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Ocurrence of Human Bocavirus associated with acute respiratory infections in children up to 2 years old in the City of Belém, Pará State, Brazil

Ocorrência de Bocavírus Humano associado às infecções respiratórias agudas em crianças de 0 a 2 anos de idade na Cidade de Belém, Pará, Brasil

La aparición de Bocavirus Humano asociado con las infecciones respiratorias agudas en niños de 0 a 2 años de edad en Belém (Estado de Pará, Brasil)

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ABSTRACT

INTRODUCTION: Acute Respiratory Infections (ARI) are one of the main public health problems in the world. Most of these infections are associated with several pathogens, and viruses are the most prevalent agents. Recently, a new parvovirus named Human Bocavirus (HBoV) has been described. Investigations on the relationship between this new agent and cases of ARI in individuals are still scarce. Herein, we review a study of HBoV in a population segment in the Amazon. MATERIALS AND METHODS: In this study, samples of nasopharyngeal aspirates from patients with ARI treated in Health Care Units in Belém, Brazil, were analyzed. Identification of the virus was carried out by polymerase chain reaction using pairs of specific oligonucleotides, followed by phylogenetic analysis of the nucleotide sequences obtained. RESULTS: Of the 397 samples studied, three specimens were HBoV-positive, and one presented as a co-infection with the respiratory syncytial virus. DISCUSSION: The positivity rate obtained in this investigation was lower than that described in other studies; however, previous studies involved hospitalized patients, which constitute a different population group. The phylogenetic analyses revealed a significant similarity between the virus strains found and those previously described. CONCLUSION: This is the first report associating HBoV with ARI in the Amazon.

Keywords: Respiratory Tract Infections; Bocavirus; Parvoviridae Infections.

INTRODUCTION

Due to their high mortality, Acute Respiratory Infections (ARI) are still one of the main public health problems worldwide, especially in developing countries[7,28]. These infections are generally associated with various pathogens with viruses being the most prevalent[6,13]. Among these viruses are Influenza A and B (Flu A and Flu B), Parainfluenza 1, 2 and 3 (HPIV), Adenovirus (AdV), Respiratory Syncytial Virus (RSV), Human Rhinovirus (HRV), Coronavirus (HCoV) and Human Metapneumovirus (hMPV)[3,18].

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Several studies are currently being conducted in order to determine the etiology of respiratory tract infections. In 12% to 39% of cases, no known agent is found. Under this premise, Allander et al[2] have described the development of a novel methodology based on molecular biology techniques in order to investigate new agents possibly related to respiratory tract infections. Using this methodology, these authors detected a parvovirus, previously called Human Bocavirus (HBoV), which is taxonomically classified within the genus *Bocavirus*, subfamily *Parvovirinae*, family *Parvoviridae* [2,14].

HBoV is a non-enveloped virus with icosahedral symmetry, a diameter of 18 to 26 nanometers and a capsid formed by approximately 60 capsomeres. The viral genome is non-segmented, composed of a single-stranded deoxyribonucleic acid (DNA) of both positive and negative sense and is approximately 5.3 Kb. The virus has three open reading frames (ORFs) that encodes four proteins: VP1 and VP2, virion structural proteins; NS1, non-structural protein;

and the nucleoprotein NP1, which has an unknown function.

Studies have shown that HBoV apparently has a worldwide distribution[5,9,11,16,23,25,27], with an incidence ranging from 1.5%[5] to 19%[1]. Few studies have been conducted in Latin America to determine the association of HBoV with other respiratory infections. The high percentage of cases in which the virus is found in the presence of another infection (5 – 85%), either with another virus or other pathogens[12,25,27], is noteworthy.

The transmission of HBoV is undefined because it has been found several times in the respiratory tract[5,9,23,27], serum[15], blood[1,17], and urine[24], as well as in cases of gastroenteritis[4,24]. Nevertheless, studies have shown that in patients younger than 2 years of age[1,24] hospitalized for a respiratory tract infection, HBoV is the fourth most frequently detected agent, suggesting that the respiratory tract is the main route of transmission.

The symptoms observed in cases of HBoV infection include those of a common cold with fever and rhinorrhea as well as wheezing and dyspnea. In the event of complications, the occurrence of bronchitis, bronchiolitis and pneumonia have been noted[11,20,23]. Studies also suggest that HBoV can establish latent or persistent infections in mucosal lymphocytes or contribute to tonsillar hyperplasia[22].

The seasonal profile of HBoV infection cases has yet to be well established. However, in countries with a temperate climate, the occurrence of this virus is more pronounced in the winter and early spring[11,27].

To date, studies have demonstrated the existence of three types of HBoV (HBoV1, HBoV2 and HBoV3). Type 3 has only been found in feces4, and type 2 has been found in both blood and feces[4,17]. HBoV1 is the only type associated with ARI.

As for the genetic variability, Allander et al[2] described two very conserved lineages (ST1 and ST2). This seems to be a very stable virus, and few mutations are found in the VP1 and VP2 genes sequences. Divergences in nucleotide sequences are caused by point mutations, which result in a few changes in the amino-acid chain[16,25].

In countries with a tropical climate, such as Brazil, little research has been conducted on the occurrence and genetic variability of HBoV. Thus, there is a clear need to conduct studies that will generate epidemiological data on this virus in order to better define the role of HBoV in cases of ARI in Amazônia.

MATERIALS AND METHODS

STUDY POPULATION

Between 2004 and 2007, combined swab samples (nasal/throat) and nasopharyngeal aspirates were collected in an outpatient setting by the *Sistema de Vigilância Virológica da Rede Influenza* (Virological Surveillance System of the Influenza Network), the *Laboratório de Vírus Respiratórios* - LVR (Respiratory Virus Laboratory) of the Instituto Evandro Chagas - IEC. A screening procedure allowed selection of children younger than 2 years of age who presented with signs and symptoms of ARI lasting no longer than 5 days.

SAMPLE PROCESSING

The collected samples were centrifuged at 1000 rpm for 10 min. The pellets from the centrifuged samples were used to prepare slides according to the protocol in the commercial kit *Light Diagnostics™ Respiratory Panel I Viral Screening and Identification* IFA for Indirect Immunofluorescence (IDIF) that was used to identify the viruses Influenza A and B, Parainfluenza 1-3, Adenovirus and RSV. The supernatant was used in an attempt to isolate HBoV using molecular biology techniques.

IDENTIFICATION USING MOLECULAR BIOLOGY

For the extraction, we standardized a protocol in which we initially added 150 L of TNE (Tris-Na-EDTA) buffer, 20 L of 10% sodium dodecyl sulfate (SDS) and 10 L of proteinase K (10 mg/mL) to 120 L of the sample in a microtube. This mixture was incubated at 56° C for 30 min in a thermal block (Eppendorf). We then added 200 L of saturated phenol, vortexed the sample for 1 min and centrifuged it at 20° C for 3 min at 10,000 rpm. We transferred 150 L of the supernatant to a microtube containing 150 L of phenol, chloroform and isoamyl alcohol. This mixture was then vortexed for 1 min and centrifuged at 20° C for 3 min at 10,000 rpm. A total of 100 L of the resulting supernatant was transferred to a microtube containing 100 L of chloroform, vortexed for 1 min and centrifuged at 20° C for 3 min at 10,000 rpm. Finally, we carefully removed 40 L of the supernatant and stored it at -20° C.

A polymerase chain reaction (PCR) was conducted using the following pairs of specific oligonucleotides: BoV 118F and BoV 542R (NP1), VP/+/1 and VP/-/726 (VP1) and VP/+/1005 and VP/-/2072 (VP2). The primers and the amplicon sizes can be found in table 1.

Table 1 – Primer descriptions

Oligonucleotide	Sequence	Amplicon size
BoV 118F	5'GAGCTCTGTAAGTACTATTAC3'	354pb
BoV 542R	5'CTCTGTGTTGACTGAATACAG 3'	
VP/+/1	5'GCTGCTGAAAGCATGGAAGCA3'	725pb
VP/-/726	5'GGCGCTGCCAATCCTGTGGT3'	
VP/+/1005	5'GCTGGAGGCAATGCTACAGAA3'	1067pb
VP/-/2072	5'TCCGCTTGTCCATTGAGGAGG3'	

For all the genes, we used a reaction with a final volume of 50 L containing: 5 L of extracted DNA, 0.5 L of each primer (50 pmol/ L), 5 L of reaction buffer (10x), 2 L of dNTPs (5 mM), 5 L of MgCl2 (25 mM), 1.25 U of Taq DNA polymerase and 31.5 L of DNAse/RNAse-free

water. The adopted program was specified by Allander et al[2].

PCR samples positive for HBoV were tested for the VP1/VP2 gene, prepared for partial sequencing with the Kit Big Dye® terminator Cycle Sequencing (Applied Biosystem) according to the manufacturer's instructions and sequenced with the ABIPrism 3130xl automated sequencer (Applied Biosystem).

All the reactions were performed in a Mastercycler ep Gradient S (Eppendorf) automated thermocycler. Negative and positive controls were always performed to avoid contamination by exogenous DNA.

SEQUENCE ANALYSIS AND EDITING AND PHYLOGENETIC TREE CONSTRUCTION

The nucleotide sequences obtained from HBoV were analyzed and edited using the program BioEdit v. 7.0 and compared with sequences of other viral isolates available in the GenBank database (http://www.ncbi.nlm.nih.gov) using the programs Clustal W v. 1.7[26] and Mega v.3.1[21]. The neighbor joining (NJ) method was used to construct phylogenetic trees on the program Mega v 3.1. The distance matrix in the NJ method was calculated based on the aligned sequences by using Kimura's two-parameter formula[19]. We used a Bootstrap analysis with two thousand replicates to enhance reliability of cluster analysis[10].

RESULTS

We analyzed 397 samples, 3 (0.76%) of which were positive for HBoV infection, as confirmed by sequencing.

For all the positive cases, the infection occurred during the dry season and affected male children (on average, 29 weeks old) who presented rhinitis and cough. In one case, a HBoV/RSV coinfection was found. This child also presented fever and nasal congestion (Table 2).

Table 2 – Data for patients with HBoV infection

Patient	Date of sample collection	Age	Gender	Symptoms	Coinfection
099-04	12/8/2004	9 months	Male	Rhinitis, cough	No
934-07	14/8/2007	6 months	Male	Rhinitis, cough ,fever and obstruction	VRS
619-07	25/10/2007	8 months	Male	Rhinitis, cough	No

For the phylogenetic analysis, the NP1 segment was 100% similar to the strains described by Allander et al[2]. For the sequence analysis of the VP1/ VP2 gene, one sample was sequenced and was 98.3% to 99.7% similar to strains available in GenBank (Figure 1).

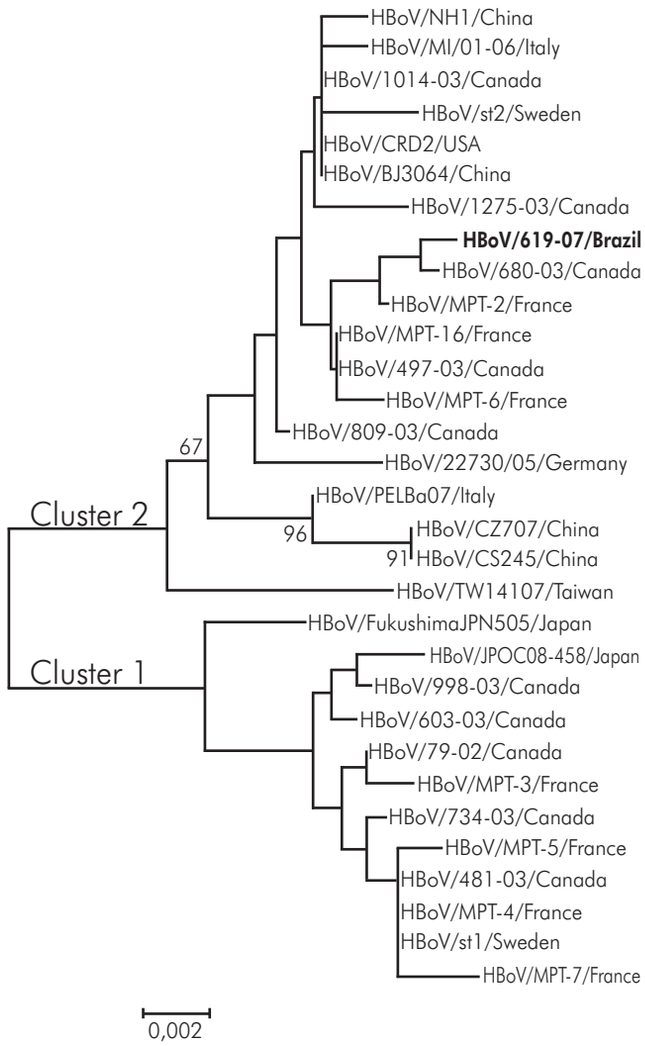

Figure 1 – Phylogenetic analysis of the partial sequence of the HBoV gene encoding the surface proteins VP1/VP2. The tree was generated using the NJ method with the program Mega v. 3.1. *Bootstrap* values (X 2000) were given to the selected nodes.

DISCUSSÃO

In the City of Belém, Pará, from January 2004 to December 2007, three (0.76%) cases of Human Bocavirus-associated acute respiratory infection were diagnosed from a total of 397 samples collected locally. Two positive samples were detected in 2004 and another in 2007.

These data reveal a low percentage of HBoV-positive cases when compared to the data available in the literature, which report detection in up to 19% of cases[1]. However, we should emphasize that the samples used in this study originated from patients in an outpatient setting, whereas the studies with higher rates of association included samples from hospitalized patients[9,27].

The low positivity rates did not lead to conclusions relative to the epidemiological patterns of prevalence and seasonality caused by the virus in the study population.

However, we emphasize the occurrence of the virus in the period of lowest seasonal rainfall.

One of the samples presented coinfection with RSV. In fact, a high incidence of association between HBoV and other viruses, frequently in association with RSV, is often described in the literature[2,8].

When comparing our sequences with those from GenBank, we observed 100% similarity for NP1 and 98.6% to 99.3% similarity for the partial sequences of VP1/VP2. These data demonstrate the high level of conservation for NP1 and reinforces the low genomic mutability of VP1/VP2[16,25].

CONCLUSIONS

This study shows a low index (0.76%) of HBoV in 0 to 2 years of age children with a coinfection with other respiratory viruses receiving ambulatory care in the capital of Pará.

The strains circulating in the region of the study had few mutations. When analyzed using their non-structural protein, an overall similarity was found relative to the strain lineage from a study conducted in Sweden, designated ST1.

In this study, we emphasize that this is the first incidence of HBoV-associated respiratory infections detected in the Legal Amazon.

Ocorrência de Bocavírus Humano associado às infecções respiratórias agudas em crianças de 0 a 2 anos de idade na Cidade de Belém, Pará, Brasil

RESUMO

INTRODUÇÃO: As Infecções Respiratórias Agudas (IRA) permanecem como um dos principais problemas de saúde pública em todo o mundo. Essas infecções são associadas a diversos patógenos sendo os vírus os prevalentes. Recentemente, foi descrito na literatura um novo parvovírus denominado Bocavírus Humano (HBoV). Investigações ainda são escassas na associação deste novo agente a casos de IRA na população em geral. Neste contexto, o presente artigo relata a pesquisa do HBoV em um segmento populacional da Amazônia. MATERIAIS E MÉTODOS: Neste estudo, foram analisadas amostras de aspirado nasofaríngeo de pacientes com diagnóstico de IRA atendidos ambulatorialmente na Cidade de Belém, Pará, Brasil. A pesquisa, com a identificação laboratorial do vírus, foi realizada mediante o emprego da técnica de reação em cadeia mediada pela polimerase, utilizando pares de oligonucleotídeos específicos, seguida da análise filogenética das sequências nucleotídicas encontradas. RESULTADOS: Das 397 amostras clínicas analisadas, encontrou-se positividade em amostras de três pacientes, sendo um destes em coinfecção com o vírus respiratório sincicial. DISCUSSÃO: O percentual de positividade obtido na investigação se revelou inferior ao descrito na literatura. Entretanto, vale ressaltar que os estudos já publicados envolveram pacientes hospitalizados, diferentemente do grupo populacional presentemente abordado. As análises filogenéticas realizadas evidenciaram expressiva similaridade dos vírus encontrados com as cepas virais já descritas. CONCLUSÃO: A presente pesquisa se caracteriza como o primeiro relato associando o HBoV à IRA na Região Amazônica.

Palavras-chave: Infecções Respiratórias; Bocavírus ; Infecções por Parvoviridae.

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RESUMEN

INTRODUCCIÓN: Las infecciones respiratorias agudas (IRA) siguen siendo uno de los principales problemas de salud pública en todo el mundo. La gran mayoría de estas infecciones están asociadas con diversos patógenos, entre los que los virus son prevalentes. Recientemente, un nuevo parvovirus llamado Bocavirus Humano (BovH) fue descrito. Las investigaciones son todavía escasas sobre la asociación de este nuevo agente con casos de IRA en la población en general. En este contexto, este artículo relata la investigación de BovH en un segmento de la población de la Amazonía. MATERIALES Y MÉTODOS: Este estudio analizó las muestras de aspirados nasofaríngeos de pacientes con diagnostico de IRA atendidos ambulatoriamente en Belém (Pará, Brasil). La investigación e identificación de laboratorio del virus se realizó empleando la técnica de reacción en cadena de polimerasa, utilizando pares de oligonucleotideos específicos, seguida de un análisis filogenético de las secuencias de nucleótidos encontradas. RESULTADOS: De las 397 muestras clínicas analizadas, dieron positivo las muestras de tres pacientes, de las que una era una coinfección con el virus respiratorio sincicial. DISCUSIÓN: El porcentaje de resultados positivos obtenidos con la investigación demostró ser inferior a lo descrito por la literatura. Sin embargo, cabe señalar que los estudios anteriores se hicieron con pacientes hospitalizados, a diferencia del grupo de población aquí estudiado. El análisis filogenético reveló una considerable similitud de los virus encontrados con las cepas de virus ya descritos. CONCLUSIÓN: Esta investigación, se caracteriza por ser el primer informe que asocia el BovH con el IRA en la Región Amazónica.

Palabras claves: Infecciones del Sistema Respiratorio; Bocavirus; Infecciones por Parvoviridae.

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