 60% of samples detected with HBoV showed detection of other respiratory pathogens. The co-infection of HBoV with other respiratory pathogens has emerged as a prevalent feature in HBoV infections, raising uncertainties regarding whether HBoV acts as the primary causative agent or merely a secondary virus present in cases of respiratory tract infections. (Kang *et al.,* 2018). However, currently, there have been numerous reported cases where HBoV has been found as the sole pathogen detected in severe respiratory tract infections without co-infection with other respiratory pathogens (Akça *et al.,* 2016; Liao *et al.,* 2022; Moesker *et al.,* 2015; Moreno *et al.,* 2014; Ziemele *et al.,* 2019). Co-infection with other pathogens can occur due to the extended shedding of HBoV1 in the nasopharynx, persisting for weeks or even months (Trapani *et al.,* 2023). This is supported by several studies indicating persistent HBoV1 infection lasting for months without showing symptoms after the initial infection (Mohammadi, 2023). Based on the study by Schildgen *et al.* (2013), the DNA of HBoV can persist as episomal covalently closed circular DNA (cccDNA) and merge with the host genome, possibly leading to persistent infection. Chronic inflammation due to persistent HBoV infection may indirectly contribute to the development of lung cancer (Schildgen *et al.,* 2013). On the other hand, lung cancer also acts as a comorbid condition alongside other comorbidities such as congenital heart disease, chronic lung disease, neuromuscular disorders, and immunological conditions, potentially amplifying the risk associated with respiratory infections caused by HBoV (Christensen *et al.,* 2019). Nevertheless, the mechanisms underlying HBoV latency, persistence, and reinfection remain inadequately comprehended, primarily due to the absence of permissive cell lines and effective animal models (Bhat and Almajhdi, 2021).

The clinical manifestations of the respiratory system in children with ARI detected with HBoV are generally similar or comparable to infections caused by other respiratory viruses, such as cough, nasal congestion and discharge, pharyngeal hyperemia, and wheezing, which may resolve spontaneously upon reaching the post-infection phase (self-limiting) (Mohammadi, 2023; Trapani *et al.,* 2023). However, in recent years, clinical manifestations of HBoV infection have been widely reported in various countries, associated with severe respiratory symptoms in children. One such case

reported by Liao J *et al.* (2022) illustrates severe clinical manifestations of pneumonia and respiratory distress syndrome (ARDS) caused by HBoV infection in a 13-month-old child, resulting in death (Liao *et al.,* 2022). Furthermore, in another study by Moekser *et al.* (2015), HBoV was also shown to cause severe acute respiratory tract infections (SARI) with clinical manifestations of ARDS, severe atelectasis, and respiratory failure necessitating mechanical ventilation support and requiring treatment in the pediatric intensive care unit in children in the Netherlands (Moesker *et al.,* 2015). Several studies conducted in Turkey, Argentina, and Latvia have also found similar findings, where HBoV causes severe respiratory tract infections in children requiring hospitalization (Akça *et al.,* 2016; Moreno *et al.,* 2014; Ziemele *et al.,* 2019).

However, despite the high number of HBoV cases worldwide, including in Indonesia, and the existence of several reports of HBoV cases causing severe respiratory symptoms without co-infection, the pathogenesis of HBoV remains poorly understood to date due to the lack of suitable animal models for HBoV infection. Current studies on HBoV are still limited to culturing primary human airway epithelia cultured at the air-liquid interface (HAE-ALI), which mimics the human respiratory tract and allows direct HBoV infection (Yan *et al.,* 2020). HBoV infection in HAE-ALI, based on the study by Yan *et al.* (2020), indicates that virus receptors are expressed both on ciliated apical cells and in the laterobasal regions of the respiratory epithelium (Yan *et al.,* 2020).

Specific management or antiviral HBoV infection therapy is unavailable (Mohammadi, 2023). Symptoms resulting from HBoV infection are managed with supportive therapy to control symptoms, such as administration of antipyretics, oxygen supplementation during hypoxia, and bronchodilator administration in patients experiencing wheezing (Alkhalf *et al.,* 2022). Studies on antivirals to combat HBoV infection have not been conducted thus far, similar to the unavailability of specific antivirals for parvovirus B19V, which shares close relatedness with HBoV and both belong to the *Parvoviridae* family infecting humans (Alkhalf *et al.,* 2022; Hu *et al.,* 2022). Presently, there is no vaccine for HBoV; hence, specific preventive measures cannot be implemented. (Bhat & Almajhdi, 2021; Mohammadi, 2023).

Although no antiviral agents or vaccines are currently available for HBoV infection, accurate diagnostic tools for detecting HBoV are necessary to monitor the spread of infection and the clinical manifestations. Various molecular detection methods for HBoV have been developed using polymerase chain reaction (PCR) targeting the NP1, NS1, or VP1/2 genes, which are proteins or genes of HBoV (Alkhalf *et al.,* 2022). PCR methods that can be used for HBoV detection include in-house PCR, quantitative PCR (qPCR), and reverse transcription PCR (RT-PCR) (Mohammadi, 2023; Trapani *et al.,* 2023). Multiplex PCR can simultaneously detect multiple etiological agents in one reaction, as utilized in detecting HBoV by LMK FK UI. In addition to Vitro Master Diagnostica from Spain, Multiplex PCR has also been developed by several companies to detect HBoV, including the RespiFinder assay (PathoFinder, Maastricht, Netherlands) and the Luminex xTAG Respiratory Viral Panel (RVP) FAST v2 assay (Luminex Molecular Diagnostics, Toronto, ON, Canada) (Alkhalf *et al.,* 2022). Detection of HBoV using rapid antigen tests with nasopharyngeal swab samples has also been developed to detect the VP2 capsid protein of HBoV (Bruning *et al.,* 2016). Furthermore, detection of HBoV infection through the presence of IgM and IgG antibodies against HBoV in patient serum can also be performed. Detection of infection using these antibodies has higher specificity compared to qPCR-based HBoV detection (Mohammadi, 2023). In detecting antibodies against HBoV, diagnostic tools have been developed using antigens from VP1 and VP2 proteins of HBoV based on yeast-derived virus-like particles (Tamošiunas *et al.,* 2016).

5. Conclusions

HBoV was detected at a considerable rate among children with ARI in Jakarta during the transitional period of the Covid-19 era (2022-2023). The pediatric patients identified with HBoV were predominantly children under 5 years of age (90%), with others falling within the age range of 5-7 years. HBoV was detected as a co-infection with other respiratory pathogens and as a single agent. To date, numerous reports have linked severe respiratory tract infections in children to positive HBoV detection without the presence of other respiratory pathogens. This underscores HBoV as a pathogenic factor in ARI among children, warranting heightened vigilance and attention. Continuous research on HBoV at the molecular level, including investigations into antiviral agents and vaccines for therapeutic and preventive purposes against HBoV infections, is imperative for effectively managing HBoV infections in children.

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Conflict of interest

All authors have no conflict of interest in this article.

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