Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Pediatr Infect Dis J. Author manuscript; available in PMC 2017 May 01.

Published in final edited form as:

Pediatr Infect Dis J. 2016 May ; 35(5): 471–476. doi:10.1097/INF.0000000000001058.

Determining Persistence of Bocavirus DNA in the Respiratory Tract of Children by Pyrosequencing

Johana Castro Wagner, MD[1], **Richard B. Pyles, PhD**[1], **Aaron L. Miller, MS**[1], **J. Nokso-Koivisto, MD, PhD**[1], **Michael J. Loeffelholz, PhD**[2], and **Tasnee Chonmaitree, MD**[1,2]

[1]Departments of Pediatrics, University of Texas Medical Branch. Galveston, Texas. USA

[2]Departments of Pathology, University of Texas Medical Branch. Galveston, Texas. USA

Abstract

Background—Although human bocavirus type 1 (HBoV1) is a respiratory pathogen, presence of HBoV-DNA in secretions of asymptomatic children raised the question on the significance of HBoV-positive results.

Methods—Archived specimens from a prospective, longitudinal study were tested for HBoV. A total of 94 children (aged 6 – 36 m) were HBoV(+) during 172 upper respiratory tract infection (URI) and/ or acute otitis media (AOM) episodes. We used pyrosequencing of NP1, VP1 and VP2 genes to type HBoV and subtype HBoV1 in these specimens.

Results—Of the specimens tested, HBoV-DNA were successfully sequenced in 128 (74%) samples from 70 children; all were HBoV type 1. Subtypes identified (n=108) were: LWK/TW (63%), LWK/BJ (20%), Bonn/BJ (16%) and LWK/KU3 (1%). Of 46 children for whom shedding pattern could be determined, viral clearance within 30d (13-29d) occurred in 28%; another 22% of children had no recurrence after 32 to 267d. Prolonged virus presence of >30 d (34 to 181d+) occurred in 22%; intermittent detection (61+ to 170d+) in 20%. Infection with the same HBoV1 subtype after 4-5 negative samples (244 and 265d interval) occurred in 4%. Infection with 2 different HBoV1 subtypes (29 and 87d apart) occurred in only 4%. Newly acquired HBoV1-URI resulted in AOM in 53% of cases.

Conclusions—Children with HBoV1 infection commonly shed for a prolonged period leading to repeated viral DNA detection. Recurrence after 8-9 m suggests possible persistence and reactivation. Infections with 2 different HBoV1 subtypes within one-year period are uncommon. Newly acquired HBoV1-URI is often complicated by AOM.

Keywords

acute otitis media; bocavirus subspecies; virus persistence; common cold; virus pyrosequencing

Corresponding author and reprints: Tasnee Chonmaitree, MD, Department of Pediatrics, University of Texas Medical Branch, 301 University Blvd., Galveston, Texas 77550-0371, Phone: (409) 772-4876; Fax: (409) 747-1753, tchonmai@utmb.edu.
Dr. Wagner is currently at the University of South Florida, St. Petersburg, Florida, USA. Dr. Nokso-Koivisto is currently at the University of Helsinki, Finland.

Disclosures: The authors have no conflict of interest to disclose

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

INTRODUCTION

Human Bocavirus (HBoV) is the second member of the parvovirus species known to be pathogenic in humans. ([1]) Four different HBoV types have been described thus far, including HBoV type 1 (HBoV1) predominately found in the respiratory tract. ([1–3]) HBoV1 is commonly detected in association with other respiratory viruses during respiratory infections. Because of this finding in addition to frequent prolong shedding in asymptomatic children, its pathogenic role has been questioned. ([4]) Nonetheless, increasing evidence confirms the association of HBoV1 with both upper and lower respiratory tract infections (URI and LRI), and acute otitis media (AOM) in young children. ([2, 4–9]) A recent study suggested that acquired immunity could be achieved by age 6, with a median of 2.3 years, ([5]) making an acute HBoV1 infection more likely during childhood. Prolonged HBoV1 presence in nasopharyngeal secretions has been described for up to 6 months ([8,10]) and identified in healthy asymptomatic children. ([2,4,5,11,12])

In a previous study, ([13]) we detected HBoV DNA in nasopharyngeal secretions of children during acute URI. Herein, we further analyzed HBoV using pyrosequencing, a rapid, simple and cost-effective method targeting specific regions of DNA. The aim was to characterize the nucleic acid sequences detected, to differentiate prolong presence from a newly acquired infection and to compare the latter to AOM development.

MATERIALS AND METHODS

Specimens analyzed in this study were archived nasopharyngeal secretions that were HBoV positive (+). The specimens were collected from a prospective, longitudinal study performed between January 2003 and March 2007 at the University of Texas Medical Branch in Galveston, Texas, USA. ([14]) Healthy children (6 to 36 months of age) with no associated co-morbidities were enrolled and followed for one year each for occurrences of viral URI and AOM. The Institutional Review Board approved the study; informed consent was obtained from the parents or guardians of all the study subjects. Specimens were collected only during URI visits and when AOM were diagnosed; the specimens were tested for respiratory viruses by viral culture and polymerase chain reaction (PCR) as previously described. ([13, 14]) In the original study ([14]), 988 specimens obtained from 214 children were tested for viruses; 707 archived specimens were available for HBoV-PCR testing. ([13])

Archived DNA from HBoV positive specimens were recovered; PCR prior to each pyrosequencing assay was achieved using three different sets of primers designed in order to target 3 different regions of the HBoV genome including NP1, VP1 and VP2. By targeting a highly conserved region within the HBoV genome, the NP1 region, ([15]) we differentiated HBoV type 1 from other less common types occasionally identified in the respiratory tract (2, 3, or 4). Conversely, VP1 and VP2 regions are highly variable regions in the virus and several sequences have been described by different authors, ([15–17]) potentially allowing differentiation of more than one HBoV1. By targeting these areas, we were able to identify and subtype various types of HBoV1. Primers used in this study include: NP1 primers: Forward: Biotin-CGCATTGCTAGAGATGGTACTAA and Reverse: ACATTAGCTAAGTGCCTACGGTA, sequencing: ATTACATCTTTCACAATCAG. VP1

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

primers: Forward: Biotin-CTAAACCAGGAACCTCAAAAATGT and Reverse: TCCTCCTCCAATACTTCCTGTTC, sequencing: TGTTCCTCCCCCTGA. VP2 primers: Forward: Biotin-GTGAATGGRTTAAYAATGAAAGAGC and Reverse: TGCTGTGCTTCCGTTTTGTC, sequencing: CTTATGTACTGAACTCTTCT. A c1000 thermocycler was used to carry out PCR using the following protocol: Master mix per sample composed of 12.5 μl Bio-Rad Supermix, 1.0 μl forward primer (5 μM), 1.0 μl reverse primer (5 μM), 6.5 μl of TOC water and 3 μl of the DNA template. The reactions were carried out as follows: cycle 1: 95°C (1 minute and 30 seconds); cycle 2: 95°C (30 seconds), 58°C (30 seconds), 72°C (30 seconds) and repeated 49 times. These were followed by 5 minutes at 72°C and a hold step at 4°C. The resulting biotinylated PCR products were pyrosequenced using a Pyromark ID 96 instrument with PyroMark Gold reagents following the manufacture's instructions. For each assay the final sequencing primer concentration was 0.3 μM with a cyclic dispensation: 12 (GTAC). Resulting sequences were compared using a HBoV1 library built from GenBank/BLAST and Clustal Omega alignment functions, based on the most variable regions targeted (VP1 and VP2), subtypes within HBoV type 1 were described based on existing worldwide sequences: hBoV1_Irish, KU3, BJ3722, Bonn-1, LWK, TW674, st1. ([1, 16–21]) We assigned a unique identifier for each strain based on the sequence (both VP1 and VP2) that showed the greatest homology to the sample: KU3, BJ, Bonn, LWK, TW.

RESULTS

Patients and specimens

A total of 201 children contributed 707 archived specimens that were available for HBoV-PCR testing. The number of children, specimens and the process of sample analysis are illustrated in Figure 1. HBoV-PCR was positive in 172 specimens (94 children); these specimens were first subjected to pyrosequencing of NP1 region. Of these, 128 (74%) specimens (70 children) were successfully sequenced; all were confirmed to be HBoV type 1 (HBoV1).

Subtype identification of HBoV1 were successful in 108 (63%) specimens (53 children), including children with single and multiple HBoV1-positive samples. Demographic characteristics and risk factors of HBoV1-positive children and those with successful HBoV1 sequencing are shown in Table 1.

HBoV1 subtypes

By targeting the two most variable regions in the genome: VP1 and VP2; we identified HBoV1 subtypes. For VP1 region, two main sequences were obtained and identified by matching the previously described LWK and Bonn-1 sequences within this region ([16,21]) available in GenBank. In contrast, for VP2, three main sequences were obtained and matched the described TW674, BJ3722, and KU3 genomes. ([17, 19, 20]) The data obtained was paired and allowed recognition of four main HBoV1 subtypes in the area of Galveston, TX, USA. A unique identifier was assigned corresponding to the sequence of reference (KU3, BJ3722, Bonn-1, LWK, TW674) based on VP1/VP2 region as follows: LWK/TW, LWK/BJ, LWK/KU3 and Bonn/BJ (Table 2).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Patterns of HBoV1 shedding

We determined the pattern of HBoV1 shedding in children using available longitudinal data from follow-up samples of the same patient for presence or absence of HBoV1 and compared HBoV1 subtypes. From 94 children, we were able to determine the shedding pattern in 46; 17 cases were in the group with single HBoV1-URI episode, compared with subsequent absence of the virus. In 29 children with multiple HBoV1-URI episodes, we compare the nucleotides from the sequencing results. The remaining 48 children were considered indeterminate as we were not able to call a pattern due to lack of follow-up samples or sequenced data.

To determine a pattern of HBoV1 shedding among the group of children with multiple HBoV1 (+)-URI episodes, we compared sequences from each URI episode to determine similarity. If the nucleotide sequence was the same on both targeted regions (VP1 and VP2), it was deemed the same HBoV1 subtype. If a variation was noted at either targeted region, the HBoV1 subtype was considered to be different and thus, a *newly acquired infection*. We used the longitudinal data, especially the successive negative samples, to determine the duration of the infection. For lack of better alternative, we considered the initial HBoV1 (+)-URI from enrollment a *new infection*. The viral load in the new infection category ranged from $9.64x10^3$ to $1.73x10^{12}$ copies/mL of samples.

Overall, we identified 6 main patterns of HBoV1 shedding (Table 3): *Viral clearance within 30 days* (Pattern 1) was identified in 13 children (28%). All but three of the children in this group had 2-7 HBoV1-negative follow-up samples lasting 54 to 290 days, suggesting no recurrence. Three children had only one negative follow-up sample each 14–19 days later. Similarly, a pattern of *no recurrence after positive episode* (Pattern 2) was identified in 10 children; all with viral clearance after 30 days and at least two (2 – 6) negative follow-up samples after 32 to 267 days. *Prolonged presence* (Pattern 3) was defined as repeated presence of the same HBoV1 strain for more than 30 days. Of these, 5 children had 1 or more negative episodes suggesting the presence of virus <60 days; the remaining had persistent infection over 60 days. *Intermittent detection* (Pattern 4) was defined as the presence of a specific HBoV1 subtype in subsequent URI episodes with at least one negative HBoV1 (+)-URI in between. A *recurrent HBoV1 infection* (Pattern 5) was identified when a specific subtype was present during an URI episode then reappeared after several (4-5) HBoV-negative URI episodes. Finally, 2 subjects (4%) were infected with *two different HBoV1 subtypes* (Bonn/BJ and LWK/BJ in one subject and Bonn/BJ and LWK/TW in the other) within 1-year-period (Pattern 6).

Figure 2 illustrates different patterns of HBoV1 shedding. Subject 1 (28-month old), with 7 symptomatic URI episodes had HBoV1-LWK/TW subtype identified in two episodes and corresponding to newly acquired infection with clearance in less than 30 days. HBoV1 co-infected with rhinovirus and adenovirus/rhinovirus in this case. Subject 2 (6-month old) had 7 URI episodes with only the initial being HBoV1-Bonn/BJ (+), co-infecting with enterovirus. This was followed by several negative samples for a total of 260 days. Subject 3 (14-month old) had 5 URI episodes, with 2 being HBoV1-LMK/BJ consecutively for 53 days (pattern of prolonged presence); HBoV1 co-infected with enterovirus and parainfluenza. Subject 4 had 7 URI episodes from HBoV1-LWK/BJ (+) and exhibited a

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

pattern of prolonged presence for 99 days; HBoV1 co-infected with influenza and adenovirus/rhinovirus. Subject 5 (30-month old) had 6 URI episodes with two HBoV1-LWK/TW (+) intermittently detected with 2 negative samples in between; HBoV1 co-infected with adenovirus and enterovirus. Subject 6 (11-month old) had 7 URI episodes with 3 being HBoV1-LWK/TW (+), with the last episode occurring after an interval of 323 days from the previous HBoV1-URI episode, representing recurrence or virus reactivation; HBoV1 co-infected with enterovirus and influenza. Lastly, subject 7 (8-month old) had 10 URI episodes with 3 HBoV1 (+) of two different subtypes: one HBoV1-Bonn/BJ (+) and two HBoV1-LWK/BJ (+); HBoV1 co-infected with cytomegalovirus and RSV in this case.

HBoV1 infection and acute otitis media

Of 172 HBoV1 (+)-URI episodes, 128 (74%) were HBoV1 co-infected with other viruses; of these, 55 (43%) were complicated by AOM. In 44 (26%) episodes, HBoV alone was detected (23 children). Twenty six of 44 samples were fully sequenced. Of these, 13 (50%) HBoV1-URI episodes were complicated by AOM. Table 4 compares the pattern of HBoV1 detection in cases without other virus co-infection by occurrence of AOM complication. For newly acquired HBoV1 infection (n=15), the rate of AOM following HBoV1-URI was 53% (8 of 15). The rate of AOM following HBoV1-URI in persistent and recurrent detections was 46%. From the HBoV1-URI episodes with AOM complication, two main subtypes were identified: five LWK/TW and three Bonn/BJ.

DISCUSSION

In this study, we used pyrosequencing to characterize HBoV1 detected in successive specimens of children with URI and described different shedding patterns. Our data showed that at least 46% of 46 children with HBoV1 infection shed the virus for a prolonged period leading to detection of HBoV1 in respiratory secretions repeatedly or intermittently for up to 265 days. Infection with 2 different HBoV subtypes in a one-year period was uncommon. Importantly, we identified a high incidence of AOM complicating URI (53%) among children with newly acquired HBoV1 infection (HBoV1 as the sole virus, without other co-infecting virus. Our data support the possible ototropic nature of HBoV1, and agree with the findings from previous studies. ([5, 22, 23])

From 108 fully sequenced HBoV1 (+) specimens, not only we isolated HBoV type 1 as the only type present in the respiratory mucosa as described in previous studies, ([2,3]) but we also differentiated 4 main subtypes in the Galveston, TX, USA area with the majority being LWK/TW (63%), followed by LWK/BJ, Bonn/BJ and less commonly LWK/KU3. No literature available thus far has proposed HBoV subtype classification.

The use of molecular diagnostics has been associated with prolonged duration of viral (nucleic acid) detection in respiratory specimens. Using viral culture, common cold viruses have been described to shed in the respiratory tract from 2 – 7 days (maximum 10 days) following an acute infection. ([24]) More recent data suggest that rhinovirus, the most common URI virus, exhibits a relative rapid clearance of viral nucleic acids in less than 30 days; ([25]) whereas adenovirus exhibits a prolonged presence for up to 203 days. ([26]) For HBoV1, our data on viral persistence are similar to findings from other studies and may help

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

explain the relative high incidence of HBoV1 in some populations and its common association with other respiratory viruses. ([8, 10, 27]) In contrast, unlike other studies, ([8, 10]) we also identified cases when HBoV1 was cleared within 13 – 29 days and had no recurrence.

In our children who experienced either single or multiple HBoV1 (+)-URI episodes, we described different shedding patterns characterized mainly by repeated detection (Patterns 3 – 5, 46%) followed by those with viral clearance within 30 days (Pattern 1, 28%). The proportion of our cases with short duration of viral clearance may be overestimated because the children may have had HBoV1 infection prior to enrollment into the study. Nevertheless, 62% (8 of 13) of children in Pattern 1 had at least one HBoV1-negative URI samples prior to becoming HBoV1-positive. Although viral load data were available, we were not able to use them to help differentiate new infection, recurrent infection or prolonged shedding because of lack of sequential samples from birth and lack of serological data.

Repeated HBoV1 detection was identified continuously or intermittently in the respiratory mucosa (Patterns 3 – 4). No recurrence after an HBoV1 infection was observed in 22% (Pattern 2); this supports the proposal that stable protective antibody levels occur in most children after initial HBoV1 infection.([5]) In our study, infection with two different subtypes within one-year period is uncommon (Pattern 6) demonstrating HBoV1's limited genetic variability as shown by other studies. ([28]) Interestingly, we identified 2 subjects with presence of the same HBoV1 subtype for up to a 265-day interval (Pattern 5), with successive negative samples in between. This raised the possibility of virus latency followed by reactivation, as described for the *Parvoviridae* family. ([27])

AOM is one of the most common diseases during infancy in childhood; the disease mostly occurs as a complication of viral URI. ([14, 29]) Specific respiratory viruses such as RSV, coronavirus, and adenovirus are associated with increased AOM risk. ([14]) For HBoV1, increasing evidence supports its role as a respiratory pathogen associated with acute URI, LRI and AOM in young children. ([4–6, 9, 22, 23, 27, 29]) In this study, even with lack of serology to confirm acute infection, the absence of other co-infecting respiratory viruses during URI and previously HBoV1-negative samples suggest that new HBoV1 infection is highly associated with AOM following URI (8 out of 15 episodes). This finding supports a possible association with AOM development as described by others. ([22, 23]) Not only we found that newly acquired HBoV1 infection was associated with AOM complication, but persistent and recurrent detections were also associated with AOM complicating URI.

Our study limitations included the inability to sequence many specimens previously positive by PCR, thus reducing the sample size. This could possibly be from repeat thawing of archived samples resulting in the denaturation of DNA materials or from low viral load. The study design was to collect samples only during URI or AOM visits but not at regular intervals. Therefore, we were not able to identify primary HBoV1 infection with certainty and follow-up samples in many children were not sufficient. Our strengths include the longitudinal study design, with relatively a large sample size, and the use of next generation sequencing to identify HBoV1 subtypes. Not only we were able to compare virus subtypes in sequential samples, we also described the epidemiologic characteristics of HBoV1

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

subtypes in our community during the study period. In a recently published study, Martin et al ([30]) reported HBoV1 primary infection and shedding in 66 infants. Data from their study agree with ours on the prolonged and intermittent shedding patterns of HBoV1.

We conclude that HBoV1 DNA detection in the respiratory tract is often the result of a prolonged presence or intermittent shedding, and not due to newly acquired infection. Children are rarely infected with two different subtypes of HBoV1 within 1 year. For these reasons, positive PCR findings for HBoV1 need to be interpreted carefully.

Acknowledgments

This study was supported by the grants R01 DC 005841 and UL1 TR001439 from the National Institutes of Health. We thank Krystal Revai, Janak Patel, Sangeeta Nair, M. Lizette Rangel, Kyralessa B. Ramirez, Syed Ahmad, Michelle Tran, Liliana Najera, Rafael Serna, Carolina Pillion, and Ying Xiong for assistance with study subjects and specimen collection and processing.

Source of support: This study was supported by the grants R01 DC 005841 and UL1 TR001439 from the National Institutes of Health.

References

1. Allander T, Tammi MT, Eriksson M, et al. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci USA. 2005; 102:12891–12896. [PubMed: 16118271]
2. Peltola V, Söderlund-Venermo M, Jartti T. Human bocavirus infections. Pediatr Infect Dis J. 2013 Feb; 32(2):178–179. [PubMed: 23328822]
3. Tran DN, Nguyen TQ, Nguyen TA, et al. Human bocavirus in children with acute respiratory infections in Vietnam. J Med Virol. 2014 Jun; 86(6):988–994. [PubMed: 24123072]
4. Malecki M, Schildgen V, Schildgen O. Human bocavirus: still more questions than answers. Future Virology. 2011; 6(9):1107–1114.
5. Meriluoto M, Hedman L, Tanner L, et al. Association of human bocavirus 1 infection with respiratory disease in childhood follow-up study, Finland. Emerg Infect Dis. 2012; 18:264–271. [PubMed: 22305021]
6. Moriyama Y, Hamada H, Okada M, et al. Distinctive clinical features of human bocavirus in children younger than 2 years. Eur J Pediatr. 2010; 169:1087–1092. [PubMed: 20383526]
7. Chieochansin T, Samransamruajkit R, Chutinimitkul S, et al. Human bocavirus (HBoV) in Thailand: clinical manifestations in a hospitalized pediatric patient and molecular virus characterization. Journal of Infection. 2008; 56(2):137–142. [PubMed: 18164764]
8. Martin ET, Fairchok MP, Kuypers J, et al. Frequent and prolonged shedding of bocavirus in young children attending daycare. J Infect Dis. 2010; 201:1625–1632. [PubMed: 20415535]
9. Uršič T, Jevšnik M, Zigon N, et al. Human bocavirus and other respiratory viral infections in a 2-year cohort of hospitalized children. J Med Virol. 2012 Jan; 84(1):99–108. [PubMed: 22028039]
10. Lehtoranta L, Söderlund-Venermo M, Nokso-Koivisto J, et al. Human bocavirus in the nasopharynx of otitis-prone children. Int J Pediatr Otorhinolaryngol. 2012; 76:206–211. [PubMed: 22119148]
11. Jartti T, Hedman K, Jartti L, et al. Human bocavirus-the first 5 years. Rev Med Virol. 2012; 22:46–64. [PubMed: 22038931]
12. Allander T. Human bocavirus. J Clin Virol. 2008 Jan; 41(1):29–33. [PubMed: 18055252]
13. Nokso-Koivisto J, Pyles RB, Miller AL, et al. Role of Human Bocavirus in Upper Respiratory Tract Infections and Acute Otitis Media. J Ped Infect Dis. 2014; 3.2:98–103.
14. Chonmaitree T, Revai K, Grady JJ, et al. Viral upper respiratory tract infection and otitis media complication in young children. Clin Infect Dis. 2008; 46:815–823. [PubMed: 18279042]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

15. Chieochansin T, Chutinimitkul S, Payungporn S, et al. Complete coding sequences and phylogenetic analysis of Human Bocavirus (HBoV). Virus Res. 2007 Oct; 129(1):54–57. [PubMed: 17532505]
16. Dijkman R, Koekkoek SM, Molenkamp R, et al. Human bocavirus can be cultured in differentiated human airway epithelial cells. J Virol. 2009 Aug; 83(15):7739–7748. [PubMed: 19474096]
17. Lin JH, Chiu SC, Lin YC, et al. Clinical and genetic analysis of Human Bocavirus in children with lower respiratory tract infection in Taiwan. J Clin Virol. 2009 Mar; 44(3):219–224. [PubMed: 19208496]
18. Sadeghi M, Kantola K, Finnegan DP, et al. Possible involvement of human bocavirus 1 in the death of a middle-aged immunosuppressed patient. J Clin Microbiol. 2013 Oct; 51(10):3461–3463. [PubMed: 23903541]
19. Huang Q, Deng X, Yan Z, et al. Establishment of a reverse genetics system for studying human bocavirus in human airway epithelia. PLoS Pathog. 2012; 8(8):e1002899. [PubMed: 22956907]
20. Zhao L, Qian Y, Zhu R, et al. Preliminary studies suggest that a novel parvovirus called human bocavirus (HBoV) is related to acute respiratory infections in paediatric patients in Beijing. Chin. J. Microbiol. Immunol. 2006; 26:385–388.
21. Liu WK, Chen DH, Liu Q, et al. Detection of human bocavirus from children and adults with acute respiratory tract illness in Guangzhou, southern China. BMC Infect Dis. 2011 Dec.14(11):345. [PubMed: 22168387]
22. Beder LB, Hotomi M, Ogami M, et al. Clinical and microbiological impact of human bocavirus on children with acute otitis media. Eur J Pediatr. 2009 Nov; 168(11):1365–1372. [PubMed: 19221788]
23. Rezes S, Söderlund-Venermo M, Roivainen M, et al. Human bocavirus and rhino-enteroviruses in childhood otitis media with effusion. J Clin Virol. 2009 Nov; 46(3):234–237. [PubMed: 19736042]
24. Cherry, JD.; Nieves, DJ. Upper respiratory tract infections. In: Feigin, RD.; Cherry, JD., editors. Textbook of Pediatric Infectious Diseases. 4th. Philadelphia: Lippincott Williams & Wilkins; 1998. p. 128-143.
25. Loeffelholz MJ, Trujillo R, Pyles RB, et al. Duration of rhinovirus shedding in the upper respiratory tract in the first year of life. Pediatrics. 2014 Dec; 134(6):1144–1150. [PubMed: 25404719]
26. Kalu SU, Loeffelholz M, Beck E, et al. Persistence of adenovirus nucleic acids in nasopharyngeal secretions: a diagnostic conundrum. Pediatr Infect Dis J. 2010; 29:746–750. [PubMed: 20308936]
27. Christensen A, Nordbø SA, Krokstad S, et al. Human bocavirus in children: mono-detection, high viral load and viraemia are associated with respiratory tract infection. J Clin Virol. 2010; 49:158–162. [PubMed: 20833582]
28. Ditt V, Viazov S, Tillmann R, et al. Genotyping of human bocavirus using a restriction length polymorphism. Virus Genes. 2008 Feb; 36(1):67–69. [PubMed: 18071891]
29. Ede LC, O'Brien J, Chonmaitree T, et al. Lactate dehydrogenase as a marker of nasopharyngeal inflammatory injury during viral upper respiratory infection: implications for acute otitis media. Pediatr Res. 2013 Mar; 73(3):349–354. [PubMed: 23202721]
30. Martin ET, Kuypers J, McRoberts JP, et al. Human bocavirus-1 primary infection and shedding in infants. J Infect Dis. 2015 Aug 15; 212(4):516–524. [PubMed: 25632039]

Abbreviations

Neg	HBoV PCR (-)
L/T	LWK/TW strain
L/BJ	LWK/BJ strain
B/BJ	Bonn/BJ strain
L/BJ	LWK/BJ strain

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Para	parainfluenza
Rh	rhinovirus
Ad	adenovirus
En	enterovirus
Flu	Influenza
Echo	echovirus
hMPV	human metapneumovirus
CMV	cytomegalovirus
RSV	respiratory syncytial virus

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

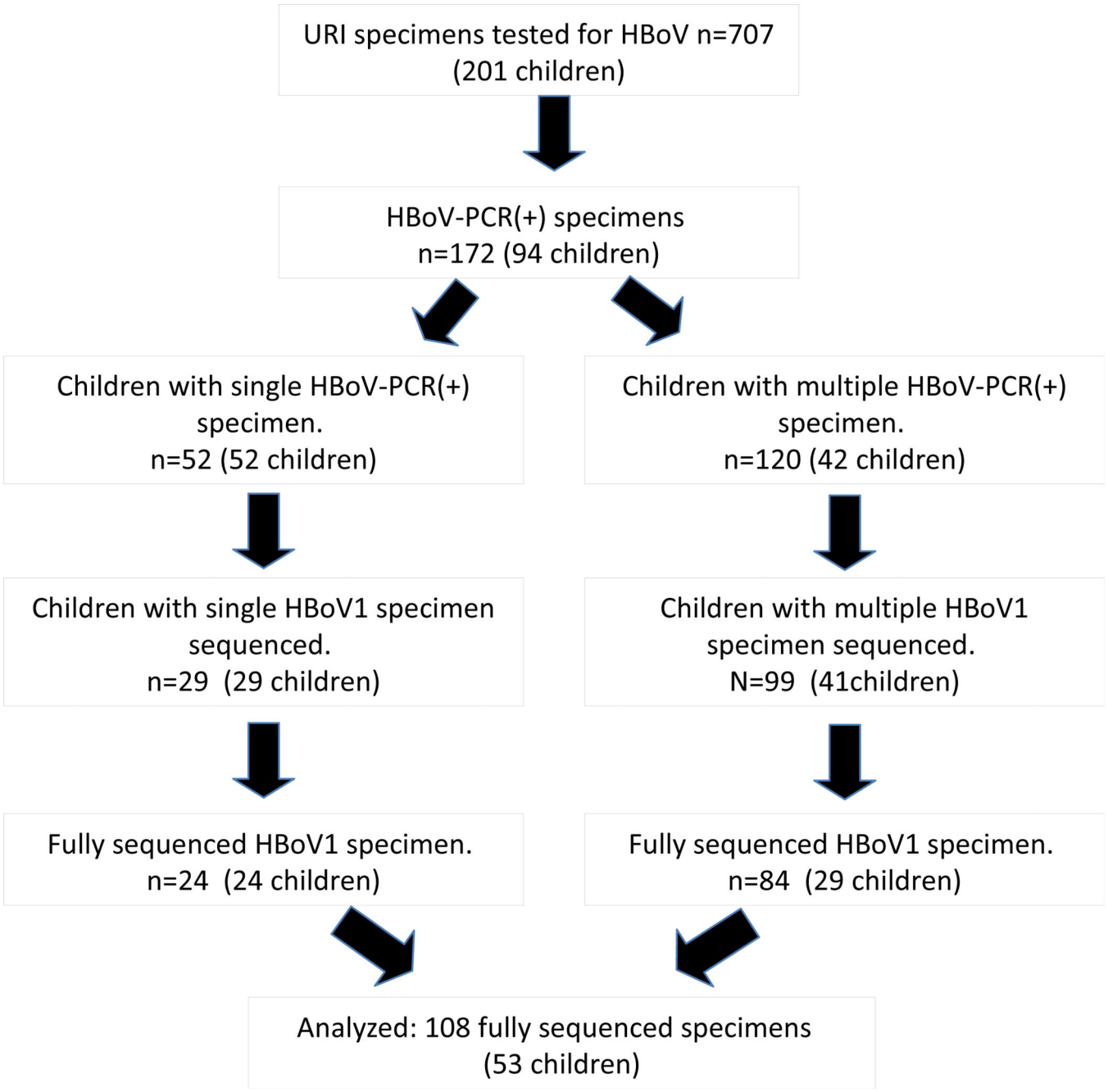

Figure 1.
Flow diagram for subjects and specimens included in the study.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

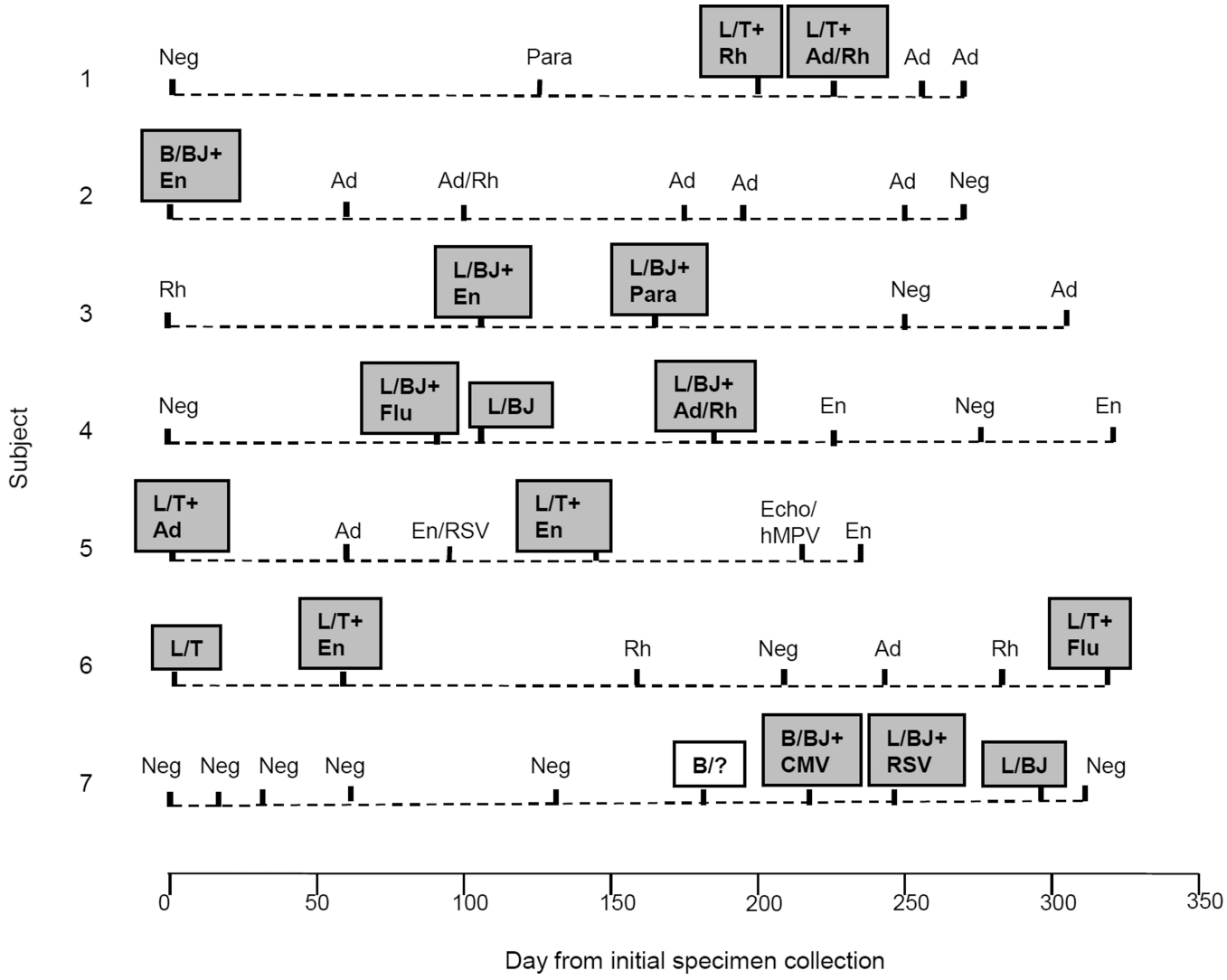

Figure 2.

Examples of multiple HBoV1 cases. Each specimen collected during symptomatic URI is represented by a vertical hash mark. HBoV1 subtypes are displayed in grey-boxes. HBoV1 co-infected with other respiratory virus (es) indicated with '+other'. Cases: 1: *Newly acquired infection with clearance < 30 days*; 2: *No recurrence on successive sample* (32 to 260d); 3: *Prolonged presence of 30 – 60 days*; 4: *Prolonged presence of >60 days*; 5: *Intermittent detection* with negative samples in between; 6: *Recurrence or reactivation* (323 and 244 days); 7: *Different HBoV1 subtypes in <1 year*.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1

Demographic characteristics and risk factors of study children:

	All Children with HBoV1 (+)[1] (n=94)	%	Children with HBoV1 sequenced (n=70)	%
Female	45	48%	31	45%
Age at enrollment in months (range)	12 (6–34)		13.8 (6–34)	
Race:				
-Asian	3	3%	1	1%
-Black	28	30%	22	31%
-Biracial	8	9%	6	9%
-White	55	59%	41	59%
Ethnicity: Hispanic/Latino	37	39%	27	39%
Child care arrangements:				
-Home				
-Home day care	60	64%	46	66%
-Day Care center	7	7%	6	8%
	26	28%	18	26%
Breastfeeding[2]	50	53%	31	44%
Cigarette smoke exposure	34	36%	23	33%

[1] One or more human bocavirus (HBoV1) PCR positive result(s) during one year study period.

[2] Any breast-feeding irrespective of the duration

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 2

Human bocavirus type 1 (HBoV1) subtypes (n=108 fully sequenced specimens).

HBoV subtype	Nucleotide sequence in VP1 region	Nucleotide sequence in VP2 region	Specimens (n=108)	%
LWK/TW	GGTGTTCTGTGGTGCG TCCACAGTATCA (LWK)	TGTTGGAACTTTTGGA TTGAACATCAATCCA (TW)	68	63
LWK/BJ	GGTGTTCTGTGGTGCG TCCACAGTATCA (LWK)	TGTTGGGACTTTTGGA TTAAACATTAGTCCAG GAGGAA (BJ)	22	20
LWK/KU3	GGTGTTCTGTGGTGCG TCCACAGTATCA (LWK)	TGTTGGAACTTTTGGA TTAAACATTAGTCCAG GAGGAA (KU3)	1	1
Bonn/BJ	GGTGTTTGTGGTGCGT CCACAGTATCAGGTT (Bonn)	TGTTGGGACTTTTGGA TTAAACATTAGTCCAG GAGGAA (BJ)	17	16

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 3

HBoV1 shedding patterns in children with single and multiple detections.

Pattern	N	%	Mean Age* (m)	Description
1	13	28	15.7	Viral clearance within 30 days (13 to 29d)
2	10	22	20.6	No recurrence after positive episode (32 to 267d)
3	10	22	17.9	Prolonged presence: 30–60 days (34 to 53d) and >60 days (68 to 181d)
4	9	20	18.2	Intermittent detection (61 to 170d)
5	2	4	10.7	Recurrence after 4–5 successive negative sample (244 to 265d interval)
6	2	4	10.9	Detection of 2 different HBoV1 subtypes (29 and 87d apart)
Total	46	**100**		

*Age at the first HBoV1 detection

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 4

Type of HBoV1 detected in 26 URI episodes (without co-infection), with and without AOM complication.

HBoV1 detection Type	URI with AOM (n)	URI without AOM (n)	Total
New[1]	8	7	15
Persistent[2]	4	4	8
Possible persistent/recurrent[3]	1	2	3
Total	13	13	26

[1] New infection: HBoV1 (+)-URI episode preceded by negative HBoV1 sample and no recent HBoV1 infection. Initial URI HBoV1 (+) since enrollment.

[2] Persistent detection: sample preceded by HBoV1 (+) URI

[3] Possible persistent/recurrent detection: sample preceded by recent URI with same HBoV1 (+) sequence although with HBoV1 (−) sample in between.