Human Bocavirus Infection Among Children with Respiratory Tract Infection in Ibadan, Nigeria

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Research article

Keywords: Human Bocavirus 1, Childhood Respiratory Tract Infection, Ibadan, Nigeria

Posted Date: June 25th, 2019

DOI: https://doi.org/10.21203/rs.2.10676/v1

Abstract

Background: Human Bocavirus (HBoV) which is a single stranded DNA virus of the family Parvoviridae, is responsible for 21.5% of childhood respiratory tract infections (RTI) annually. Among the four genotypes currently known, HBoV1 has been associated with acute respiratory tract infection. Although, there have been studies on HBoV in some countries, there is limited information on this virus in sub-Saharan Africa where there is the highest burden of RTI. This study aimed to characterize the circulating strains of HBoV in Ibadan, Nigeria. Methods: Nasopharyngeal and oropharyngeal swab samples were collected from 333 children ≤5 years old presenting with RTI attending hospitals in Ibadan, whose parents assented from 2014 – 2015. HBoV isolates were sequenced after a nested PCR and phylogenetic analysis was carried out using Mega 6 software. The HBoV isolates of this study has been assigned the Genbank accession numbers KY701984 – KY702006. Results: A total of 27 children tested positive for HBoV by PCR and 23 of the 27 isolates were successfully sequenced. The 23 HBoV isolates from this study were type as HBoV-1 genotype by phylogenetic analysis. Phylogram analysis indicated that the isolates belong to the same clades. Six Isolates aligned closely to the reference strains ST1 and ST2, while 17 isolates showed a high divergence to the reference isolates. Conclusion: This study shows the contribution on HBoV in respiratory tract infections in Nigeria and that HBoV1 strain is the strain associated with the infection.

Background

Respiratory tract infections (RTIs) are responsible for about 25% of annual childhood deaths worldwide (1). *Human Bocavirus* (HBoV) has been linked with 21.5% childhood RTIs annually (2). HBoV is a relatively new DNA (Deoxyribonucleic acid) virus which belongs to the family *Parvoviridae* (3). The virions are small, icosahedral and non-enveloped, with a negative sense, linear, single-stranded genome of approximately 5.3 kilobases (4). The HBoV genome codes for two nonstructural proteins (NS1 and NP1) and two structural proteins (VP1 and VP2). The virus has been classified into four strains; HBoV1, 2, 3, and 4, Based on the nucleotide divergence of VP1 capsid region (4).

HBoV1 infection in children is often identified by wheeze, whereas, HBoV2 – 4 have been found to be involved in childhood diarrhea cases and acute flaccid paralysis (5). HBoV1 has been associated with childhood RTIs in both developed and developing countries, causing some severe cases of bronchopneumonia and bronchiolitis (6). Studies have shown that the virus is prevalent in children age five and below, with the four genotypes often causing different clinical symptoms (7). Also, since the discovery of the virus from nasopharyngeal aspirates in 2005 by Allander and colleagues, it been observed that HBoV1 is the fourth most common virus in respiratory samples (8).

Although, there has been studies carried out in some parts of Asia, Australia, Europe and America, there is still a dearth of information on the circulating strains of the virus in Sub-Saharan Africa, especially Nigeria. Therefore, this study aimed to characterize the circulating strains of HBoV among children ≤ 5 years old in Ibadan, presenting with symptoms of respiratory tract infections.

Methods

Ethical approval was obtained from the UI – UCH Ethical Review Committee and permission was obtained from some selected hospital boards before the commencement of this study. A total of 333 children ≤ 5 years old, showing signs of acute respiratory tract infection, whose parents had given consent, were recruited for this study. Sterile swab sticks were used to collect Oropharyngeal (OP) and nasopharyngeal (NP) samples from each child, and transported immediately to the laboratory in sterile vials containing 2mL of viral transport medium (VTM). DNA was extracted from the VTM using commercial kit by Jena Bioscience (Germany) according to the manufacturer's instruction. HBoV was detected from the DNA in a nested PCR procedure (9) using primers (Table 1) targeting the VP1/2 region of the virus genome and Red Load Taq Master kit (Jena bioscience, Germany) in a 25µl reaction (9).

For the first round PCR assay, the cycling conditions were 95^0C for 2 min, 10 cycles at 95^0C for 35 sec, 58^0C for 60 sec and 72^0C for 60 sec with a decrease of 0.5^0C in annealing temperature after each cycle and 30 cycles at 95^0C for 30 sec, 54^0C for 45 sec, and 72^0C for 45 sec, and a final extension of 72^0C for 10 min. For the second round PCR assay, cycling conditions similar (but with modifications) to that of the first round assay were used. Particularly, instead of the 58^0C annealing temperature used for the first round assay, 60^0C was used (9). All samples that are positive for HBoV (showing the expected 575 base pairs) at the end of the PCR assay were purified and sequenced.

Sequencing was done at Inqaba Biotec using primers for the second round PCR assay. Sequence similar to the HBoV sequences in the GenBank was found using BLAST (Basic Local Alignment Search Tool) program in the National Center for Biotechnology Information (NCBI) database. Reference HBoV sequences were downloaded from GenBank and a multiple sequence alignment (MSA) was done using the Clustal W programme in MEGA 6 software (10). Maximum Likelihood trees were generated after correcting for multiple substitutions, complete removal of positions that contained gaps and estimating reliability by 1000 bootstraps. MSA and phylogenetic analysis of the sample sequences with previous sequences from Nigeria and around the world was also done. The sequences have been deposited in GenBank with the following accession numbers KY701984-KY702006.

Data entry, cleaning, and data analysis were performed using SPSS statistical software, and descriptive statistics were presented as tables.

Results

A total of 333 children (Male = 178; Female = 155) aged five years and below presenting with cough, wheeze, breathlessness, fever, nasal congestion, catarrh, vomiting, tonsillitis, otitis media, rash, diarrhea, lack of appetite, bronchiolitis, and bronchopneumonia were recruited from September 2014 to August 2015 from hospitals and health centers in Ibadan, Oyo State Nigeria.

Twenty-seven (8.1%) of the 333 children tested were positive for HBoV infection, 16 (59.3%) of which were female. The months of February and December had the highest number of HBoV isolate (Table 2). Age group 1 – 2 years had the highest prevalence. Twenty-three out of the 27 HBoV positive isolates were sequenced and characterized (Table 3 - 5). All the isolates were HBoV1 genotype. Also, the maximum likelihood tree shows that 17 (out of 23) isolates were highly diverged from the others although all the 23 isolates are HBoV1 (Figure 1 & 2).

Phylogenetic tree constructed with the sequenced isolates and WHO reference strains using MEGA6 (10), showed that the 23 isolates of this study clustered around HBoV1 WHO reference strains, although 17 of the isolates showed a higher divergence (Figure 1 & 2). The divergence of the sequences of the isolates and the reference strains sequences revealed an overall mean distance of 0.0 within the isolates and 0.1 (Table 6 & 7) when compared with WHO reference strains sequences.

Discussion

In this study HBoV was detected in 8.1% of the 333 children recruited. The detection rate observed in this study is higher than 6.8% reported by Moreno *et al*. in Argentina, 7.2% reported by Tran *et al.* in Vietnam and much higher than 1.2% reported by Niang *et al*. in Senegal but lower than 16.8% reported by Symekher *et al*. among Kenyan children (7,11–13). This difference could be as a result of sampling strategy, severity of illness in the children recruited and different climates. The months of January (41%) and December (30%) recorded the highest number of HBoV infections among the children recruited in the study (Table 2), and this is similar to the finds of Goktas *et al.* (14) which reveals that HBoV infection activity has a peak period in the months of December to January, which are cold seasonal. This seasonal character could be a result of the cold temperature observed in these months of the year as supported by the findings of Erling *et al.* (15). Although not significant, females (59.3%) were found to have a higher prevalence of HBoV in this study (Table 3). This is similar to what was previously reported in India by P. Bharaj, *et al.* and in Saudi-Arabia by Abdel-Moneim, *et al*., but different from reports by Symekher *et al*. among Kenyan children and Salmon-Mulanovich *et al*. among children in South America (13,16–18). Children of age group >1 – 2 years had the highest detection rate and showed symptoms of highest severity (Table 3). This is similar to the findings of (9). Among the symptoms observed in children with HBoV in this study, cough (100%) and catarrh (100%) had the highest frequency, whereas vomiting (11%) tonsillitis (3.7%), and diarrhea (3.7%) were the least observed symptoms (Table 4). The symptoms observed in children positive for HBoV infection in this study, are similar to the reports of Hengst *et al*., Korner *et al*., Akca *et al*. and Moesker *et al*. (2,6,19,20). This suggests that laboratory screening for HBoV should be included in the management of in the children ≤ 5 years old presenting with cough, catarrh and other respiratory tract symptoms in Nigeria.

Of the 27 HBoV isolate detected in this study, 23 were successfully sequenced. The phylogram generated from the nucleotide sequence of the 23 HBoV isolates in this study and that of HBoV reference strains

showed all the isolates clustering with the reference strain of HBoV1 (Figure 1 & 2). This is similar to the findings of Nokso-Koivisto *et al*., Abdel-Moneim *et al*. and Principi *et al*., where they reported HBoV-1 as the genotypes mostly found to be associated with respiratory distress in children (17,21,22).

The phylogenetic analysis revealed that six of the 23 isolates in this study have a very high level of similarity with ST1 and ST2 which are the WHO references strains for HBoV-1 genotype (3) while the remaining 17 isolates showed lower similarity to the reference strains. The six isolates that clustered closely to the references strains for HBoV-1 (Figure 1 & 2) were gotten from children that exhibited symptoms of high severity (Table 4 & 5). This is similar to the findings of Kenmoe *et al.* and Pogka *et al*., whose study indicated that HBoV-1 isolates that aligns with the HBoV-1 reference strains are often gotten from children showing symptoms of severe acute respiratory tract infections (23,24). This could be because HBoV -1 reference strains were isolated from children with severe acute respiratory tract illness by Allander *et al*. (3). The remaining 17 isolates that showed a higher divergence to the reference strains where obtained from children who exhibited less severe symptoms of respiratory tract illness (Table 4).

There was low level of diversity in the nucleotide and amino acid differences within the HBoV-1 isolates of this study (that is; an overall mean distance of 0.0 within the isolates and 0.01 when aligned with the WHO reference strain sequences) (Table 6 & 7). This shows the high level of genetic homogeneity exhibited by the HBoV1 isolates of this study, and this supports the findings of Allander *et al*., Arthur *et al*., Cheng *et al*. and Ghietto *et al*. (3,4,25,26).

When comparing the isolates obtained in this study to other isolates in the Gen Bank it was observed that majority of the isolates were closely related to those from Pakistan. This finding supports those of Kantola *et al*. and Salmón-Mulanovich *et al*., which suggests that there is frequent importation of foreign strains of HBoV-1 genotype, and thereby indicates tourism might plays an important role in the transmission of the virus (18,27).

Finally, the 8.1% prevalence of HBoV1 that was found in this study showed that this virus strain is involved in childhood respiratory tract illnesses in Ibadan, and is therefore of public health importance in Nigeria.

Conclusions

Respiratory tract infections are implicated in high number of childhood deaths globally, and HBoV1 has been shown to be involved in the respiratory illness of children ≤ 5 years old. The finding of this study reveals that *Human Bocavirus* type 1 (HBoV1) is endemic in Ibadan and might be actively circulating among children in Nigeria. Therefore, this study shows the need for continuous surveillance of HBoV and laboratory investigation of the role it plays in disease process.

Declarations

Ethics approval and consent to participate: UI – UCH Ethical Review Committee and permission was obtained from some selected hospital boards before the commencement of this study.

Consent for publication: Not applicable

Availability of data and material: The sequence information generated and analyzed during the current study are available in the GenBank and have been assigned accession numbers KY701984-KY702006.

Competing interests: The authors declare that they have no competing interests

Funding: This study was self-funded

Authors' contributions: JOO collected samples, carried out molecular study, analyzed sequence data and wrote manuscript. FAO assisted in molecular study, sequence data analysis and manuscript writing. AJA supervised molecular study, sequence data analysis and manuscript writing. All authors read and approved the final manuscript.

Acknowledgements: Not applicable

References

1. Akinloye OM, Esa R, Savolainen-kopra C, Ziegler T, Iwalokun BA, Deji-Agboola MA, et al. Specific Viruses Detected in Nigerian Children in Association with Acute Respiratory Disease. J Trop Med. 2011;2011:Article ID: 690286.
2. Moesker FM, van Kampen JJA, van der Eijk AA, van Rossum AMC, de Hoog M, Schutten M, et al. Human bocavirus infection as a cause of severe acute respiratory tract infection in children. Clin Microbiol Infect [Internet]. 2015;21(10):964.e1-964.e8. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1198743X1500659X
3. Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci U S A [Internet]. 2005;102(36):12891–6. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1200281%7B&%7Dtool=pmcentrez%7B&%7Drendertype=abstract
4. Cheng W, Chen J, Xu Z, Yu J, Huang C, Jin M, et al. Phylogenetic and recombination analysis of human bocavirus 2. BMC Infect Dis. 2011;11.
5. Chow BDW, Ou Z, Esper FP. Newly recognized bocaviruses (HBoV, HBoV2) in children and adults with gastrointestinal illness in the United States. J Clin Virol. 2010;47(2):143–7.
6. Körner RW, Söderlund-Venermo M, van Koningsbruggen-Rietschel S, Kaiser R, Malecki M, Schildgen O. Severe human bocavirus infection, Germany. Emerg Infect Dis. 2011;17(12):2303–5.
7. Moreno L, Eguizábal L, Ghietto LM, Bujedo E, Adamo MP. Infección respiratoria por bocavirus humano en lactantes de Córdoba, Argentina TT - Human bocavirus respiratory infection in infants in

córdoba, Argentina. Arch Argent Pediatr [Internet]. 2014 Feb [cited 2018 Jan 17];112(1):70–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24566786

8. Allander T, Jartti T, Gupta S, Niesters HGM, Lehtinen P, usterback R, et al. Human Bocavirus and Acute Wheezing in Children. Clin Infect Dis [Internet]. 2007;44(7):904–10. Available from: https://academic.oup.com/cid/article-lookup/doi/10.1086/512196
9. Kapoor A, Simmonds P, Slikas E, Li L, Bodhidatta L, Sethabutr O, et al. Human bocaviruses are highly diverse, dispersed, recombination prone, and prevalent in enteric infections. J Infect Dis [Internet]. 2010 Jun 1 [cited 2018 Jan 17];201(11):1633–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20415538
10. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013;30(12):2725–9.
11. Tran DN, Nguyen TQN, Nguyen TA, Hayakawa S, Mizuguchi M, Ushijima H. Human bocavirus in children with acute respiratory infections in Vietnam. J Med Virol [Internet]. 2014 Jun [cited 2018 Jan 24];86(6):988–94. Available from: http://doi.wiley.com/10.1002/jmv.23789
12. Niang MN, Diop OM, Sarr FD, Goudiaby D, Malou-Sompy H, Ndiaye K, et al. Viral etiology of respiratory infections in children under 5 years old living in tropical rural areas of Senegal: The EVIRA project. J Med Virol [Internet]. 2010 May [cited 2018 Jan 24];82(5):866–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20336732
13. Symekher SML, Gachara G, Simwa JM, Gichogo J, Rotich M, Ng'ayo MO, et al. Human Bocavirus Infection in Children with Acute Respiratory Infection in Nairobi, Kenya. Open J Med Microbiol [Internet]. 2013 Dec 20 [cited 2018 Jan 24];3(4):234–8. Available from: http://www.scirp.org/journal/doi.aspx?DOI=10.4236/ojmm.2013.34035
14. Goktas S, Sirin MC. Prevalence and Seasonal Distribution of Respiratory Viruses During the 2014 - 2015 Season in Istanbul. Jundishapur J Microbiol [Internet]. 2016 Sep [cited 2018 Feb 17];9(9):e39132. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27800148
15. Erling V, Jalil F, Hanson L a, Zaman S. The impact of climate on the prevalence of respiratory tract infections in early childhood in Lahore, Pakistan. J Public Health Med [Internet]. 1999;21(3):331–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10528962
16. Bharaj P, Sullender WM, Kabra SK, Broor S, Preeti B, Sullender WM, et al. Human bocavirus infection in children with acute respiratory tract infection in India. J Med Virol. 2010;82:812–6.
17. Abdel-Moneim a S, Kamel MM, Al-Ghamdi S, Al-Malky MIR. Detection of Bocavirus in Children Suffering from Acute Respiratory Tract Infections in Saudi Arabia. PLoS One. 2013;8(1):e55500.
18. Salmón-Mulanovich G, Sovero M, Laguna-Torres VA, Kochel TJ, Lescano AG, Chauca G, et al. Frequency of human bocavirus (HBoV) infection among children with febrile respiratory symptoms in Argentina, Nicaragua and Peru. Influenza Other Respi Viruses. 2011;5(1):1–5.
19. Hengst M, Hausler M, Honnef D, Scheithauer S, Ritter K, Kleines M. [Human Bocavirus-infection (HBoV): an important cause of severe viral obstructive bronchitis in children]. Klin Padiatr [Internet]. 2008;220(5):296–301. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18716982

20. Akça H, Tuygun N, Polat E, Karacan CD. Severe Respiratory Tract Infections with Human Bocavirus in Children. J Microbiol Infect Dis [Internet]. 2016 [cited 2018 Jan 24];6(3):145–7. Available from: http://dergipark.gov.tr/download/article-file/325665
21. Nokso-Koivisto J, Pyles RB, Miller L, Jennings K, Loeffelholz M, Chonmaitree T. Role of Human Bocavirus in Upper Respiratory Tract Infections and Acute Otitis Media. J Pediatric Infect Dis Soc [Internet]. 2014;3(2):98–103. Available from: http://jpids.oxfordjournals.org/cgi/doi/10.1093/jpids/pit061
22. Principi N, Piralla A, Zampiero A, Bianchini S, Umbrello G, Scala A, et al. Bocavirus Infection in Otherwise Healthy Children with Respiratory Disease. PLoS One [Internet]. 2015;10:e0135640. Available from: http://dx.doi.org/10.1371/journal.pone.0135640
23. Kenmoe S, Vernet M-A, Njankouo-Ripa M, Penlap VB, Vabret A, Njouom R. Phylogenic analysis of human bocavirus detected in children with acute respiratory infection in Yaounde, Cameroon. BMC Res Notes [Internet]. 2017;10(1):293. Available from: http://bmcresnotes.biomedcentral.com/articles/10.1186/s13104-017-2620-y
24. Pogka V, Moutousi A, Kossyvakis A, Kalliaropoulos A, Sgouras DN, Giannaki M, et al. Genetic variability of human metapneumo- and bocaviruses in children with respiratory tract infections. Influenza Other Respi Viruses. 2014;8(1):107–15.
25. Arthur JL, Higgins GD, Davidson GP, Givney RC, Ratcliff RM. A Novel Bocavirus Associated with Acute Gastroenteritis in Australian Children. Münger K, editor. PLoS Pathog [Internet]. 2009 Apr 17 [cited 2018 Jan 24];5(4):e1000391. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19381259
26. Ghietto LM, Cámara A, Cámara J, Adamo MP. High frequency of human bocavirus 1 DNA in infants and adults with lower acute respiratory infection. J Med Microbiol. 2012;61(4):548–51.
27. Kantola K, Hedman L, Arthur J, Alibeto A, Delwart E, Jartti T, et al. Seroepidemiology of human bocaviruses 1-4. J Infect Dis. 2011;204(9):1403–12.

Tables

Table 1: Oligonucleotide primers used for the amplification and sequencing of HBoV

Primers	Sequence 5´ – 3´	Sense	Reference
HBoV 1F	CGC CGT GGC TCC TGC TCT	+	(9)
HBoV 1R	TGT TCG CCA TCA CAA AAG ATG TG	-	
HBoV 2F	GGC TCC TGC TCT AGG AAA TAA AGA G	+	
HBoV 2R	CCT GCT GTT AGG TCG TTG TTG TAT GT	-	

Table 2: Showing seasonal distribution of HBoV

Month of Sample Collection	HBoV Positive		Total	P Value
	Yes	No		
JANUARY	11	29	40	0.000
FEBRUARY	2	23	25	
MAY	2	7	9	
JUNE	0	27	27	
JULY	0	17	17	
AUGUST	2	80	82	
SEPTEMBER	1	79	80	
OCTOBER	0	6	6	
NOVEMBER	1	6	7	
DECEMBER	8	32	40	
Total	27	306	333	

Table 3: Characteristics of the study population

Variable	Category	Frequency	Gender		Total positive HBoV	percentage	STD	P Value
			Male / No Positive	Female / No Positive				
Age	0-6 months	133	68 / 0	65 / 2	2	39.9	1.636	0.001
	>6months -1 year	51	28 / 1	23 / 1	2	15.3		
	>1-2 years	67	37 / 8	30 / 3	11	20.1		
	>2-3 years	27	12 / 0	15 / 3	3	8.1		
	>3-4 years	29	19 / 2	10 / 3	5	8.7		
	>4-5 years	26	14 / 0	12 / 4	4	7.8		
Total		333	178 / 11	155 / 16	27			

Table 4: Frequency of symptoms among HBoV positive children

Symptom	Frequency
Cough	27
Wheeze	7
Breathlessness	5
Fever	9
Nasal Congestion	16
Catarrh	27
Vomiting	3
Itches	0
Tonsillitis	1
Diarrhea	1
Otitis Media	2
Rash	2
Anorexia	7
Restlessness	0

Table 5: List of isolates with their observable symptoms

Sample ID	Symptoms
OLA17	Cough, Nasal Congestion, Catarrh
ADO40	Cough, Wheeze, Catarrh
OLA21	Cough, Nasal Congestion, Catarrh, Anorexia
ADO38	Cough, Wheeze, Catarrh
ADO27	Cough, Wheeze, Breathlessness, Nasal Congestion, Catarrh
ADO45	Cough, Catarrh
ADO34	Cough, Wheeze, Catarrh
OLA16	Cough, Fever, Nasal Congestion, Catarrh, Anorexia
OLA18	Cough, Nasal Congestion, Catarrh, Otitis
ADO28	Cough, Catarrh
OLA23	Cough, Fever, Nasal Congestion, Catarrh
ADO37	Cough, Wheeze, Catarrh
ADO43	Cough, Catarrh
ADO49	Cough, Wheeze, Catarrh
ADO29	Cough, Wheeze, Catarrh
ADO44	Cough, Wheeze, Catarrh
OLA36	Cough, Fever, Nasal Congestion, Anorexia
OLA34	Cough, Nasal Congestion, Catarrh, Vomiting
OLA33	Cough, Nasal Congestion, Catarrh, Tonsillitis, Anorexia
OLA24	Cough, Fever, Nasal Congestion, Catarrh, Vomiting
OLA32	Cough, Breathlessness, Fever, Nasal Congestion, Catarrh
OLA46	Cough, Breathlessness, Fever, Nasal Congestion, Catarrh, Anorexia
OLA19	Cough, Breathlessness, Fever, Nasal Congestion, Catarrh

Table 6 and Table 7 - Due to technical limitations, Table 6 and Table 7 have been placed in the supplementary files section.

Figures

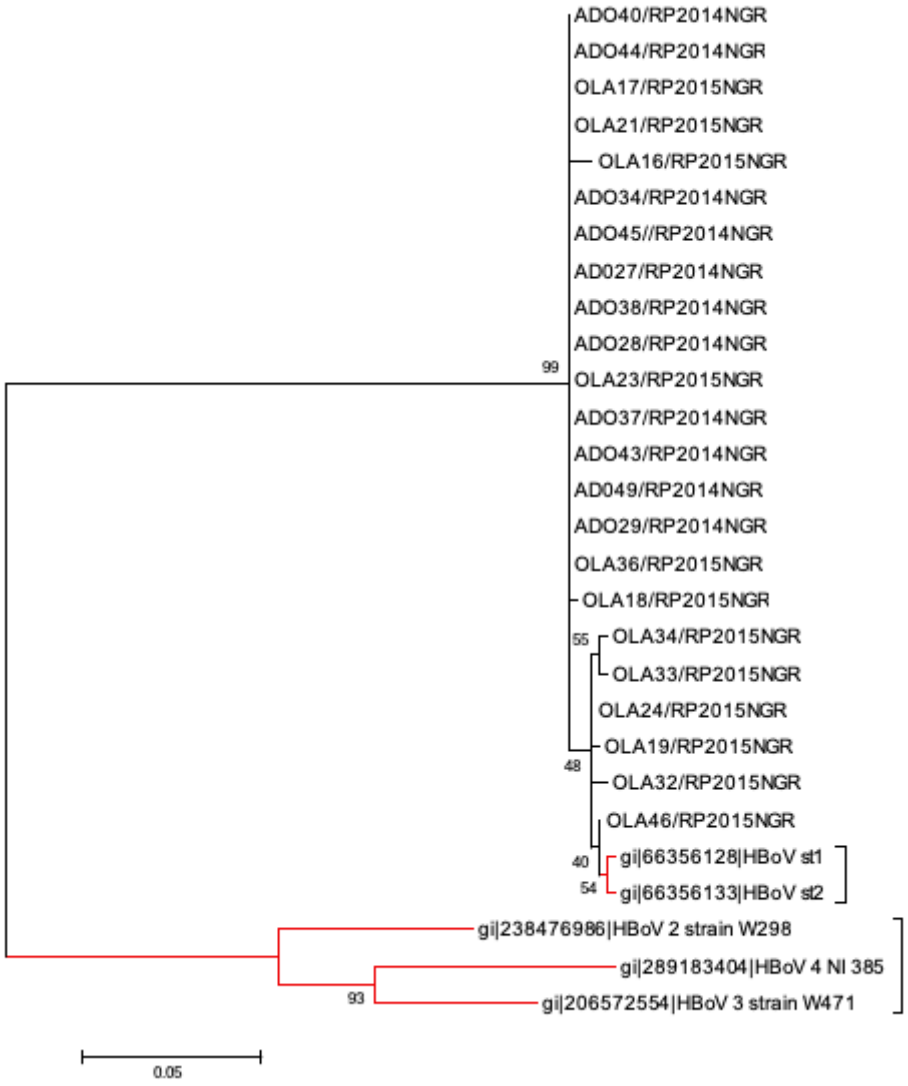

Figure 1

Phylogenetic Analysis of VP1/VP2 Nucleotide Sequences of Isolates of this Study with Reference Strains.

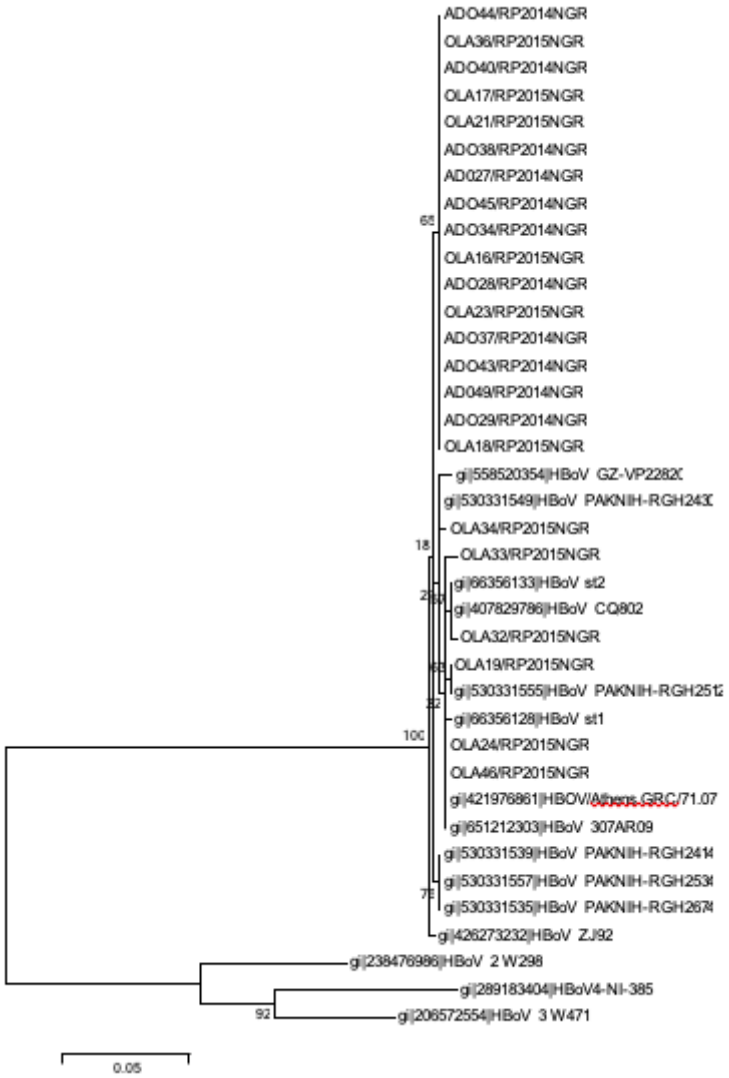

Figure 2

Phylogenetic Analysis of VP1/VP2 Nucleotide Sequences of the study Isolates with some other Identical Strains.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table7.png
- Table6.png