

Detection of Human Bocavirus Infection in Children with Lower Respiratory Tract Infections in Baghdad

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ABSTRACT

Background: Respiratory tract diseases are one of the leading causes of morbidity and mortality in young children, and a great variety of viruses are responsible of these infection.

Objectives: To determine the infection rate of human bocavirus in children with lower respiratory tract infection and related with different variable.

Patients and methods: Cross sectional study consists of 122 children under five years old their age ranged between 1 and 60 months and 75 males and 47 females; suffering from lower respiratory tract infection. They were attending respiratory wards in Fatima Al-Zahraa Hospital, Al-Elwiya Pediatrics Hospital, Ibn Al-Baladi Hospital and Pediatrics Protection Hospital in Baghdad, during the period from December 2017 to February 2018. Full information were collected from parents or relevant of each patient use specific formula sheet as well as nasopharyngeal samples were collected and used for DNA extraction and amplification with specific primers by PCR.

Results: Out of all 122 samples, eight samples were positive for HBoV (6.6%). Most infections were recorded in males 5(62.5%) patients aged 1-30 months 7(87.5%) but statistically non-significant. Significant differences ($p=0.02$) were noticed with nasal discharge 100% and wheezing 100% in HBoV-positive children, while non-significant with other parameter so higher proportion of HBoV-positive children had asthma (62.5%). Exactly 50% of HBoV-positive children were suffering from diarrhea. Nervous manifestation did no differ significantly between positive and negative groups (37.5% and 38.59% respectively). The results of phylogenetic analysis for HBoV DNA isolated from nasopharyngeal swabs revealed that all local isolates (8 isolates) are with HBoV type 1.

Conclusions: Infection rate of human bocavirus was compared with rates of infection from neighboring countries, no significant differences were notice between infection rate and different parameters except with nasal discharge and wheezing.

Keywords: Human Bocavirus, Children, Lower Respiratory Tract Infections.

الكشف عن فيروس بوكا البشري بين الأطفال المصابين بعدوى قناة الجهاز التنفسي السفلية

في بغداد

الملخص

خلفية الدراسة: تعد عدوى قناة الجهاز التنفسي المسبب الرئيسي للمرض والوفيات خصوصا بين اطفال. والمسببات المرضية عديدة ومتنوعة.

الهدف من الدراسة: لتحديد نسبة الإصابة بفيروس بوكا عند الاطفال الذين يعانون من عدوى الجهاز التنفسي السفلية وعلاقتها مع بعض المتغيرات.

المرضى وطرائق العمل: دراسة المقطعية شملت 122 طفلاً دون سن الخامسة، تراوحت أعمارهم بين شهر واحد لغاية 60 شهراً، (75 ذكر، 47 إناث) من الذين يعانون من إصابات الجهاز التنفسي السفلية والمراجعين لمستشفى فاطمة الزهراء، مستشفى العلوية للأطفال، مستشفى ابن البلدي و مستشفى حماية الاطفال في بغداد للفترة من كانون الأول عام 2017 ولغاية شباط عام 2018. معلومات كاملة جمعت من ذوي او مرافقي المريض باستخدام استمارة خاصة. إضافة إلى المسحات الانفية البلعومية التي استخدمت لاستخلاص الدنا وتضخيمه بواسطة بادئ خاص باستخدام تضاعف البلمرة المتسلسل.

النتائج: سجلت الدراسة الحالية ثمانية إصابات بفيروس بوكا من بين 122 عينة، وبنسبة إصابة بلغت 6,6% اذ كانت أكثر شيوعاً عند الذكور (62.5%) و الاطفال دون السنة 7(87.5%). لم تسجل نتائج التحليل الإحصائي علاقة ذات دلالة إحصائية بين العلامات السريرية المختلفة والإصابة بفيروس بوكا الا في حالة الصفير والسيلان الانفي 122(100%) لكل منهم، 62.5% كانوا يعانون من الربو و50% الاسهال كما لوحظ عدم وجود اختلافات ذات دلالة احصائية مع الاختلاجات العصبية ضمن الحالات الموجبة 37.5% والسالبة 38.5% على التوالي. اظهرت التحاليل الجينية ان جميع العزلات تعود الى فيروس بوكا البشري النوع الاول. **الاستنتاجات:** نسبة فيروس بوكا البشري كانت مقارنة للدول المجاورة. لم تسجل نتائج التحليل الاحصائي علاقة ذات دلالة إحصائية بين العلامات السريرية المختلفة والإصابة بفايروس بوكا الا في حالة الصفير والسيلان الانفي.

الكلمات الدالة: فيروس بوكا البشري، الأطفال المصابين ، قناة الجهاز التنفسي السفلية.

INTRODUCTION

Acute Respiratory Infection (ARI) are the major cause of morbidity and mortality worldwide, especially in young children. but is also a concern for elderly subjects and immune compromised patients (Zhang *et al.*, 2009). The major viral etiological agents of ARI in all age groups include influenza viruses, parainfluenza viruses, Respiratory Syncytial Virus (RSV), adenoviruses, human rhino virus (hRV), human Metapneumovirus (hMPV), human Bocavirus (HBoV), corona viruses and picornaviruses (Bezerra *et al.*, 2011).

Human bocavirus like other parvovirus genera, is a small (18-26 nanometers), non-enveloped, icosahedral virus with approximate 5.3 kb single-stranded incomplete DNA (Huang *et al.*, 2012). Human bocavirus 1 to 4 are structurally similar (Kailasan *et al.*, 2016). They differ in the major capsid protein VP2 by only 10 to 20%. The high similarity in virion capsid structures and capsid protein sequences among HBoV1 to 4 results in considerable cross-reactivity of IgG antibodies (Guo *et al.*, 2012).

Human bocavirus1 is transmitted most likely by the respiratory route; it causes respiratory illnesses (Jartti *et al.*, 2012). Its presence in feces suggests that this virus is transmitted by the fecal-oral route (Zhou *et al.*, 2014). Human bocavirus 1 is more frequently detected in clinical samples from patients with respiratory diseases, whereas HBoV-2, HBoV-3, and HBoV-4 are more frequently associated with gastrointestinal infections, although their exact role as entero-pathogens still remains unclear (Schildgen *et al.*, 2013).

Human bocavirus 1-4 pathogenicity has been examined in patients with respiratory disease, gastroenteritis, heart disorders, meningitis/encephalitis, fetal hydrops or death, and cancer as well as transplant recipients/ immunosuppressed subjects (Broccolo *et al.*, 2015). A few reports of HBoV1

outbreaks infection are available, such as in March 2014 outbreak of HBoV1 infection in young children in Toyama, Japan (Obuchi *et al.*, 2015). A recent study in Egypt suggest a high rate of nosocomial infection with HBoV-1 which was detected among 56.8% of children suffering from lower respiratory tract infections (Abdel-Moneim *et al.*, 2016).

Human bocavirus studies in neighboring countries revealed different percentage of infection ranging from 6-22, Turkey, 6.7% (Demirci *et al.*, 2016). Jordan 9.1% (Al-Rousan *et al.*, 2011). Iran, 10.7 % (Tabasi *et al.*, 2016) and Saudi Arabia, 22.5% (Abdel-Moneim *et al.*, 2013). In Iraq a recent study done by (Atyah *et al.*, 2017) revealed an infection rate (24.6%) using real time PCR. The aims of this study is to determine the infection rate of human bocavirus among children with lower respiratory tract infection and its relation with different soci-demographic and clinical parameters.

PATIENTS AND METHODS

A cross sectional study which consists of 122 children under five years old suffering from lower respiratory tract infection after an examination by a practitioner physician; their age ranged between 1 to 60 months (75 males and 47 females). They were attending respiratory wards in Fatima Al-Zahraa Hospital, Al-Elwiya Pediatrics Hospital, Ibn Al-Baladi Hospital and Pediatrics Protection Hospital in Baghdad, during the period from December 2017 to February 2018.

Nasopharyngeal samples were collected from each participant by specific swabs from Sigma Virocult Company in UK with viral transport medium and stored as frozen at -70 °C until use, nasopharyngeal swab specimens DNA was extracted by using specific kit (Instant virus DNA/RNA, Cat. No. 845-KS-4500250, German). Full information were collected from each participant by using specific formula sheet.

Two set of primers listed in (Table 1) were used in the present study to amplify the fragments of human bocavirus VP1/2 capsid gene (Smuts and Hardie, 2006). Kenmoe in Cameroon (2017) found them to be very efficient for detection of HBoV1-4 (Kenmoe *et al.*, 2017). Primers were dissolved in TBE buffer to prepare a stock solution of a final concentration of (100 pmol / μ l) and was kept at (-20). Working solution 10 pmol / μ l concentration was prepared by adding 10 μ l of the stock solution to 90 μ l of the distilled water.

Table 1: Sequence of primers utilized in the present study

First round PCR				
Primer	Sequence 5'-3'	TM °C	GC %	Product size
VP-A	5'- GCACTTCTGTATCAGATGC CTT-3'	57.5	45.5	904bp
VP-B	5 CGTGGTATGTAGGCGTGTA G-3'	56.9	55.0	
Second round PCR				
VP-C	5'- CTTAGAACTGGTGAGAGCA CTG-3'	57.5	50.0	850bp
VP-B	5'- CGTGGTATGTAGGCGTGTA G-3'	56.9	55.0	

35A semi-nested PCR was performed to amplify the VPI/2 gene. For the first round PCR, a (904bp) fragment was amplified by using forward primer (VP-A) and reverse primer (VP-B), PCR amplification mixture was performed in (50 μ l) final volume, (8 μ l) of template DNA, (2 μ l) of each primer and (38 μ l) distilled water was added to master mix according to the protocol then thermal cycling was done as shown in (Table 2).

A second round PCR was performed to improve the sensitivity, in which (2 μ l) of reverse primer (VP-B) and (2 μ l) of the other forward primer (VP-C) and (4 μ l) of primary PCR product were mixed with (42 μ l) distilled water to reach a (50 μ l) final volume according to the previous protocol (Chieochansin *et al.*, 2007).

Table 2: Thermal cycling condition for amplification of the DNA

Steps	Temperature °c	Time	No. of cycle
Initial denaturation	95	5 minute	1
Denaturation	95	30 second	35
Annealing	54	45 second	
Extension-1	72	1,5 minute	
Final Extension	72	7 minute	1

After successful amplification of the target regions of HBoV1-4 by using primers, (25 μ l) of PCR product along with primers, they were sent abroad to MacroGen Company in South Korea for direct sequencing.

RESULTS

Over 60% of the study population are males, the most predominate age group is 1-12 months (53.2%), over than 62.3% were living in urban area. All children with LRTI were suffering from wheezing. Of note, 37.7% of children had a history of asthma. Nasal discharge are present in 71.3% while 38.6% were suffering from diarrhea and over 38% had some kinds of nervous manifestations as shown in (Table 3).

Table 3: Demographic and clinical details of children with LRTI

Variable factors		Samples (%)
Gender type	Male	75 (61.4%)
	Female	47 (38.6%)
Age groups	(1-12) months	65 (53.2%)
	(13-24) months	38 (31.1%)
	(25-36) months	6 (4.9%)
	(37-48) months	8 (9.7%)
	(49-60) months	5 (6.1%)
Residence	Rural	46 (37.7%)
	Urban	76 (62.3%)
Wheezing	Yes	122 (100%)
	No	0 (0%) 0
History of asthma	Yes	46 (37.7%) 37.7
	No	76 (62.3%) 62.3
Nasal discharge	Yes	87 (71.3)
	No	35 (28.7)
Diarrhea	Yes	47 (38.6%) 38.6
	No	75 (61.4%) 61.4
Nervous manifestation	Yes	47 (38.6%) 38.6
	No	75 (61.4%)
Total		122 (100%)

According to the result of seminested-PCR, there are 8 (6.6%) samples that gave positive result (Fig. 1) for the first round PCR with a fragment length of 980bp as shown in Fig. (2).

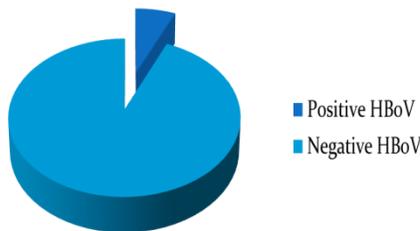


Fig. 1: Positive and negative result of HBoV according to seminested- PCR in children with LRTI

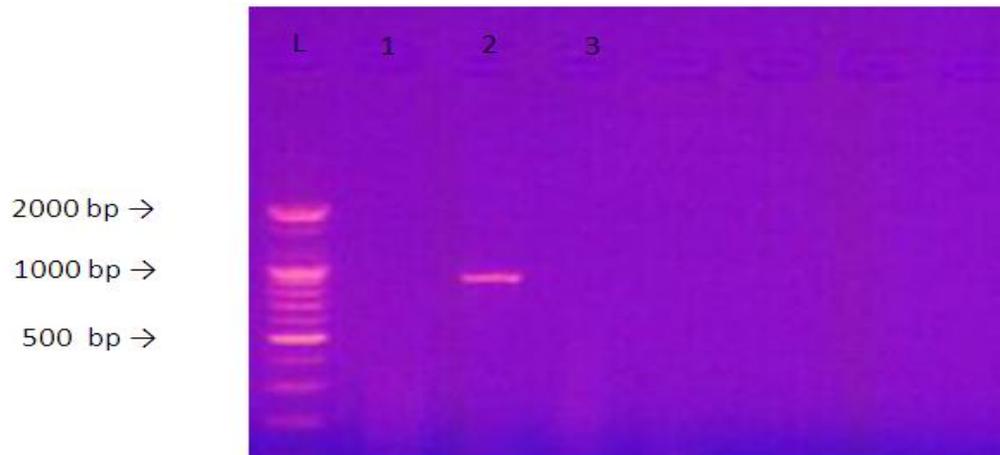


Fig. 2: Gel electrophoresis of the first round PCR with product size 980bp stained with ethidium bromide and illustrated under UV light

A second round of PCR was performed to confirm the correct amplification of the first round product. All samples positive for the first round were subjected to second round. The result is shown in Fig. (3). All these samples gave positive results with fragment length of 850 bp.

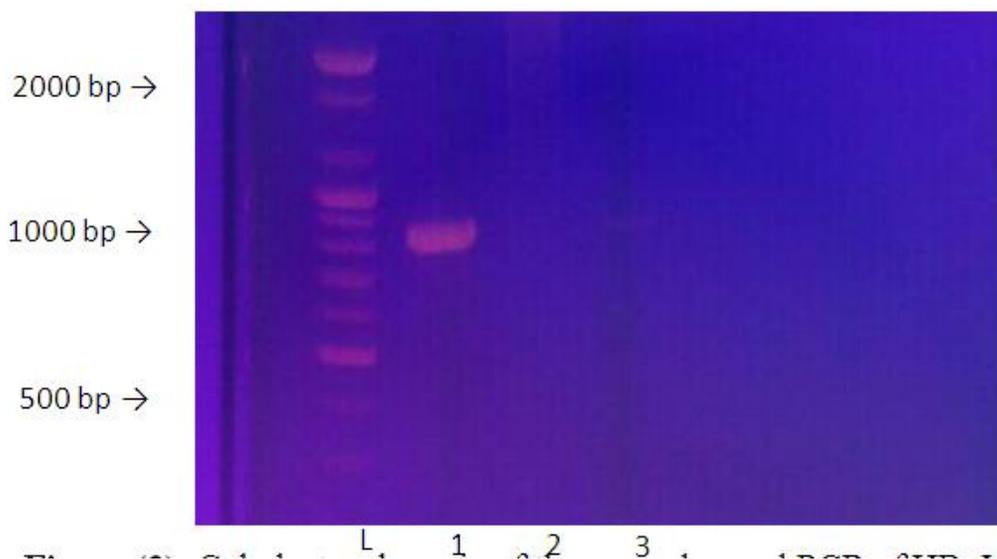


Fig. 3: Gel electrophoresis of the second round PCR of HBoV with product size 850bp stained with ethidium bromide and illustrated under UV light.

On studying the demographic factors between positive and negative groups in (Table 4), the present study had found that the gender groups did not differ significantly between positive and

negative groups, 62.55% of HBoV-positive males compare to 61.4% among HBoV- negative males. Similarly, 1-30 months almost similar proportion between positive and negative group at the same age group (87.5% and 84.2% respectively). Exactly 25% of HBoV-positive were rural residence compared to 38.59% HBoV- negative at the same residence, although the two-residence groups were not significant differences.

Table 4 : Human bocavirus infections rate among children according to different demographic data

Variable factors		Positive (8)	Negative (114)	Total	P-value
Gender type	Male	5(62.5%)	70(61.4%)	75	0.156
	Female	3 (37.5%)	43(29.82%)	47	
Age groups	(1-30) m	7(87.5%)	96 (84.21%)	103	0.805
	(31-60)m	1(12.5%)	18(15.78%)	19	
Residence	Rural	2(25%)	44(38.59%)	46	0.430
	Urban	6(75%)	70(61.40%)	76	

In regard to the clinical features (Table 5), higher proportion of HBoV-positive children had history of asthma (62.5%), compared to 36.84% of HBoV- negative children with the same symptom. According to HBoV-positive with nasal discharge were 100% versus 70% of HBoV- negative with the same symptom, with significant differences (p=0.02). Exactly 50% of HBoV-positive children were suffering from diarrhea compared to 37.71% of HBoV- negative children with diarrhea. Statistical analysis showed that there were no significant differences between the two groups. Nervous manifestation did not differ significantly between positive and negative groups (37.5% and 38.59% respectively). Of note, all children with LRTI were suffering from wheezing (100%). All strain belong to human bocavirus type 1 according to result of sequence.

Table 5: Distribution of HBoV patients according to clinical features in the present study

Variable factors		Positive	Negative	Total	P. Value
History of asthma	Yes	5(62.5%)	42(36.84%)	47	0.156
	No	3(37.5%)	72(63.15%)	75	
Nasal discharge	Yes	8(100%)	80(70.17%)	88	0.020
	No	0	34(29.82%)	34	
Diarrhea	Yes	4(50%)	43(37.71%)	47	0.496
	No	4(50%)	71(62.28%)	75	
Nervous manifestation	Yes	3(37.5%)	44(38.59%)	47	0.951
	No	5(62.5%)	70(61.40%)	75	
Wheezing		8(100%)	114(100%)	122	1.0

DISCUSSION

According to the results of nasopharyngeal swabs, the current study revealed 6.6% prevalence of HBoV infection in pediatric children with LRTI, this percentage is lower than the reported by Atyah *et al.*, (2017) who found HBoV infection is (24%) in children under 15 years old. And higher than reported by Zhao *et al.*, (2014) they detected human bocavirus in 0.80%, study of Al-Rousan *et al.*, (2011) showed that 1.5%, and with Schildgen *et al.*, (2008) they revealed that (3.1%) positive result during HBoV DNA. These variation in prevalence of HBoV in different studies can be attributed to several factors, first of all is the detection method, viral load, season of sampling, age, immune status and sample size.

Based on statistical analysis, there was no significant association of demographic or clinical characteristics with the HBoV infection except for nasal discharge. This may ascribed to the limited number of samples and very few positive results. These results are compatible with many other international studies (Schildgen *et al.*, 2008; Demirci *et al.*, 2016; Moradi *et al.*, 2017). Unlike current study, many studies revealed significant association of the different demographic and clinical characteristic with HBoV infection. According to gender, it has been found that the infection in males are more than females seems to be similar with those participated in other studies such as (Symekher *et al.*, 2013; Tran *et al.*, 2014). While current study was inconsistent with study conducted by Bubshait *et al.*, (2015) which revealed the gender females (60%) was higher than males among Saudi Arabia patients. Concerning the age groups, many studies reported a significant association. In an Iraq study, the percentage of age groups (1-24 month) were 70% with significant differences between positive and negative age groups. In a Korean study involved 1528 children with acute RTI, there were 178 patient positive for HBoV. The mean age of the patients were 24 months (Ahn *et al.*, 2014).

Data obtained from this study indicate that most patients from urban area, and exactly 25% of positive HBoV live in rural area, which agreed with similar study that has reported increase infections in Iranian urban area (Tabasi *et al.*, 2016). In contrast, this result disagreed with study done by Mitui *et al.*, (2012), who stated that half positive HBoV were from urban area. The reason of high prevalence in the urban residence could be related with crowded and bad hygiene in the big city. Wheezing is another clinical manifestation, which was found to be significantly associated with HBoV in some studies. In a Chinese study conducted in children with severe LRTI, wheezing was present in 90% of patients with significant differences between positive and negative groups (Deng *et al.*, 2012). In the Korean study, 40% of positive patients had wheezing with significant differences between positive and negative groups. More than one study are compatible with the current study, on the other hand a study conducted on hospitalized Iranian adults with respiratory tract infections showed 100% wheezing which is compatible with the current study. However, even patient negative for HBoV had wheezing (Mortazavi *et al.*, 2015). In the other hand, this study revealed a high number of patients with history of asthma in 5 cases (62.5%) agreed with a study done by Del Rosal *et al.*, (2016), who revealed about 50% of HBoV-positive patients had history of asthma in Spain. Another study in Saudi Arabia was showed no difference between positive and negative group regarding a history of asthma (Abdel-Moneim *et al.*, 2013). In contrast, a study in china revealed 21% of HBoV-positive was with history of asthma (Xu *et al.*, 2012). Elevated percent age of HBoV-positive with asthma may contribute to destroy the respiratory tract by asthma and make a good receptor to the virus. Regarding the nasal discharge, all children with HBoV infection had nasal discharge, this result agrees with a study conducted on outpatient children from china suffering from nasal discharge with HBoV-positive (Xu *et al.*, 2012). In contrast, nasal discharge in a study conducted in Chinese children suffering from severe respiratory infection with rhinorrhea was 19% (Deng *et al.*, 2012). In another study conducted in infants with lower respiratory disease in Argentina, HBoV was 12% (Ghietto *et al.*, 2012).

Interestingly, in these studies, there was no significant association of nasal discharge with HBoV infection.

This study has shown that 4 cases (50%) of HBoV-positive children were suffering from diarrhea which is comparable with other studies such as Lasure and Gopalkrishna, 2017 and Amr *et al.*, 2017; they found that 47% and 38% respectively. Otherwise, about 15% of Saudi children had diarrhea with HBoV-positive in RTI (Abdel-Moneim *et al.*, 2013). The main reason for different results regarding demographic and clinical characteristic in the current, local and international studies may be due to sample size.

According to phylogenetic analysis of HBoV, to the best of our knowledge, the current study is the first study in Iraq to determine type of human bocavirus, so the results of phylogenetic analysis for HBoV DNA isolated from NPS revealed 8 isolates. After the alignment of these local isolates sequence with 18 references isolates by software mega 6 software, all these isolates were found to be related to HBoV type 1. Many studies in neighboring countries showed similar results with the current study. In 80 children with RTI from Saudi Arabia reported that HBoV infections was only type 1 (Abdel-Moneim *et al.*, 2013). Another study in Iran involved 140 children with acute RTI less than two years old during fall and winter, had the same result (Tabasi *et al.*, 2016).

In conclusions, infection rate of human bocavirus was comparable with infection rate in neighboring countries, no significant differences were notice between infection rate and different parameters except with nasal discharge. Further studies with large sample size must be done to clarify this issue.

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