PREVALENCE OF HUMAN BOCAVIRUS AND ADENOVIRUS AMONG CHILDREN WITH RESPIRATORY TRACT INFECTIONS IN ILORIN, NIGERIA

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Abstract
Human adenovirus type 7 (HAdV7) and human bocavirus (HBoV1) are associated with mild to severe upper and lower respiratory infections in children. HAdV7 co-infections with HBoV1 have been implicated in wide-spread morbidity in sub-Saharan Africa. The study aimed to detect these viruses in children under 5-year-old with Respiratory Tract infections attending the University of Ilorin Teaching Hospital Ilorin, Nigeria using polymerase chain reaction (PCR). Two Hundred (200) children under 5- years old were recruited with confirmed symptoms of respiratory tract infections, nasopharyngeal (NP) and oropharyngeal (OP) samples were collected using sterile flocked swab. The socio-demographic information/ risk factors and clinical presentations were obtained with the aid of well-structured questionnaire. Viral detection was done using real-time polymerase chain reaction; the genes amplified were Hexon for HAdV7 and VP1 for HBoV1. Out of the 200 samples, 35 (17.5%) were positive with 7% (14/200) prevalence recorded for HAdV7 and 10.5% (21/200) for HBoV1 respectively. Of the 35 positive samples, co-infection was observed in 15 (42.9%) of the samples. It was observed that 111 subjects were male, and there was no significance difference in the prevalence of the viruses with respect to gender. The prevalence was significantly higher amongst 0-1year age group. There was statistical significance for some of the socio-demographic and risk factors. According to findings from this study, HAdV7 and HBoV1 are important cause of infection in the respiratory system of children. It is therefore important to carry out more research on these viruses and highlight the transmission patterns and the severity of the disease in Nigeria among this susceptible age group.
Keywords*:* Children, Human Adenovirus, Human Bocavirus, Respiratory infection, Co-infection

1. Introduction

Respiratory tract infections (RTIs) are any infectious disease of the upper or lower respiratory tract. Worldwide, respiratory diseases consisting of various conditions caused by infectious and non-infectious factors represent a significant contributor to childhood morbidity and mortality especially in the under-five years [1,2].

Human adenoviruses (HAdVs) constitute a group of double-stranded, non-enveloped DNA viruses classified within the genus Mastadenovirus of the Adenoviridae family, in accordance with the Baltimore Classification Scheme of Viruses [3]. Adenovirus typically presents with a diameter ranging from 70 to 90nm and features an icosahedral capsid [3]. Its initial isolation in the 1950s from adenoid tissue-derived cell cultures gave rise to its name. Within the human population, there are 56 acknowledged human adenovirus types (HAdV-1 to 56) distributed across seven species (Human Adenovirus A to G) [4], eliciting a broad spectrum of illnesses ranging from mild respiratory infections in pediatric patients to life-threatening multi-organ diseases in immunocompromised individuals. Notably, HAdV-7 demonstrates heightened virulence compared to other serotypes [5-6].

Over 80% of diagnosed HAdV infections are observed in children under the age of four, largely due to their immature humoral immunity [7]. Although most cases exhibit a self-limiting course, adenoviruses have been implicated in the development of acute pneumonia [1]. The report emphasized the crucial role of secondary bacterial flora attachment, a process facilitated by immune system suppression.

Human adenovirus (HAdV) detection can be achieved from various sample types, such as nasopharyngeal aspirates, swabs, bronchoalveolar lavage, urine, stool, and blood, employing direct or indirect immunofluorescence, shell vial cultures, or PCR [8].

In contrast, Human bocavirus (HBoV), a relatively recent discovery belonging to the family Parvoviridae, is a single-stranded DNA virus. Studies have highlighted its prevalence primarily among children aged five and below, with distinct genotypes

often associated with diverse clinical presentations [9,10].

Notably, HBoV1 and HBoV3 were characterized in 2005 from nasopharyngeal aspirate pools, demonstrating associations with respiratory tract infections [11]. HBoV has been linked with approximately 21.5% of childhood respiratory tract infections annually, with HBoV1 particularly implicated in severe cases of bronchopneumonia and bronchiolitis in both developed and developing countries [12,13,14]. Symptomatology observed in HBoV-positive children aligns with previous reports [15].

Coinfection with other respiratory viruses, often observed with HBoV, may exacerbate disease severity and contribute to adverse outcomes [9]. Consequently, the rising attention on HBoV infection worldwide underscores its variable incidence, clinical presentation, and frequent association with coinfections [15].

Seasonal patterns further characterize these viral infections. HAdV exhibits increased prevalence from late winter to early summer, although variations may occur depending on geographical location, leading to respiratory infection outbreaks, particularly in densely populated environments [16,17]. Conversely, HBoV is detectable year-round, with peak incidence during winter months, particularly between November and March, accounting for approximately 80% of cases [18].

Nigeria, facing a high prevalence of risk factors implicated in acute respiratory tract infections (ARTIs) among children, including age, sex, overcrowding, nutritional status, and socioeconomic status, remains limited in its studies of ARTIs [19]. Studies have revealed that rates of acute respiratory infection per child to be at least threefold higher [20]. Previous studies in Nigeria has reported various prevalence of 4.9% in Ibadan [21], 38% in Ilorin [22] and 8.5% in Ado Ekiti [23] for Adenovirus implicated in repiratory tract infections while the prevalence rate reported for Human Bocavirus in subjects with respiratory tract infections were 2.4% and 8.1% respectively in Ibadan[23,24]. These findings indicate the circulation of these two respiratory viruses within the country and underscore the imperative for community-based initiatives aimed at surveillance and intervention for viral respiratory tract infections in children under five years of age in Nigeria [25].

It is therefore necessary to carry out more research on these respiratory viruses to further provide information on their prevaence, highlight the transmission patterns and the severity of the disease in Nigeria in order to monitor and also prevent potential underlying respiratory complications such as pneumonia, and bronchiolitis among this susceptible age group. Facilities for routine diagnostic test of respiratory viruses should be made available hospitals for appropriate screening of children before the commencement of antibiotic therapy.

2.0 Materials and Methods

2.1 Ethical Approval

The approval for this study was obtained from the Ethical Review Committee (ERC) of University of Ilorin Teaching Hospital (UITH), Kwara state. A consent form was issued to Patient's parents/guardian; this was done after a clear explanation of the purpose of the study as contained in the information sheet.

2.1.1 Sample size

Two hundred nasopharyngeal (NP) and oropharyngeal (OP) samples were collected for this study at University of Ilorin Teaching Hospital between October to December, 2021. A random sampling method was used in the study. Patients aged 0-5 years attending paediatrics unit of the University of Ilorin Teaching Hospital were enrolled. A total of 200 nasopharyngeal and oropharyngeal swabs were collected from patients with respiratory symptoms such as runny nose, cough, sneezing, mild fever, nasal congestion, and sore throat. Samples were obtained by inserting a commercially purchased sterile swab into the nostril and mouth to a depth of 2–4 cm, and retracting it in a slow rotating motion, in order to trap epithelial cells in the swab. The swabs were stored in 2ml of Viral Transport Medium (PBS), the samples were transported to the laboratory using ice packs to the molecular diagnostic laboratory at University of Ilorin Teaching hospital (UITH) and were stored at -80^{o}C until analysis.

2.2 Extraction of viral DNA from samples

Viral DNA was extracted using a Zymo viral DNA kit (Cat No: R1035, Lot No: 209610), following the manufacturer's protocol. In summary, samples were mixed with viral DNA buffer, processed through a spin column, washed twice, and eluted with DNase/RNase-free water. The principle involves selective DNA binding to the column matrix, washing away contaminants, and elution for downstream applications.

2.2.1 Primers and probes design and synthesis

Primers for HAdV7 and HBoV1 were designed from the E4 and NP1 regions respectively, using sequences available in the NCBI GenBank. Controls used for the study were: Negative Control (No Template Control) which lacked any template DNA and was included in each PCR run to monitor for contamination and ensure the absence of false-positive results and endogenous Internal Control (Glyceraldehyde phosphate (GAPDH), a commonly used reference gene was used as the internal control for the study. This control was essential for monitoring the efficiency of the PCR reactions. The fluorogenic probes for HAdV7 and HBoV1 consisted of oligonucleotides with the 3′ reporter dye

hexachlorofluorescein (HEX) and 6-carboxyfluorescein (FAM) respectively with 5′ quencher dye black hole quencher 1 (BHQ1) (Table 1). Primers and probes were synthesized by Inqaba biotech, South Africa.

Table 1: Oligonucleotide Primers and Probes used for HAdV and HBoV detection, E4 and NP1 gene amplification and sequence

Primer name	Sequence (5-3)	Target amplicon/ gene size (bp)
HAdV7-E4F	ACCACCCAAACTACACCAA	E4
HAdV7-E4R	CAGTTTCGGGGTGTGTGTTG	gene/155bp
HAdV7-E4FP	**HEX-**CGCGCAAAGCACAAGCTCTAAAGAAGC-**BHQ1**	
HBoV_NP1F	TCAGGAACACCCAATCAGCC	NP1
HBoV_NP1R	GTAGAACCCACACCACCCTG	gene/175bp
HBoV_NP1FP	**FAM-**TGCACTGCTTCGAAGACCTCAGACCA-**BHQ1**	
GAPDHF	GAAGGTGAAGGTCGGAGT	GAPDH
GAPDHR	GAAGATGGTGATGGGATTTC	human/206bp
GAPDHP	**Cy3.5-**CAAGCTTCCCGTTCTCAGCC**-BHQ2**	

Key: HAdV, Human Adenovirus; HBoV, Human Bocavirus; GAPDH, Glycealdehyde phosphate dehydrogenase.

2.2.3 Preparation of master mix

The reaction for the PCR was done using Luna Universal Probe One-Step RT-qPCR Kit (New England, BIOLabS, E3006), it was prepared according to manufacturer's instructions. The master mix was prepared using the following; One-step reaction mix, primers, probes, GAPDH and nuclease free water.

2.2.4 Multiplex polymerase chain reaction (Multiplex qPCR)

A 14µl of the master mix was pipetted into each well on the qPCR plate; which had 96 wells. 6µl of the DNA template from each sample was into the respective well containing 14µl of the mix to make up a 20µl mixture except the first well which was the qPCR control. The plate was sealed using a sealant and it was carefully placed in the qPCR machine. The channels used were HEX for HAdV7, FAM for HBoV and ROX for GAPDH fluorescence dye.

The samples were subjected to 45 cycles for a runtime of 1hour 31 minutes, hot start was for a minute at 95°C, denaturation at 95°C for 15s, annealing at 50°C for about 30s and extension at about 60°C for 30s. An AriaMx Real-time PCR System (Agilent) was used. There was a No template control (NTC) for each of the PCR runs. The channel probes emitted fluorescence when there was amplification.

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Table 2: Prepared Reaction for the Multiplex qPCR

Components	20 Reaction	Final concentration
Luna universal probes One-step reaction mix	10	1x
Forward primers	0.4µl	0.2Mm
Reverse primers	0.4µl	0.2Mm
Probes	0.2µl	0.1Mm
Nuclease free water	1	1x
Template DNA	6 µl	

2.3 Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 26. Socio-demographic data was summarized using descriptive statistics such as mean, frequency, percentage and cumulative percentage was generated. Categorical information was analyzed with parametric and non-parametric test as adequate. Binary Logistic analysis was used to analyze the association of socio-demographic factors with HAdV7 and HBoV1 infection in children less than five years as sampled.

3.0 Results

3.1 Prevalence of for HAdV7 and HBoV1among children with respiratory tract infection

A total of 200 nasopharyngeal and oropharyngeal swab samples were screened for HAdV7 and HBoV1. The threshold for the positive samples for HAdV7 is 273.966 with the CT values ranging from 19.90-33.01 while that of HBoV1 is 119.84 with a CT value ranging from 19.60-38.85.

Of the 200 samples tested, thirty-five (35) were positive for both HAdV7 and HBoV1 accounting for a prevalence of 17.5% (35/200). Fourteen (14) of the 200 samples tested positive for HAdV7 giving a prevalence of 7% (Figure 1), while 10.5% (21/200) were positive for HBoV1 (Figure 2).

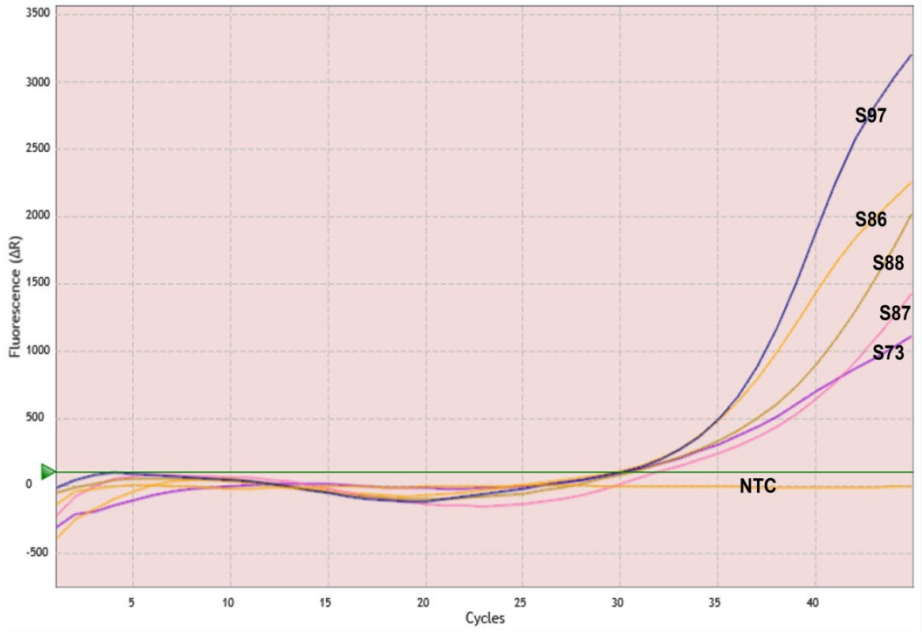

Fig 1a

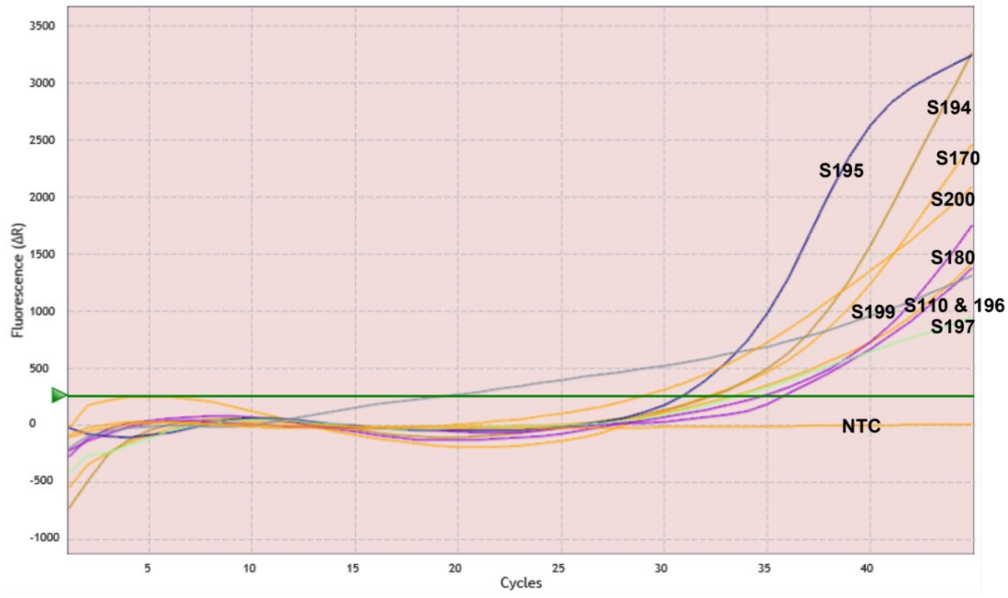

Fig 1b

FIG. 1b: The optics graph illustrates the results of real-time PCR analysis targeting Human Adenovirus 7 (HAdV7) using HEX fluorescent dye with a threshold set at 273.966. Positive signals were detected in samples 73, 86, 87, 88, 97, 110, 170, 180, 194, 195, 196, 197, 19 9, and 200, with corresponding cycle threshold (CT)

values ranging from 19.90 to 33.01. No amplification was observed in the No Template Control (NTC), indicating absence of contamination.

FIG. 2a&b: The optics graph displays Real-time PCR results for Human Bocavirus 1 using FAM fluorescent dye with a threshold set at 119.84. Positive signals were observed in samples 20, 51, 55, 57, 71, 73, 86, 87, 89, 91, 97, 99, 110, 170, 180, 194, 195, 196, 197, 199, and 200, with CT values ranging from 19.60 to 38.85. Glyceraldehyde phosphate (GAPDH) served as the internal control for PCR reactions, while no amplification was detected in the No Template Control (NTC).

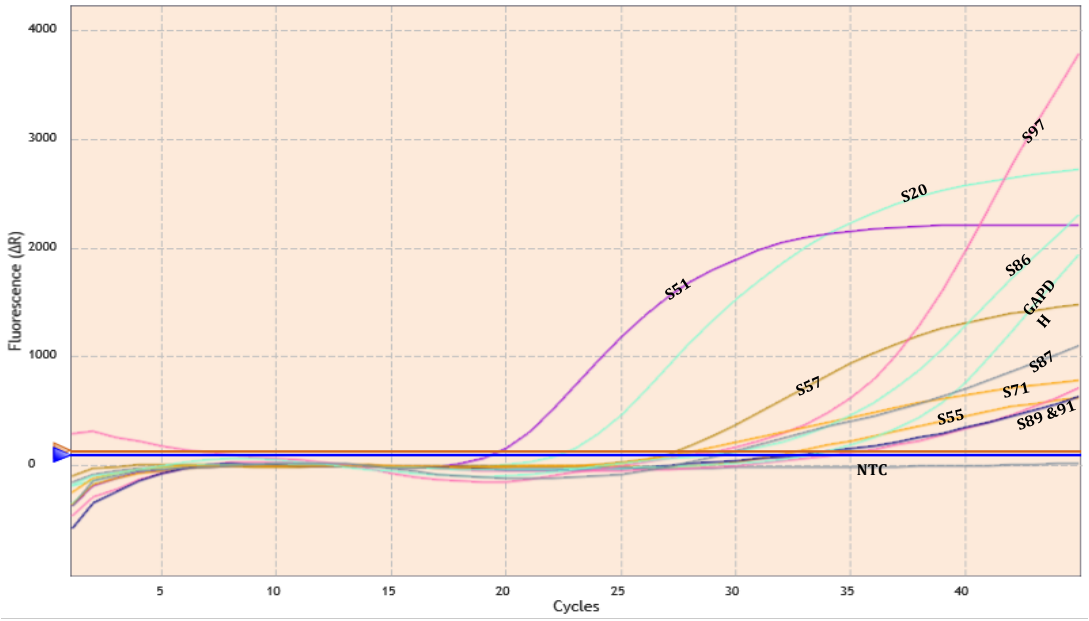

Fig 2a

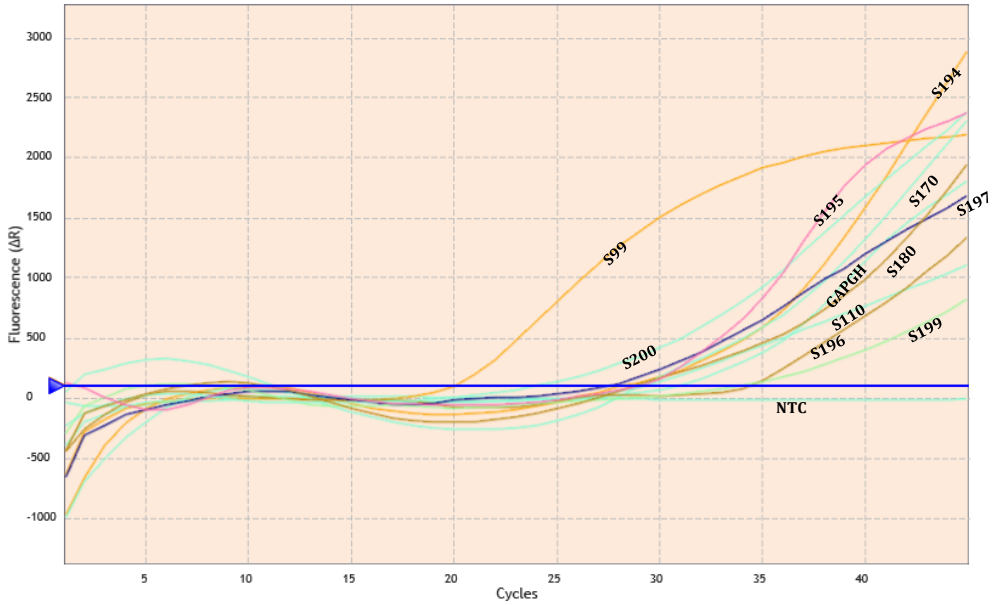

Fig 2b

3.2 Prevalence of Human adenovirus and Human Bocavirus in relation to some risk factors (age, gender, attendance of Daycare and exclusive breastfeeding)

With respect to the gender of the children enrolled in the study, out of the 14 samples that tested positive for HAdV, 71.4% (10/14) were male while 28.5% (4/14) were female. However, for HBoV1, 12/21 (57.1%) males tested positive, and the females that showed positivity were 9/21 (42.1%) as shown in Table 1.
In relation to age distribution, the highest positivity of 78.6% (11/14) was recorded in the 0-1 year age group for those that tested positive for HAdV7. Similar trend was also observed for HBoV1, with the highest positivity also recorded in age group 0-1 year 66.7% (14/21) (Table 1).
The relationship between attendance of daycare and positivity to the two respiratory viruses shows that, those that attended day care and were positive for HAdV7 were 5 (7.6%) and those who do not attend daycare had a prevalence rate of 12.1% (P = 0.008).While those postive for HBoV1 and attend daycare were 9 (6.7%) and those who do not attend day care were 13 (12.1%) (P = 0.078) (Table 1).
The association between exclusive breastfeeding and positivity was also determined, it was observed that children that were fed exclusively had a prevalence rate of 35.7% (5/14) for those that tested positive for HAdV7 and 33.3% (7/21) for HBoV1 respectively. No statistical association was observed (Table 1).

Table 1: Prevalence of Human adenovirus and Human Bocavirus in relation to age, gender, attendance of Daycare and exclusive breastfeeding

Risk factors		Human Adenovirus		Human Bocavirus		
		Positive (%)	x^2(p-value)	Positive (%)	x^2(p value)	Total
Gender	Male	10 (71.4)	0.092 (0.761)	12 (57.1)	0.562 (0.453)	22
	Female	4 (28.6)		9 (42.9)		13
Age	0-1 years	11 (78.6)	1.013 (0.152)	14(66.7)	6.875 (0.143)	25
	1-2 years	2 (14.3)		5 (23.8)		7
	2-3 years	0		0		0
	3-4 years	0		1 (4.8)		1
	4-5 years	1 (7.1)		1 (4.8)		2
Attendance of Daycare	Yes	5 (7.6)	6.974 (*0.008)	9 (6.7)	7.087 (0.078)	14
	No	8 (12.1)		13 (9.7)		21
Exclusive breastfeeding			7.794 (0.050)		8.079 (*0.044)	
	Yes	5 (35.7)		7 (33.3)		12
	No	9 (64.3)		14 (66.7)		23

X^2= wald Chi-square
P value ≤ 0.05= significance

3.3 Prevalence of human adenovirus and human bocavirus co-infection in various age groups

The co infection rate between human adenovirus and human bocavirus among the children who tested positive was 42.9% (15/35). This was further analyzed to show their distribution among age group. It was observed that children between ages of 1.1-2 years recorded the highest co-infection rate of 13.3% (2/15), this was followed by those in the age group 4.1-5 years had 1 (6.7%) while no co infection was recorded in the age group 2.1-3 years and 3.1-4 years respectively (Figure 3).

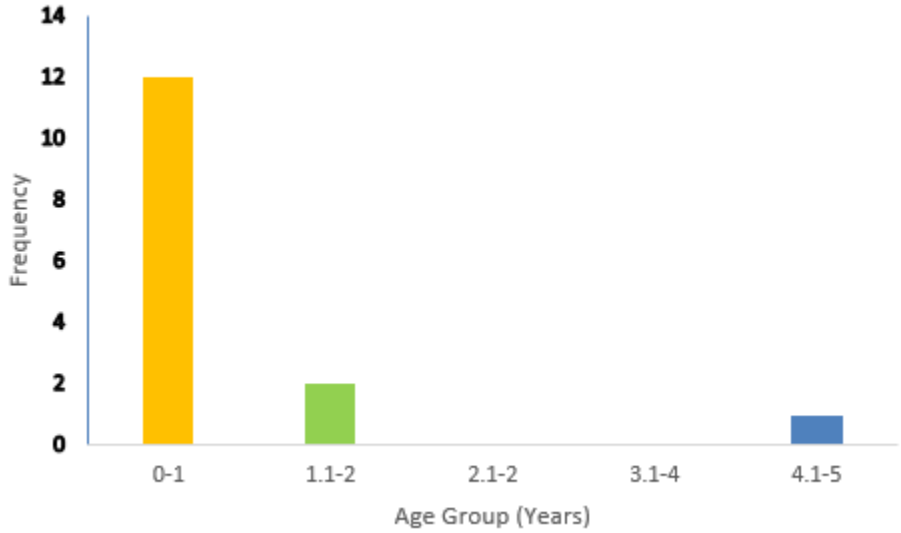

Figure 3: Rate of human adenovirus and human bocavirus co-infection in various age groups

3.4 Prevalence of human adenovirus and human bocavirus in relation to respiratory symptoms

The distribution of different symptoms of respiratory infection in children less than five years in relation with human adenovirus infections revealed that out of total number of 26 children that had runny nose, only 1(3.8%) of them tested positive for human adenovirus. Ten children had persistent sneezing, only 1 (10%) tested positive for adenovirus. Four (12.5%) children tested positive to human adenovirus out of 32 children that were coughing. Forty-nine children showed the three symptoms (runny nose, cough and sneezing), only 5 (10.2%) tested positive to human adenovirus. A p-value of 0.679 showed that there is no significant difference between respiratory symptoms and human adenovirus infection (Figure 4).

With respect to human bocavirus, the distribution of respiratory symptoms found in children less than five years are as follows: Twenty-six children had runny nose, 4 (15.4%) tested positive to human bocavirus, ten children showed symptoms of sneezing, only 1(10%) tested positive to human bocavirus and of the 32 children that were coughing only 1 (10%) tested positive to the same virus. There was no significant difference between respiratory symptoms found in children below five years at UITH and human bocavirus (p-value = 0.968) (Figure 5).

4.0 Discussion

This study found a prevalence of 7.0% for HAdV7 among children with acute respiratory tract infection, which aligns with rates of 6.3% and 8.5% reported in Nigeria[21, 22]. Comparatively, our findings are consistent with global prevalence rates, such as 6.6% in Iran[26], 6.1% in Norway[27], and 7.08% in China[28].

A prevalence of 10.5% of children tested positive for HBoV1 in this study, which is lower than the 16.8% reported among Kenyan children[29]. However, it is comparable to rates reported by Joseph *et al*.[24] in Nigeria (8.1%), Bharaj *et al*. [30] in India (7.2%), Tran *et al*. [31] in Vietnam (7.2%), Moreno *et al*. [32] in Argentina (6.8%), and Hosseininasab *et al.* [33] in Iran (6.5%). Conversely, it is higher than rates reported by Niang *et al.* [34] in Senegal (1.2%), Akinloye *et al*. [21] in Nigerian children (2.4%), and Akturk *et al*. [35] in Turkey (2.3%).

The recorded prevalences of both HAdV7 and HBoV1 in this study highlight their significance as common respiratory pathogens in Nigerian children. Variations in observed prevalence compared to other studies may stem from differences in sampling strategies, methodologies, illness severity among recruited children, populations, climates, and diagnostic techniques.

In relation to gender distribution, there was a male predominance among enrolled children, with more males testing positive for both HAdV7 and HBoV1 compared to females. This finding is consistent with previous research indicating a higher prevalence of respiratory viruses among male children[23,36,37,38]. The underlying reasons for gender differences in adenovirus and human bocavirus infections remain unclear and may involve complex interactions of biological factors such as immune responses and hormonal influences.

In addition to biological factors, behavioral and environmental influences, such as exposure to respiratory pathogens in daycare settings or household contacts, may contribute to gender-based differences in infection rates [39, 40]. The higher degree of hospitalizations and respiratory infections in males, attributed to anatomical differences like the shorter and narrower trachea, could further explain the male predominance and higher positivity rates among enrolled children.

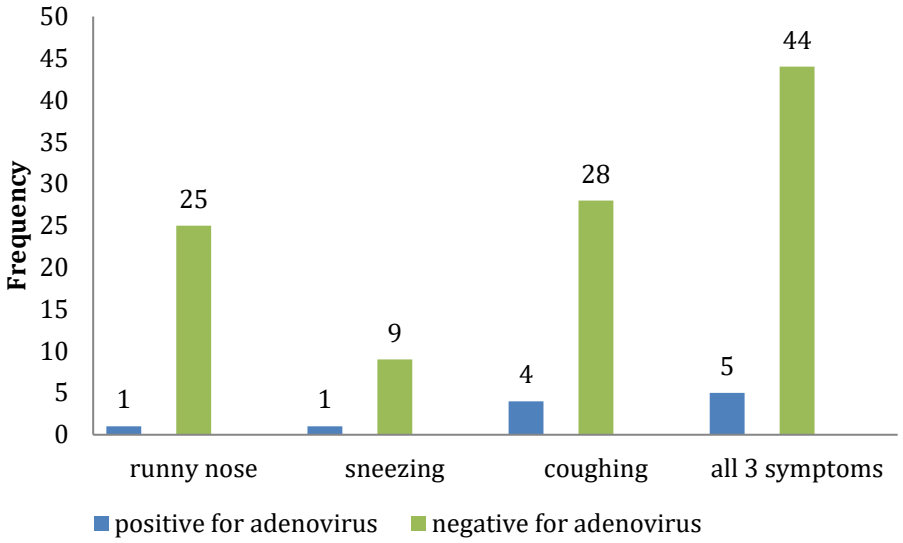

Fig. 4: Prevalence of human adenovirus in relation to respiratory symptoms

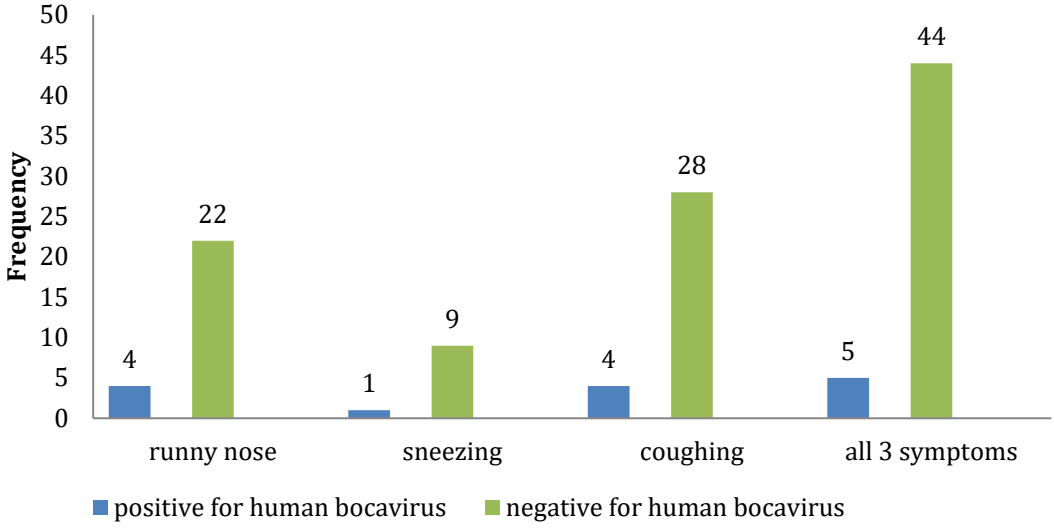

Fig. 5: Prevalence of human bocavirus in relation to respiratory symptoms

With respect to age of the enrolled children, the prevalence of HAdV7 and HBoV1 infections was notably higher among the 0-1 year age group in this study, consistent with previous research findings[23, 36, 37, 38, 40]. This underscores the significance of viruses as common causative agents of respiratory infections during infancy and early childhood, likely due to the immaturity of the immune system at this stage [37, 38, 41].
Concerning breastfeeding, a higher prevalence was observed among exclusively breastfed children compared to those who were not, although this difference was not statistically significant [42]. This suggests that breastfeeding may not confer local immunity against acquiring respiratory viruses, nor does exclusivity or duration of breastfeeding reduce the occurrence of respiratory tract infections. However, contrary findings have been reported, with some studies indicating that exclusive and prolonged breastfeeding offers protection against respiratory tract infections in the first year of life [42]. It is noteworthy that few studies have explored the effect of breastfeeding on respiratory tract infections after infancy, yielding varying results [43].
A substantial proportion (42.9%) of children who tested positive for HAdV7 and HBoV1 concurrently exhibited concomitant infection. This observation is consistent with findings from a study conducted by[45], where 62.9% of tested children demonstrated concurrent human adenovirus and human bocavirus infections. Respiratory tract infections (RTIs) are frequently associated with simultaneous infections by multiple respiratory viruses, posing a potential risk for complications[46]. Authors have documented that such co-infections elevate the risk of prolonged hospitalization and the development of severe illnesses compared to mono-infections [47]. The collective impact of these viruses can strain healthcare resources, resulting in increased hospital admissions, healthcare expenses, and absenteeism from educational or occupational settings.

Conclusion

The prevalence rates of adenovirus and human bocavirus in children with respiratory tract infections in Nigeria are consistent with global and African trends, highlighting the significant burden of these viral infections on paediatric respiratory health in the country. These findings underscore the necessity for ongoing surveillance and research initiatives to enhance comprehension and management of the epidemiology of respiratory viral infections among Nigerian children.

Limitation of Study

Positive controls for both viruses were not utilized during PCR analysis due to unsuccessful attempts to obtain them. However, an endogenous internal control was employed to ensure the reliability of the study's findings. Furthermore, the inability to sequence positive samples for HAdV7 and HBoV1 constitutes a notable limitation. Sequencing these samples could have yielded valuable insights into the genetic diversity and strain variations of these viruses circulating within the study population. Such information would have enriched our understanding of the epidemiology and potential clinical implications associated with these viral infections..

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