Olli Kesti

HUMAN BOCAVIRUSES: COMMON BUT NOT WIDELY KNOWN

Syventävien opintojen kirjallinen työ

Kevätlukukausi 2020

Olli Kesti

HUMAN BOCAVIRUSES: COMMON BUT NOT WIDELY KNOWN

Kliininen laitos

Kevätlukukausi 2020

Vastuuhenkilö: Tuomas Jartti

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
KESTI, OLLI: Human bocaviruses: common but not widely known

Syventävien opintojen kirjallinen työ, 21 s., 1 liites.
Lastentautioppi
Helmikuu 2020

Human bocavirus 1 (HBoV1), belonging to the *Parvoviridae* family was discovered in 2005 in nasopharyngeal samples from children with respiratory tract infections (RTIs). Three additional bocaviruses, HBoV2-4, were discovered in 2009-2010. These viruses have mainly been found in faecal samples, and their role in human diseases is still uncertain. HBoV1 causes a wide spectrum of respiratory diseases in children including common cold, acute otitis media, pneumonia, bronchiolitis and asthma exacerbations. HBoV1 DNA can persist in airway secretions for months after an acute infection. Consequently, acute HBoV1 infection cannot be diagnosed with standard DNA PCR; quantitative PCR and serology are better diagnostic approaches. Because of their high clinical specificity, diagnostic developments such as HBoV1 mRNA and antigen detection have shown promising results. This review summarizes the knowledge on human bocaviruses, with special focus on HBoV1.

Key words: human bocavirus, respiratory tract infection

TURUN YLIOPISTO
Lääketieteellinen tiedekunta

KESTI, OLLI: Ihmisen bokavirukset: yleisiä, mutta huonosti tunnettuja

Syventävien opintojen kirjallinen työ, 21 s., 1 liites.
Lastentautioppi
Helmikuu 2020

Ihmisen bokavirus 1 (hBoV1), joka kuuluu Parvovirusten sukuun, löydettiin vuonna 2005 ylähengitystieinfektioita sairastaneiden lasten nenänielunäytteistä. Kirjallisuuskatsaus tiivistää ajankohtaista tietoa bokaviruksien genomista ja rakenteesta, epidemiologiasta, esiintyvyydestä muiden virusinfektioiden yhteydessä, transmissiosta, patogeneesistä, persistenssistä, diagnostiikasta, kliinisestä kuvasta, vakavan hBoV1-infektion riskitekijöistä sekä hoidosta.

Katsaus on jatkoa työryhmän aiemmalle kirjallisuuskatsaukselle, jossa tarkastelun kohteena olivat ennen 23.5.2011 ilmestyneet bokavirusta koskevat julkaisut. Tämä tutkielma perustuu 508:aan aikavälillä 23.5.2011–30.4.2018 julkaistuun PubMed-tietokannassa oleviin englanninkieliseen artikkeliin, jotka löydettiin hakusanalla ”bocavirus”. Näistä artikkeleista valikoitiin merkittävät molekyylitason tutkimukset, jotka sisälsivät uutta tietoa bokaviruksen biologiasta tai diagnostiikasta. Lisäksi katsaukseen sisällytettiin kliiniset tutkimukset, joissa otoskoko oli vähintään 100 tai tutkimus sisälsi muuten arvokasta tietoa, kuten ainutlaatuisia tutkimusmenetelmiä tai potilasryhmiä.

Katsaukseen valikoituneista tutkimuksista ilmenee, että hBoV1 on yleinen ylähengitystieinfektioiden aiheuttaja lapsilla. hBoV2-4 löydettiin vuosina 2009–2010. Näitä viruksia esiintyy pääasiassa ulostenäytteissä ja niiden merkitys sairauksien aiheuttajana on vielä epäselvä. hBoV1 aiheuttaa lapsilla useita eri hengitystieinfektioiden taudinkuvia, joihin kuuluvat tavallinen flunssa, akuutti välikorvatulehdus, keuhkokuume, bronkioliitti, toistuva uloshengitysvaikeus ja astman pahenemisvaihe. hBoV1-DNA:ta voi esiintyä hengitysteiden eritteissä kuukausia akuutin infektion jälkeen. Tämän vuoksi akuutin hBoV1-infektion diagnosoinnissa ei voida käyttää tavallista DNA-PCR-menetelmää. Diagnostiikassa tarkempia menetelmiä ovat kvantitatiivinen PCR sekä serologia. Uusilla tutkimusmenetelmillä, kuten hBoV1-mRNA -tunnistuksella ja hbov1-antigeenintunnistuksella on saatu lupaavia tuloksia. Menetelmien kliininen spesifisyys on tarkempi.

Akuutin hBoV1-infektion diagnosoimiseksi suositellaan kirjallisuuskatsauksen perusteella, että ainakin kahden seuraavista kriteereistä tulisi täyttyä: suuri DNA-määrä tai mRNA:n esiintyminen nenänielueritteissä sekä positiivinen IgM, matala IgG-aviditeetti tai nelinkertainen IgG-tason kasvu pariseeruminäytteissä.

Avainsanat: Bokavirus, ylähengitystieinfektio

Human bocaviruses: common but not widely known

Andreas Christensen[1,2], Olli Kesti[3], Varpu Elenius[3], Anna L. Eskola[4], Henrik Døllner[2,5], Can Altunbulakli[6], Cezmi A. Akdis[6], Maria Söderlund-Venermo[7], Tuomas Jartti[3]

Affiliations

[1]Department of Medical Microbiology, St. Olavs Hospital, Trondheim, Norway

[2]Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway

[3]Department of Paediatrics, Turku University Hospital and University of Turku, Turku, Finland

[4]Department of Education, University of Turku, Turku, Finland

[5]Department of Pediatrics, St. Olavs Hospital, Trondheim, Norway

[6]Swiss Institute of Allergy and Asthma Research, University of Zürich and Christine Kühne-Center for Allergy Research and Education, Davos, Switzerland

[7]Department of Virology, University of Helsinki, Helsinki, Finland

Running head: Human bocavirus

Correspondence to:
Dr. Andreas Christensen, Department of Medical Microbiology, St. Olavs Hospital, P.O. Box 3250 Sluppen, 7006 Trondheim, Norway. Phone: +4772573085, Email: andreas.christensen@stolav.no

Dr. Tuomas Jartti, Department of Paediatrics, Turku University Hospital, P.O. Box 52, FIN-20520 Turku, Finland. Phone: +358 40 7270 284. Email: tuomas.jartti@utu.fi. Fax: +358 2 313 1460.

Funding: The Sigrid Jusélius Foundation and the Life and Health Medical Grant Association, Helsinki, Finland

Keywords: Parvovirus; human bocavirus; infection; parvovirus; respiratory tract infection

Summary

Human bocavirus 1 (HBoV1), belonging to the *Parvoviridae* family was discovered in 2005 in nasopharyngeal samples from children with respiratory tract infections (RTIs). Three additional bocaviruses, HBoV2-4, were discovered in 2009-2010. These viruses have mainly been found in faecal samples, and their role in human diseases is still uncertain. HBoV1

causes a wide spectrum of respiratory diseases in children including common cold, acute otitis media, pneumonia, bronchiolitis and asthma exacerbations. HBoV1 DNA can persist in airway secretions for months after an acute infection. Consequently, acute HBoV1 infection cannot be diagnosed with standard DNA PCR; quantitative PCR and serology are better diagnostic approaches. Because of their high clinical specificity, diagnostic developments such as HBoV1 mRNA and antigen detection have shown promising results. This review summarizes the knowledge on human bocaviruses, with special focus on HBoV1.

Key messages

- Human bocaviruses (HBoVs) are common in humans
- Four types of designated HBoV have been described HBoV1-4
- HBoV1 is associated with respiratory tract infections in children
- HBoV2-4 are mainly detected in fecal samples and their pathogenic potential is uncertain
- HBoV1 infection should not be diagnosed on the basis of HBoV1 DNA detection alone
- Quantitative HBoV1 DNA analysis, serology or HBoV1 mRNA detection are recommended diagnostic approaches

Introduction

Human bocavirus 1 (HBoV1) was discovered in 2005, in nasopharyngeal aspirates from children with respiratory tract infections, and belongs to the *Parvoviridae* family. The closest relatives to HBoV1 are found in animals, whereas the well-known human parvovirus B19 is more distantly related.[1] Accumulating evidence, since 2005, has been obtained in support of HBoV1 being a genuine human pathogen causing mild to severe respiratory tract infections in children.[2-13] Because of the absence of animal models for HBoV1 infection, the evidence is mainly epidemiological and clinical, including both case-control and longitudinal studies using stringent diagnostic criteria based on serology, quantitative PCR, HBoV1 monoinfection, or HBoV1 mRNA detection.[14] HBoV1 can be detected in airway samples from up to a quarter of children with upper or lower respiratory tract infections (appendix).

Since 2009, three additional bocaviruses designated HBoV2–4 have been discovered.[15-17] These viruses have mainly been detected in faecal samples with detection ranging from 1% to more than 40% in children both with and without gastrointestinal illnesses (appendix). HBoV DNA has also been detected in serum, cerebrospinal fluid, urine, tonsillar tissue, tumour

tissue and even in sewage and river water.[2,18-24] Clinical implications of these non-respiratory findings are uncertain.

From a clinical perspective, HBoV1 seems to be the most important of the human bocaviruses and should be part of a standard test repertoire for respiratory tract infections in children admitted to hospital. However, detection of HBoV1 DNA in nasopharyngeal aspirates from healthy children is also common, which leads to a low clinical specificity for the widely used HBoV1 DNA PCR method.[2,7,13,25] Therefore, accurate diagnosis of HBoV1 infections should not be based on qualitative PCR alone. During the past 10 years, improved diagnostic approaches based on serology, quantitative DNA analysis, mRNA detection, and antigen detection have been developed.[2,8,11,12,26] Despite the high prevalence of paediatric HBoV1 infections, the virus is still not recognised by many clinicians. In this Review, we give an overview of the knowledge regardingt human bocaviruses with an emphasis on clinical features and diagnostic implications. The epidemiology, basic virology, and pathogenesis of bocaviruses are briefly discussed.

Search strategy and selection criteria

We searched the PubMed database for articles published in English between May 23, 2011, and April 30, 2018, with “bocavirus” as a search term (508 hits). Of those articles, we only included major molecular studies (new data on the bocavirus biology or diagnostics) and clinical studies (sample size over 100 cases unless otherwise valuable ie, unique diagnostics or patient groups). For an overview of literature published before May 23, 2011, we refer mainly to previous reviews on human bocaviruses.[14,27]

Virus genome and structure

Parvoviruses are small (approximately 25 nm) non-enveloped viruses, with icosahedral T=1 capsid symmetry and a linear single-stranded DNA genome of 4-6 kb in length, with palindromic hairpin structures at both ends. One full-length HBoV1 genome (including hairpin ends) has been sequenced and it is 5543 nucleotides (nt) long with non-identical terminal hairpins of 140 nt and 122 nt (GenBank number JQ923422).[28] Most of the encapsidated HBoV1 DNA strands have a negative polarity.[29]

The HBoV1 genome contains two main reading frames, the left (3') half of the negative-strand genome encoding the non-structural NS1-4 proteins, and the right (5') half encoding the structural capsid proteins VP1-3, where VP3 is the major capsid protein (figure 1).[14,30-33] Unlike other parvoviruses, bocaviruses also have a smaller third middle reading frame encoding a unique nuclear protein, NP1, important in viral DNA replication and mRNA processing.[34,35] HBoV1 has been shown to express a 140 nt non-coding RNA (Boca SR) at the right-hand side of the negative-sense genome that is required for NS protein expression.[36] Intraspecies recombination has been shown for all four human bocaviruses,[17] and a recombination event between HBoV1 and a common ancestor of HBoV2 and HBoV4 might have led to the formation of HBoV3.[37]

The HBoV1 capsid follows the typical parvovirus structure comprising 60 copies of the major capsid protein motif. The capsid structure has been determined to 2.9Å resolution by cryo-electron microscopy and three dimensional image reconstruction.[38] The inner core of the capsid is formed by an α-helix and an eight-stranded β-barrel structure, typical of parvoviruses.[39] Long amino acid loops between the β-strands shape the surface of the capsid. Some features of this topology are shared with many other parvoviruses, including an open channel at the 5-fold axes with a surrounding depression, another depression at the 2-fold axes and protrusions at the 3-fold axes. The 5-fold channel is used for externalisation of VP1u, packaging, and uncoating of viral DNA. HBoV1-specific variable-loop regions, which are important for infectivity and antigenicity have also been identified on the capsid.[38] The viral capsid surface is involved in many processes, including host tropism, cell recognition, intracellular trafficking, genome packaging and the immune response.[38,39]

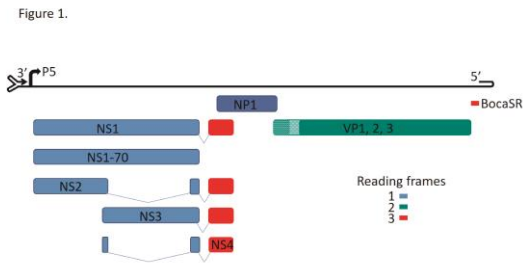

Figure 1: Human bocavirus 1 genome structure

The 5543 nucleotide human bocavirus 1 linear negative-sense single-stranded DNA genome with hairpin termini is depicted as a black line, with the P5 promoter as a black arrow. Below the genome, open reading frames are depicted in blue and red boxes for the non-structural

(NS1-4 and NP1) genes and in green for the overlapping structural (VP1-3) genes, expressed in a ratio of 1:1:10. The non-coding RNA (Boca SR) is depicted in red. Spliced introns are indicated as thin lines.

Epidemiology

In several worldwide clinical studies, HBoV1 has been one of the most commonly detected respiratory viruses in young children with respiratory tract infections (figure 2). By using qualitative PCR analysis of respiratory tract secretions, HBoV1 DNA has been detected in 0.7-25% of children with upper respiratory tract infections and 0.8-23% with lower lower respiratory tract infections (appendix). However, because of the persistence of the virus, some studies have also detected HBoV1 DNA at a similar prevalence in healthy controls.[7,13,25,40,41] HBoV1 DNA has mainly been detected in children younger than 5 years old, both with and without respiratory tract infections.[13,25,42,43] HBoV1 appears throughout the year, and no clear seasonality has been observed in epidemiological studies that controlled for sampling frequency.[7,13] In adults with respiratory illness, HBoV1 has been detected in less than 10% of cases, and in most studies, less than 1% of cases (appendix).

HBoV2-4 DNA has been detected mainly in faecal samples (Appendix). However, the prevalence of human bocaviruses in patients without diarrhoea is similar to that in patients with gastrointestinal diseases.[44] Importantly, HBoV2 DNA has been shown to be present in faecal samples for months, possibly explaining the similar prevalence.[8] HBoV1 has also been detected in faeces, presumably representing the intestinal passage of the viruses secreted from the respiratory tract in children either with or without accompanying respiratory symptoms.[44]

The prevalence of acute HBoV1 infections could only be determined after the development of serodiagnosis. In one study, maternal antibodies were shown to be present in 90% of children younger than 3 months old, after which seropositivity decreased, reaching low antibody detection when the child was between 6 and 12 months.[45] After infancy, HBoV seroprevalence increases until age 6 years, by which time 90-100 % of children have circulating antibodies against at least one of the four human bocaviruses.[8,46] HBoV1 IgG antibody concentrations remain high during adulthood, probably because of the immunity boost caused by circulating HBoV1 or by an infection of related HBoV2, HBoV3, or both.[8,9,46,47] Antibody cross- reactivity can occur between the different bocaviruses, but this problem can be overcome by blocking the antibodies recognising common epitopes by a competition enzyme immune-assay.[8,46] At age 6 years, the seroprevalence of HBoV1 is already around 80%, for HBoV2 it is 50%, for HBoV3 10%, and for HBoV4 it is close to

zero, when cross reactivity is controlled for.[8,46] However, immunological so-called original antigenic sin results in further complication by giving an underestimate of the seroprevalence. If a child has encountered an HBoV2 infection before the HBoV1 infection, a specific antibody response will not always be induced to HBoV1, but instead the child will show a boosted HBoV2 response.[8,48] An estimation can be made that no more than 20-25% of patients positive for HBoV1 DNA have an acute infection on the basis of serodiagnosis.[2,8]

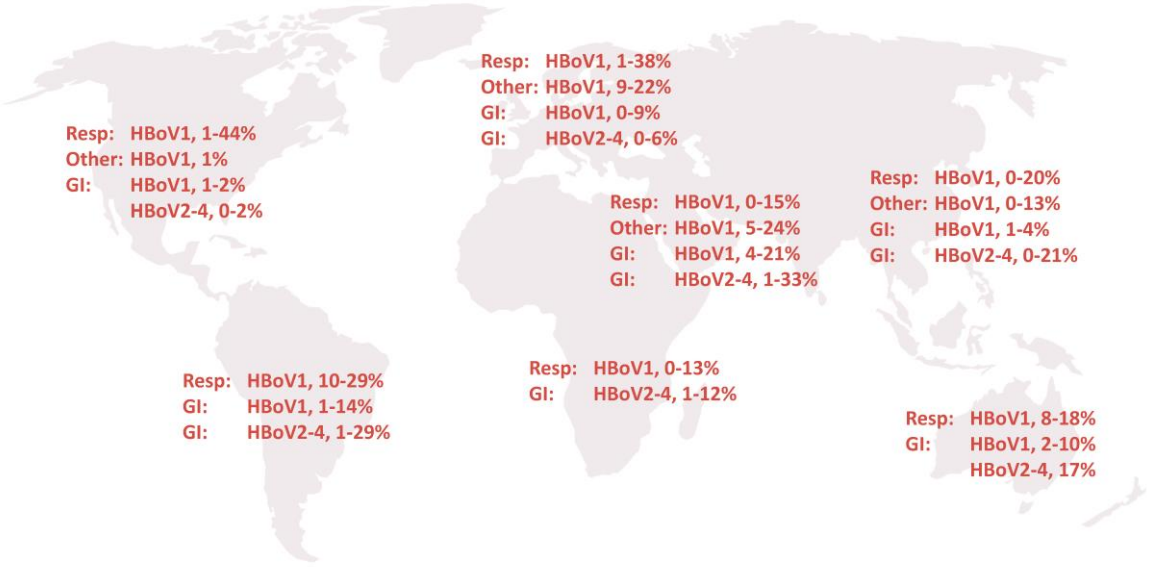

Figure 2: Geographical distribution of HBoV1-4 detected in patients with respiratory, gastrointestinal, and other diseases
HBoV=human bocavirus. Other diseases include encephalitis, hepatitis, tonsillitis, and malignant tumours. Values indicate percentages of each virus detected in each disease group for each region.

Co-detections

Multiple viral detections are common in the respiratory tract of young children, and HBoV1 is frequently detected together with other respiratory viruses (appendix). In as many as three quarters of HBoV1 DNA-positive respiratory samples, at least one other respiratory pathogen can be detected.[13,40,49-51] Prolonged shedding, lasting for months after the primary infection, has been demonstrated in a number of studies.[7,9,40,41,52,53] Additionally, according to one longitudinal study of saliva in infants, a third of the first encounters with HBoV1 might occur without associated new-onset cough or rhinorrhoea. However, whether those encounters were acute primary infections, saliva contaminations of other day-care infants, or whether these children had maternal antibodies protecting them from disease was not verified.[7] Given the prolonged shedding of HBoV1 and the fact that children younger than age 2 years can have

from six to ten respiratory infections per year,[54] DNA from HBoV1 might frequently be co-detected with other infections and also be observed in asymptomatic children.

However, even in respiratory samples containing actively transcribing HBoV1, other viruses have been detected in almost 60% of the cases.[12] This detection raises the question of whether HBoV1 also has a more active or synergetic role in multiple respiratory infections. A few on HBoV1 have been published; [2,13,43,49,55,56] however, investigating the interactions between respiratory viruses is demanding because large patient and control groups are needed to get enough samples containing each virus combination in question. Good evidence supporting the hypothesis that other viruses would modify HBoV1 symptomatology by acting either synergistically or antagonistically has not been provided. However, one study showed that HBoV1 suppressed rhinovirus-associated immune responses.[55] In the study, HBoV1 was associated with a reduction of rhinovirus' potential to cause new wheezing episodes for up to 2 years after the acute infection. This finding could be valuable and warrants further study.

Detection of other gastrointestinal viruses in HBoV2-positive and HBoV3-positive faecal samples from children with gastroenteritis have also been shown (appendix). In 44-100% of the samples, at least one other gastrointestinal virus has been detected. However, the prolonged shedding of HBoV2 in faecal samples could be interfering with the interpretation of results.[8] The occurrence of HBoV4 in studies done so far has been too low for any conclusions to be drawn.

Transmission

HBoV1 DNA has primarily been detected in samples from the respiratory tract and is most likely transmitted through the respiratory route (appendix). Conversely, HBoV2-4 has been detected in faecal samples and spread most likely faecal-oral route (appendix). Vertical transmission of human bocaviruses from mother to fetus has not been shown. HBoV DNA has been detected in plasma from healthy children and adults (including blood donors); [57-59] however, whether this detection represents transmissible viruses or circulating non-infectious HBoV DNA remains to be determined. In another study, HBoV DNA was not detected in any of 48 batches of coagulation factor products.[60]

Pathogenesis

Because of the difficulty of culturing replicative viruses with cell lines, and the absence of experimental animal models to mimic infection, the pathogenesis of human bocaviruses has been poorly studied .[27] However, studies have identified the mechanisms of HBoV1 infection in human cells. HBoV1 has been shown to disrupt epithelial barrier function [28,61] and induce a DNA damage response in in-vitro polarised airway epithelia cultures.[62] HBoV1 infection induces NLRP3 inflammasome activation and caspase 1-induced cell death mediated by pyroptosis. Additionally, epithelial cells produce high amounts of interleukin (IL)-1α and IL-18 throughout the course of infection, which also leads to the death of bystander cells. Anti-apoptotic genes *BIRC6* and *IFI6* are upregulated in epithelial cells upon HBoV1 infection. The skewing of cells into pyroptosis as opposed to apoptosis has been postulated as a mechanism for HBoV1 to establish a persistent infection.[63]

Patients with infections caused by HBoV1 show elevated concentrations of interferon (IFN)-γ, IL-2 and IL-4.[64] In-vitro stimulation of CD4 T cells with HBoV1 virus-like particles causes increased secretion of IL-10, IFN-γ and IL-13. On the other hand, the induction of T-helper-2 cytokines and pro-inflammatory molecules upon HBoV1 infection has been suggested as a factor contributing to asthma exacerbations.[65,66] On the other hand, HBoV1 has also been associated with markedly reduced rhinovirus-induced T-helper-1 and T-helper-2 proinflammatory responses.[55]

Persistence

HBoV1 DNA has been detected in the nasopharynx of children up to 12 months after the primary infection.[7]However, whether HBoV1 can establish latency by integration into the host cell genome or as an episome remains unknown.[67] As mentioned previously, the prolonged presence of the virus in the airways is a possible explanation for the high detection among healthy children observed in most clinical studies. Prolonged and intermittent HBoV1 excretion after primary infection has been documented in both immunocompetent and immunocompromised children.[7,52,53,68-70] The mechanisms of persistency, reactivation and reinfection of HBoV1 are poorly understood. However, a few studies have shown that HBoV1 DNA is common in tonsillar tissue from children with hypertrophic tonsils.[23,71,72] These

findings indicate that tonsils, and adenoids might be the source of prolonged HBoV1 shedding and that they are interesting targets for future studies on HBoV1 persistence.

Diagnosis

The diagnosis of acute viral respiratory tract infections usually relies on qualitative PCR-based assays detecting virus genomes in respiratory samples. However, for the diagnosis of HBoV1 infections, use of such assays is not feasible as the prolonged persistence of HBoV1 DNA in the airways complicates the interpretation of a positive test result.[7,9,40,41,52,53] The detection of HBoV1-specific IgM and of an increase or seroconversion of IgG provides a higher specificity than qualitative PCR-based assays.[2] Serological tests could have low sensitivity during acute infections because of late seroconversion (figure 3). However, a positive IgM together with low IgG avidity, or a 4-fold increase of IgG titre, in paired serum samples, are criteria ensuring a specific diagnosis of acute HBoV1 infection.[2,73] However, two caveats interfere with serodiagnosis of HBoV1-4: cross-reactivity and original antigenic sin, both of which need to be taken into consideration when doing HBoV1 serodiagnosis .[8,48]

Some studies have focused on the clinical value of a high HBoV1 DNA load (a load more than 10^4 or 10^6 copies per mL) in respiratory secretion, but their results have been conflicting.[2,4,7,13,40,49] The conflict results can be explained by confounding effects of varying frequencies of co-detections in the patient and control groups, type of diagnostic reference method, sample type, or varying quantitative PCR test performances. Studies that use HBoV1 serology or mRNA detection as reference standard have reached more consistent results and can provide a basis for defining a clinical cut-off quantity.[2,11,12,74] Concentrations from 10^4 to 10^8 HBoV1 DNA copies per mL of nasopharyngeal secretions have been suggested to indicate acute HBoV1 infection.[2,7,11,12,74] With HBoV1 mRNA as the reference for the performance of quantitative PCR (>10^6 copies per mL), sensitivity of 100%, specificity of 93 – 99% and positive predictive values (PPV) of 56-87% have been reported.[12,74] With serodiagnosis as standard, the performances of quantitative PCR sensitivity was 81%, specificity was 92%, and PPV was 87%.[74] Moreover, in another study, eight (38%) of 21 symptomatic children with HBoV1 DNA loads less than 10^4 copies per mL had a serologically confirmed acute infection, compared with 27 (96%) of 28 children with DNA loads more than 10^4 copies per mL.[2] Therefore, the clinical sensitivity of measuring high HBoV1 DNA loads seems to be moderate.

Presence of HBoV1 DNA in plasma or serum (DNAemia) seems to be specific for acute HBoV1 infections. DNAemia is rarely detected in controls,[2,8,13] although it has been detected in healthy blood donors.[57,58] The clinical sensitivity of this test is uncertain and in one study was found to be lower than the sensitivity for HBoV1 mRNA detection.[12] The duration of DNAemia during the acute infection is short.[2,9]

Other promising diagnostic approaches are the detection of HBoV1 mRNA by RT-PCR or HBoV1 antigen by immunodetection.[12,26] Studies on the HBoV1 mRNA test have shown high specificity for children with upper or lower respiratory tract infections.[10-12,74] The clinical sensitivity of the test depends on the duration of HBoV1 mRNA expression in the nasopharynx, and follow-up studies to address this question are needed. Antigen tests generally have lower sensitivities than tests based on nucleic acid detection. However, antigen tests are simple and robust tests that are well suited for outpatient use. Considering the very low specificity of HBoV1 DNA PCRs, antigen tests might provide an overall improvement, even with a lower sensitivity. An automated HBoV1 antigen test was released in 2014, and further investigation regarding the test's sensitivity and specificity are underway.[26]

We recommend that at least two of the following five factors should be present for the diagnosis of an acute primary HBoV1 infection: high DNA load by quantitative PCR (>10^6 HBoV1 DNA copies per mL of nasopharyngeal secretions), HBoV1 mRNA in nasopharyngeal secretions, positive IgM, low IgG avidity, or a 4-fold increase or more of IgG levels in paired serum samples. Since the role of HBoV2-4 in gastrointestinal diseases has not yet been clarified, diagnostic recommendations cannot be given for these viruses.

Clinical features

The clinical manifestations of a respiratory tract infection caused by HBoV1 are very similar to those of respiratory tract infections caused by other respiratory viruses, the most common being cough, fever, common cold, dyspnoea, diarrhoea and vomiting.[7,41] In hospital-based studies, the most frequent diagnoses have been rhinitis, acute otitis media, pneumonia, bronchiolitis and asthma exacerbations.[13,49,51,75] In case-control studies, statistically significant associations have been found between HBoV1 (DNAemia, monoinfection, or high HBoV1 DNA load in nasopharyngeal aspirate) and lower respiratory tract infections.[13,49,75] Population-based studies with control groups have shown associations between HBoV1 (high

HBoV1 load or serodiagnosis) and symptoms of upper respiratory tract infections such as cough and rhinorrhoea, and the diagnosis of acute otitis media.[7-9]

In studies comparing HBoV1 infections with respiratory syncytial virus infections, bronchiolitis seems to be more common among children infected with respiratory syncytial virus, and pneumonia among children infected with HBoV1. [2,43,51] These differences might occur because of differences in patient age. HBoV1-induced bronchiolitis ranks third after respiratory syncytial virus and rhinovirus-induced bronchiolitis, and disease severity appears similar when age differences are adjusted for.[2] 17 cases of severe, life-threatening and even fatal respiratory HBoV1 infections have been reported.[76-85] Most of these studies have been done in children younger than 2 years old with lower respiratory tract infections and respiratory failure. Ten of the children were born prematurely and seven had no known previous illnesses or risk factors. HBoV1 was the sole virus detected in all cases, and in four cases the diagnosis was confirmed by serology.[78,80,83,84] The most common clinical manifestation was an obstructive lower respiratory tract infection (figure 4). Two of the children died from respiratory failure. Furthermore, HBoV1 can exacerbate chronic pulmonary diseases like asthma, chronic obstructive pulmonary disease, and cystic fibrosis.[86,87]

C-reactive protein concentrations and white blood cell counts are usually within normal concentrations or only slightly elevated during acute HBoV1 infections.[2,43,49] Chest radiography frequently shows peribronchial or interstitial infiltrates, hyperinflation or atelectasis.[43,51,75] In one study, interstitial infiltrates were observed in 75% of children hospitalised with lower respiratory tract infections that were associated with serologically confirmed HBoV1 infections.[88]

In controlled studies on adults and patients older than 65 years, HBoV1 DNA has rarely been detected in respiratory samples from patients with or without a respiratory tract infection (appendix). Additionally, sample sizes have been too small for conclusions to be drawn on the clinical significance of the results.

HBoV2-4 DNA has been detected almost exclusively in faecal samples, but their causal role in gastrointestinal disease is uncertain. For HBoV2 and 3, equal prevalence has been found in asymptomatic children and in children with gastroenteritis (appendix). Additionally, studies investigating viral DNA load and serology have not shown associations with gastrointestinal disease.[44,89] Diagnostic challenges similar to those described for respiratory tract infections

are present because of persisting viruses. Further studies based on serology, viral DNA load, and parameters for viral gene expression are needed for elucidating the full role of human bocaviruses in gastroenteritis. For HBoV4, detection has been too low for any conclusions to be drawn on disease associations.

A few case reports have been published describing encephalitis, hepatitis, and myocarditis in patients infected with HBoV1 – 3.[18-22,90] Although no studies have proved a causal relation, the cases of encephalitis are especially intriguing because HBoV1 – 3 were the only infectious agents detected in cerebral spinal fluid.[18,20,21] In one study, whole parvovirus particles were detected in cerebral spinal fluid by electron microscopy.[18] HBoV1 DNA has been detected in tissue from hypertrophic tonsils or adenoids in children.[23,71,72] The pathogenic role of the viruses in these tissues remains uncertain.

Due to the high prevalence of HBoV antibodies in adulthood, primary infections are probably extremely rare in pregnancy. HBoV infection has not been detected in stillborn children or hydrops fetalis.[91,92]

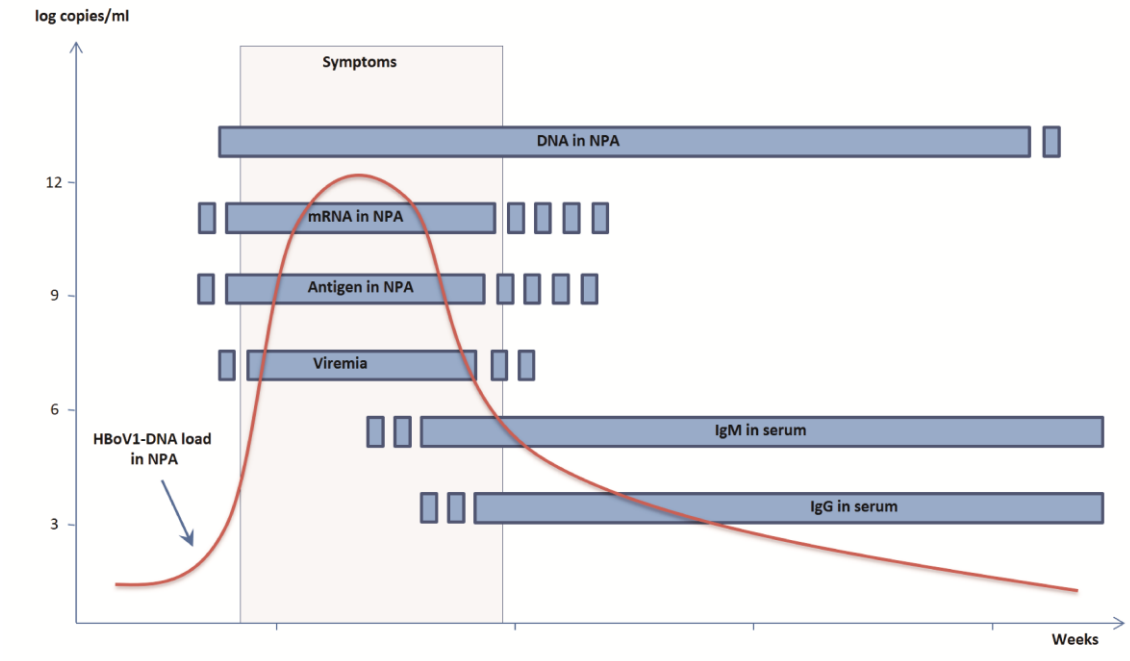

Figure 3: Temporal pattern of clinically relevant variables through the course of an acute primary HBoV1 infection

HBoV1=human bocavirus 1. NPA= nasopharyngeal aspirate. Development of HBoV1 DNA load in NPA is shown by the red curve. All other parameters are represented qualitatively by blue bars. Dotted ends of the blue bars indicate variation or uncertainty.

Risk factors

Risk factors for severe HBoV1-associated illness are similar to those for respiratory viral infections: underlying chronic medical conditions such as cardiac or pulmonary disease, prematurity with chronic lung disease, cancer or immunosuppression. Many cases of severe illness associated with HBoV1 have been published in these patient groups.[68,69,93,94] As HBoV1 infections seem to produce a long-lasting, high-avidity IgG antibody response,[2,73] a suppressed B-cell immunity would expectedly increase the risk of HBoV1 infection. In immunocompromised patients, the detection of HBoV1 DNA in respiratory samples has been reported to be associated with fever, lower respiratory symptoms, seizures, encephalitis, hepatitis, and gastrointestinal symptoms.[69,70,95,96] However, HBoV1 DNA has also been detected in the blood of asymptomatic immunocompromised children, [97] and one patient showed a prolonged DNAemia for more than 4 weeks. Further studies are needed to clarify the role of both acute and persistent HBoV infections in immunocompromised patients. Young age can be considered a risk factor for infection with HBoV1, as these infections occur most commonly in children younger than 2 years old.[7,13,27] However, maternal antibodies can protect children younger than 6 months old.[45] Other risk factors for HBoV1-induced respiratory illness among children younger than 1 year old are maternal smoking, winter birth time, and a family history of asthma.[41] Atopy (allergen-specific sensitisation) is not associated with HBoV1 infection among wheezing children.[98] Human bocaviruses have also been detected in sewage and river water, thus raising the question of whether proximity to sewage could be a further risk factor.[24] The clinical implications of these findings have not yet been elucidated.

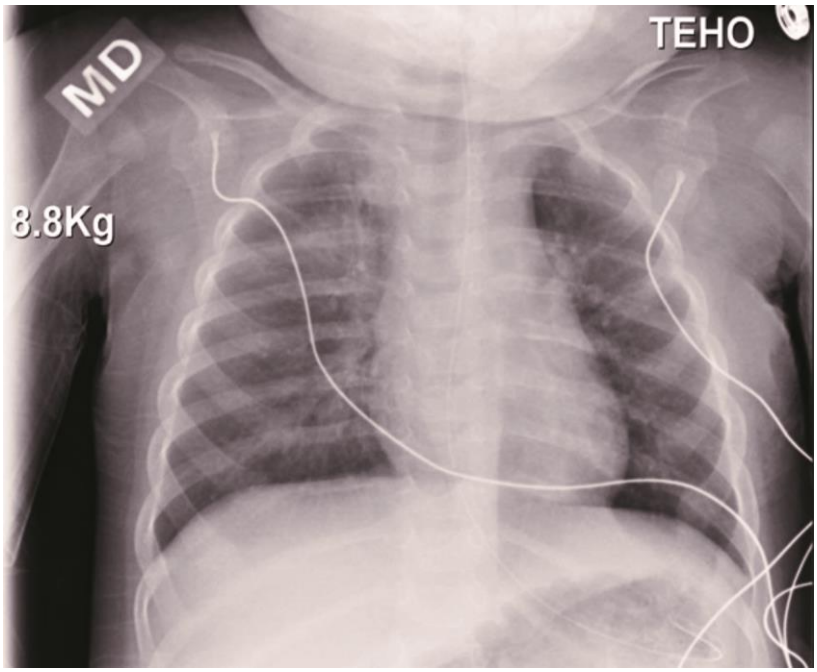

Figure 4: Chest radiograph of 10-month-old girl with bocavirus-induced pneumonia

A 10-month-old girl who had bocavirus-induced pneumonia was an A-gemini born at gestational week 28 and 6 days. Her weight at birth was 1115 g and neonatal period was non-complicated. She received propranolol hydrochloride medication to treat small skin hemangiomas. The illness started with symptoms of the common cold (stuffy nose, cough) a day before admission to the pediatric emergency room of Turku University Hospital, Turku, Finland. At examination her general condition was good and oxygen saturatio 97%, but respiratory rate was at the upper limit (48 breaths per min).Human bocavirus 1 (HBoV1) was detected by using a rapid antigen detection test (Maripoc, Turku, Finland). The mild respiratory distress was relieved by salbutamol inhalation and she was discharged. However, she came back to the emergency room later that day. Lung mucus viscosity was increased, she was tired, and the respiratory distress was advanced (respiratory rate 60 breaths per min, obstructive lung auscultation).Her C-reactive protein 1 mg/L and blood leukocyte count 10.4 10e9/l. Salbutamol helped only partially and she was admitted to the regular ward. The next day her general condition collapsed, she was hypotonic, and reporting breathing difficulties. She was admitted to the pediatric intensive care unit where her venous blood pH was 7.22,partial pressure of carbon dioxide was 7.3, base excess -5, bicarbonate was 19.3. A chest radiograph was suggestive of viral pneumonia. The complete respiratory virus panel was tested from nasopharyngeal sample with PCR in the Department of Virology, University of Turku, Turku, Finland. HBoV1 (high concentration) was the only positive finding. She was stabilized by continuous salbutamol inhalation, intravenous corticosteroid, and discontinuation of propranolol hydrochloride. HBoV1 was also detected in her plasma by PCR. After 2 days she was relocated back to regular ward where she received regular salbutamol inhalations and amoxycillin for acute otitis media infection. Finally, 2 days later, she was discharged from the hospital. In the follow-up call 3 days later, the patient appeared to have improved. Propranolol hydrochloride was continued and she did not relapse.

Treatment

HBoV infections do not yet have an approved specific treatment, and no comparative studies on antiviral drugs have been done. Prednisolone was not found effective in a pos-hoc analysis of a randomised controlled trial on wheezing children with serologically confirmed HBoV1 infection.[99] Therefore, the treatment of choice is supportive care and the most important types are bronchodilation and respiratory support for children with severe bronchiolitis, wheezing, or pneumonia. Although HBoV1 has been associated with acute respiratory infections, the

disease is often self-limiting and uncomplicated. No specific preventive measures are available.

Further research

Good progression has been made regarding the epidemiology and diagnostics of HBoV1 infections; however, several areas remain for which relatively little is known. No exact data regarding transmission of the virus or incubation time exists. Reports concerning HBoV1 pathogenesis are scare and partly contradictory, and cell tropism, cytokine responses, immunogenicity, tissue persistence, and role in asthma development remain unknown. To learn whether HBoV1 can truly modify responses of other virus infections, and via which mechanisms, is of crucial importance. Prolonged respiratory shedding of HBoV1 is clearly common, but the mechanisms and form of its persistence are not known. Although HBoV1 can cause severe and even fatal lower respiratory tract infections, specific treatment or prophylaxis remains unavailable.

Conclusions

Increasingly evidence shows that HBoV1 is an important respiratory pathogen. Overall, a substantial amount of data are now available regarding HBoV1, whereas relatively little is known about other human bocaviruses. Although most studies have been based on PCR detection of HBoV1 in respiratory tract secretions, only few have confirmed the HBoV1 diagnosis with more specific procedures. Application of more stringent diagnostic methods and criteria will be crucial for the future. Our recommendation is that at least two of the following five factors should be present in diagnosing acute primary HBoV1 infection: high DNA load in nasopharyngeal secretions, mRNA present in nasopharyngeal secretions, positive IgM, low IgG avidity, or 4-fold increase in IgG titre in paired serum samples.

Contributors

All authors participated in the critical evaluation of literature search and in writing the Review.

Declaration of interests

CAA reports grants from Allergopharma, grants from Actellion, grants from Swiss National Science Foundation, grants from Christine Kühne-Center for Allergy Research and Education, from European Commission's Horison's 2020 Framework Programme, Cure, (outside the submitted work).

TJ reports grants from The Sigrid Jusélius Foundation during the conduct of the study.

AC, OK, VE, ALE, HD, CA and MSV have no conflicts of interest to declare.

Acknowledgements

This work was funded by the Sigrid Jusélius Foundation and the Life and Health Medical Grant Association, Helsinki, Finland.

References

1 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 USA* 2005; **102**: 12891-6.

2 Soderlund-Venermo M, Lahtinen A, Jartti T, et al. Clinical assessment and improved diagnosis of bocavirus-induced wheezing in children, Finland. *Emerg Infect Dis* 2009; **15**: 1423-30.

3 Nascimento-Carvalho CM, Cardoso MR, Meriluoto M, et al. Human bocavirus infection diagnosed serologically among children admitted to hospital with community-acquired pneumonia in a tropical region. *J Med Virol* 2012; **84**: 253-8.

4 Zhao B, Yu X, Wang C, et al. High Human Bocavirus Viral Load Is Associated with Disease Severity in Children under Five Years of Age. *PLoS One* 2013; **8**: e62318.

5 Zhou L, Zheng S, Xiao Q, et al. Single detection of human bocavirus 1 with a high viral load in severe respiratory tract infections in previously healthy children. *BMC infectious diseases* 2014; **14**: 424.

6 Jiang W, Yin F, Zhou W, Yan Y, Ji W. Clinical significance of different virus load of human bocavirus in patients with lower respiratory tract infection. *Scientific reports* 2016; **6**: 20246.

7 Martin ET, Kuypers J, McRoberts JP, Englund JA, Zerr DM. Human Bocavirus 1 Primary Infection and Shedding in Infants. *J Infect Dis* 2015; 212: 516-24.

8 Kantola K, Hedman L, Tanner L, et al. B-Cell Responses to Human Bocaviruses 1-4: New Insights from a Childhood Follow-Up Study. *PLoS One* 2015; **10**: e0139096.

9 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**: 264-71.

10 Schlaberg R, Ampofo K, Tardif KD, et al. Human Bocavirus Capsid Messenger RNA Detection in Children With Pneumonia. *J Infect Dis* 2017; **216**: 688-96.

11 Proenca-Modena JL, Gagliardi TB, Escremim de Paula F, et al. Detection of human bocavirus mRNA in respiratory secretions correlates with high viral load and concurrent diarrhea. *PLoS ONE* 2011; **6**: e21083.

12 Christensen A, Dollner H, Skanke LH, Krokstad S, Moe N, Nordbo SA. Detection of spliced mRNA from human bocavirus 1 in clinical samples from children with respiratory tract infections. *Emerg Infect Dis* 2013; **19**: 574-80.
13 Christensen A, Nordbo SA, Krokstad S, Rognlien AG, Dollner H. Human bocavirus in children: mono-detection, high viral load and viraemia are associated with respiratory tract infection. *J Clin Virol* 2010; **49**: 158-62.
14 Qiu J, Soderlund-Venermo M, Young NS. Human Parvoviruses. *Clin Microbiol Rev* 2017; **30**: 43-113.
15 Arthur JL, Higgins GD, Davidson GP, Givney RC, Ratcliff RM. A novel bocavirus associated with acute gastroenteritis in Australian children. *PLoS pathogens* 2009; **5**: e1000391.
16 Kapoor A, Slikas E, Simmonds P, et al. A newly identified bocavirus species in human stool. *J Infect Dis* 2009; **199**: 196-200.
17 Kapoor A, Simmonds P, Slikas E, et al. Human Bocaviruses Are Highly Diverse, Dispersed, Recombination Prone, and Prevalent in Enteric Infections. *The Journal of Infectious Diseases* 2010; **201**: 1633-43.
18 Mitui MT, Tabib SM, Matsumoto T, et al. Detection of human bocavirus in the cerebrospinal fluid of children with encephalitis. *Clin Infect Dis* 2012; **54**: 964-7.
19 Akturk H, Sik G, Salman N, et al. Atypical presentation of human bocavirus: Severe respiratory tract infection complicated with encephalopathy. *J Med Virol* 2015; **87**: 1831-8.
20 Mori D, Ranawaka U, Yamada K, et al. Human bocavirus in patients with encephalitis, Sri Lanka, 2009-2010. *Emerg Infect Dis* 2013; **19**: 1859-62.
21 Yu JM, Chen QQ, Hao YX, et al. Identification of human bocaviruses in the cerebrospinal fluid of children hospitalized with encephalitis in China. *J Clin Virol* 2013; **57**: 374-7.
22 Brebion A, Vanlieferinghen P, Déchelotte P, Boutry M, Peigue-Lafeuille H, Henquell C. Fatal Subacute Myocarditis Associated with Human Bocavirus 2 in a 13-Month-Old Child. *Journal of Clinical Microbiology* 2014; **52**: 1006-8.
23 Proenca-Modena JL, Buzatto GP, Paula FE, et al. Respiratory viruses are continuously detected in children with chronic tonsillitis throughout the year. *International journal of pediatric otorhinolaryngology* 2014; **78**: 1655-61.
24 Blinkova O, Rosario K, Li L, et al. Frequent detection of highly diverse variants of cardiovirus, cosavirus, bocavirus, and circovirus in sewage samples collected in the United States. *J Clin Microbiol* 2009; **47**: 3507-13.
25 Byington CL, Ampofo K, Stockmann C, et al. Community Surveillance of Respiratory Viruses Among Families in the Utah Better Identification of Germs-Longitudinal Viral Epidemiology (BIG-LoVE) Study. *Clin Infect Dis* 2015; **61**: 1217-24.
26 Bruning AH, Susi P, Toivola H, et al. Detection and monitoring of human bocavirus 1 infection by a new rapid antigen test. *New microbes and new infections* 2016; **11**: 17-9.
27 Jartti T, Hedman K, Jartti L, Ruuskanen O, Allander T, Soderlund-Venermo M. Human bocavirus-the first 5 years. *Reviews in medical virology* 2011; **22**: 46-64.
28 Huang Q, Deng X, Yan Z, et al. Establishment of a reverse genetics system for studying human bocavirus in human airway epithelia. *PLoS pathogens* 2012; **8**: e1002899.
29 Böhmer A, Schildgen V, Lusebrink J, et al. Novel application for isothermal nucleic acid sequence-based amplification (NASBA). *J Virol Methods* 2009; **158**: 199-201.

30 Cecchini S, Negrete A, Virag T, Graham BS, Cohen JI, Kotin RM. Evidence of prior exposure to human bocavirus as determined by a retrospective serological study of 404 serum samples from adults in the United States. *Clin Vaccine Immunol* 2009; **16**: 597-604.
31 Dijkman R, Koekkoek SM, Molenkamp R, Schildgen O, van der Hoek L. Human bocavirus can be cultured in differentiated human airway epithelial cells. *J Virol* 2009; **83**: 7739-48.
32 Chen AY, Cheng F, Lou S, et al. Characterization of the gene expression profile of human bocavirus. *Virology* 2010; **403**: 145-54.
33 Shen W, Deng X, Zou W, et al. Identification and Functional Analysis of Novel Nonstructural Proteins of Human Bocavirus 1. *J Virol* 2015; **89**: 10097-109.
34 Fasina OO, Dong Y, Pintel DJ. NP1 Protein of the Bocaparvovirus Minute Virus of Canines Controls Access to the Viral Capsid Genes via Its Role in RNA Processing. *J Virol* 2016; **90**: 1718-28.
35 Zou W, Cheng F, Shen W, Engelhardt JF, Yan Z, Qiu J. Nonstructural Protein NP1 of Human Bocavirus 1 Plays a Critical Role in the Expression of Viral Capsid Proteins. *J Virol* 2016; **90**: 4658-69.
36 Wang Z, Shen W, Cheng F, et al. Parvovirus Expresses a Small Noncoding RNA That Plays an Essential Role in Virus Replication. *J Virol* 2017; **91:** e02375-16.
37 Cheng W, Chen J, Xu Z, et al. Phylogenetic and recombination analysis of human bocavirus 2. *BMC infectious diseases* 2011; **11**: 50.
38 Mietzsch M, Kailasan S, Garrison J, et al. Structural Insights into Human Bocaparvoviruses. *J Virol* 2017; **91:** e00261-17.
39 Halder S, Ng R, Agbandje-McKenna M. Parvoviruses: structure and infection. 2012; **7**: 253-78.
40 Martin ET, Fairchok MP, Kuypers J, et al. Frequent and Prolonged Shedding of Bocavirus in Young Children Attending Daycare. *The Journal of Infectious Diseases* 2010; **201**: 1625-32.
41 von Linstow M-L, Høgh M, Høgh B. Clinical and Epidemiologic Characteristics of Human Bocavirus in Danish Infants. *The Pediatric Infectious Disease Journal* 2008; **27**: 897-902.
42 Feng L, Li Z, Zhao S, et al. Viral etiologies of hospitalized acute lower respiratory infection patients in China, 2009-2013. *PLoS One* 2014; **9**: e99419.
43 Ursic T, Jevsnik M, Zigon N, et al. Human bocavirus and other respiratory viral infections in a 2-year cohort of hospitalized children. *J Med Virol* 2012; **84**: 99-108.
44 Paloniemi M, Lappalainen S, Salminen M, et al. Human bocaviruses are commonly found in stools of hospitalized children without causal association to acute gastroenteritis. *Eur J Pediatr* 2014; **173**: 1051-7.
45 Endo R, Ishiguro N, Kikuta H, et al. Seroepidemiology of human bocavirus in Hokkaido prefecture, Japan. *J Clin Microbiol* 2007; **45**: 3218-23.
46 Kantola K, Hedman L, Arthur J, et al. Seroepidemiology of human bocaviruses 1-4. *J Infect Dis* 2011; **204**: 1403-12.
47 Zhao LQ, Qian Y, Zhu RN, et al. Human bocavirus infections are common in Beijing population indicated by sero-antibody prevalence analysis. *Chin Med J (Engl)* 2009; **122**: 1289-92.
48 Li X, Kantola K, Hedman L, Arku B, Hedman K, Soderlund-Venermo M. Original antigenic sin with human bocaviruses 1-4. *J Gen Virol* 2015; 96: 3099-108.
49 Fry AM, Lu X, Chittaganpitch M, et al. Human bocavirus: a novel parvovirus epidemiologically associated with pneumonia requiring hospitalization in Thailand. *J Infect Dis* 2007; **195**: 1038-45.

50 Arden KE, McErlean P, Nissen MD, Sloots TP, Mackay IM. Frequent detection of human rhinoviruses, paramyxoviruses, coronaviruses, and bocavirus during acute respiratory tract infections. *J Med Virol* 2006; **78**: 1232-40.

51 Calvo C, Garcia-Garcia ML, Pozo F, Carballo D, Martinez-Monteserin E, Casas I. Infections and coinfections by respiratory human bocavirus during eight seasons in hospitalized children. *J Med Virol* 2016; **88**: 2052-8.

52 Castro Wagner J, Pyles RB, Miller AL, Nokso-Koivisto J, Loeffelholz MJ, Chonmaitree T. Determining Persistence of Bocavirus DNA in the Respiratory Tract of Children by Pyrosequencing. *Pediatr Infect Dis J* 2016; **35**: 471-6.

53 Nokso-Koivisto J, Pyles RB, Miller AL, Jennings K, Loeffelholz M, Chonmaitree T. Role of Human Bocavirus in Upper Respiratory Tract Infections and Acute Otitis Media. *Journal of the Pediatric Infectious Diseases Society* 2014; **3**: 98-103.

54 Kusel MM, de Klerk NH, Holt PG, Kebadze T, Johnston SL, Sly PD. Role of respiratory viruses in acute upper and lower respiratory tract illness in the first year of life: a birth cohort study. *Pediatr Infect Dis J* 2006; **25**: 680-6.

55 Lukkarinen H, Soderlund-Venermo M, Vuorinen T, et al. Human bocavirus 1 may suppress rhinovirus-associated immune response in wheezing children. *The Journal of allergy and clinical immunology* 2014; **133**: 256-8 e1-4.

56 Sun HM, Sun JM, Ji WM, et al. Impact of RSV Coinfection on Human Bocavirus in Children with Acute Respiratory Infections. *Journal of tropical pediatrics* 2018.

57 Tong R, Shen L, Yin W, et al. Prevalence of human parvovirus B19, bocavirus, and PARV4 in blood samples from the general population of China and lack of a correlation between parvovirus and hepatitis B co-infection. *PLoS One* 2013; **8**: e64391.

58 Bonvicini F, Manaresi E, Gentilomi GA, et al. Evidence of human bocavirus viremia in healthy blood donors. *Diagn Microbiol Infect Dis* 2011; **71**: 460-2.

59 Li H, He M, Zeng P, et al. The genomic and seroprevalence of human bocavirus in healthy Chinese plasma donors and plasma derivatives. *Transfusion* 2015; **55**: 154-63.

60 Modrow S, Wenzel JJ, Schimanski S, et al. Prevalence of nucleic acid sequences specific for human parvoviruses, hepatitis A and hepatitis E viruses in coagulation factor concentrates. *Vox Sang* 2011; **100**: 351-8.

61 Deng X, Yan Z, Luo Y, et al. In vitro modeling of human bocavirus 1 infection of polarized primary human airway epithelia. *J Virol* 2013; **87**: 4097-102.

62 Deng X, Yan Z, Cheng F, Engelhardt JF, Qiu J. Replication of an Autonomous Human Parvovirus in Non-dividing Human Airway Epithelium Is Facilitated through the DNA Damage and Repair Pathways. *PLoS pathogens* 2016; **12**: e1005399.

63 Deng X, Zou W, Xiong M, et al. Human Parvovirus Infection of Human Airway Epithelia Induces Pyroptotic Cell Death by Inhibiting Apoptosis. *J Virol* 2017; **91:** 01533-17.

64 Chung JY, Han TH, Kim JS, Kim SW, Park CG, Hwang ES. Th1 and Th2 cytokine levels in nasopharyngeal aspirates from children with human bocavirus bronchiolitis. *J Clin Virol* 2008; **43**: 223-5.

65 Lindner J, Zehentmeier S, Franssila R, et al. CD4+ T helper cell responses against human bocavirus viral protein 2 viruslike particles in healthy adults. *J Infect Dis* 2008; **198**: 1677-84.

66 Kumar A, Filippone C, Lahtinen A, et al. Comparison of Th-cell immunity against human bocavirus and parvovirus B19: proliferation and cytokine responses are similar in magnitude but more closely interrelated with human bocavirus. *Scandinavian journal of immunology* 2011; **73**: 135-40.

67 Schildgen O, Qiu J, Soderlund-Venermo M. Genomic features of the human bocaviruses. *Future virology* 2012; **7**: 31-9.

68 Schenk T, Maier B, Hufnagel M, et al. Persistence of Human Bocavirus DNA in Immunocompromised Children. *Pediatr Infect Dis J* 2011; **30**: 82-4.

69 Koskenvuo M, Mottonen M, Waris M, Allander T, Salmi TT, Ruuskanen O. Human bocavirus in children with acute lymphoblastic leukemia. *Eur J Pediatr* 2008; **167**: 1011-5.

70 Srinivasan A, Gu Z, Smith T, et al. Prospective detection of respiratory pathogens in symptomatic children with cancer. *Pediatr Infect Dis J* 2013; **32**: e99-e104.

71 Proenca-Modena JL, Pereira Valera FC, Jacob MG, et al. High rates of detection of respiratory viruses in tonsillar tissues from children with chronic adenotonsillar disease. *PLoS ONE* 2012; **7**: e42136.

72 Gunel C, Kirdar S, Omurlu IK, Agdas F. Detection of the Epstein-Barr virus, Human Bocavirus and novel KI and KU polyomaviruses in adenotonsillar tissues. *International journal of pediatric otorhinolaryngology* 2015; **79**: 423-7.

73 Hedman L, Söderlund-Venermo M, Jartti T, Ruuskanen O, Hedman K. Dating of human bocavirus infection with protein-denaturing IgG-avidity assays—Secondary immune activations are ubiquitous in immunocompetent adults. *Journal of Clinical Virology* 2010; **48**: 44-8.

74 Xu M, Arku B, Jartti T, et al. Comparative Diagnosis of Human Bocavirus 1 Respiratory Infection With Messenger RNA Reverse-Transcription Polymerase Chain Reaction (PCR), DNA Quantitative PCR, and Serology. *J Infect Dis* 2017; **215**: 1551-7.

75 Allander T, Jartti T, Gupta S, et al. Human bocavirus and acute wheezing in children. *Clin Infect Dis* 2007; **44**: 904-10.

76 Moesker FM, van Kampen JJ, van der Eijk AA, et al. Human bocavirus infection as a cause of severe acute respiratory tract infection in children. *Clin Microbiol Infect* 2015; **21**: 964.e1-8.

77 Ursic T, Steyer A, Kopriva S, Kalan G, Krivec U, Petrovec M. Human bocavirus as the cause of a life-threatening infection. *J Clin Microbiol* 2011; **49**: 1179-81.

78 Korner RW, Soderlund-Venermo M, van Koningsbruggen-Rietschel S, Kaiser R, Malecki M, Schildgen O. Severe human bocavirus infection, Germany. *Emerg Infect Dis* 2011; **17**: 2303-5.

79 Edner N, Castillo-Rodas P, Falk L, Hedman K, Soderlund-Venermo M, Allander T. Life-threatening respiratory tract disease with human bocavirus-1 infection in a 4-year-old child. *J Clin Microbiol* 2012; **50**: 531-2.

80 Jula A, Waris M, Kantola K, et al. Primary and secondary human bocavirus 1 infections in a family, Finland. *Emerg Infect Dis* 2013; **19**: 1328-31.

81 Ursic T, Krivec U, Kalan G, Petrovec M. Fatal human bocavirus infection in an 18-month-old child with chronic lung disease of prematurity. *Pediatr Infect Dis J* 2015; **34**: 111-2.

82 Calvo C, Garcia-Garcia ML, Blanco C, et al. Human bocavirus infection in a neonatal intensive care unit. *J Infect* 2008; **57**: 269-71.

83 Oikawa J, Ogita J, Ishiwada N, et al. Human bocavirus DNA detected in a boy with plastic bronchitis. *Pediatr Infect Dis J* 2009; **28**: 1035-6.

84 Eskola V, Xu M, Soderlund-Venermo M. Severe Lower Respiratory Tract Infection Caused by Human Bocavirus 1 in an Infant. *Pediatr Infect Dis J* 2017; **36**: 1107-8.

85 Ziyade N, Sirin G, Elgormus N, Das T. Detection of Human Bocavirus DNA by Multiplex PCR Analysis: Postmortem Case Report. *Balkan medical journal* 2015; **32**: 226-9.

86 Zheng XY, Xu YJ, Guan WJ, Lin LF. Regional, age and respiratory-secretion-specific prevalence of respiratory viruses associated with asthma exacerbation: a literature review. *Archives of virology* 2018; **163**: 845-53.

87 Flight W, Jones A. The diagnosis and management of respiratory viral infections in cystic fibrosis. *Expert Rev Respir Med* 2017; **11**: 221-7.

88 Don M, Soderlund-Venermo M, Valent F, et al. Serologically verified human bocavirus pneumonia in children. *Pediatr Pulmonol* 2010; **45**: 120-6.

89 Jin Y, Cheng WX, Xu ZQ, et al. High prevalence of human bocavirus 2 and its role in childhood acute gastroenteritis in China. *J Clin Virol* 2011; **52**: 251-3.

90 Haytoglu Z, Canan O. Bocavirus Viremia and Hepatitis in an Immunocompetent Child. *Balkan medical journal* 2017; **34**: 281-3.

91 Riipinen A, Väisänen E, Lahtinen A, et al. Absence of human bocavirus from deceased fetuses and their mothers. *Journal of Clinical Virology* 2010; **47**: 186-8.

92 Enders M, Lindner J, Wenzel JJ, et al. No detection of human bocavirus in amniotic fluid samples from fetuses with hydrops or isolated effusions. *J Clin Virol* 2009; **45**: 300-3.

93 Longtin J, Bastien M, Gilca R, et al. Human bocavirus infections in hospitalized children and adults. *Emerg Infect Dis* 2008; **14**: 217-21.

94 Volz S, Schildgen O, Klinkenberg D, et al. Prospective study of Human Bocavirus (HBoV) infection in a pediatric university hospital in Germany 2005/2006. *J Clin Virol* 2007; **40**: 229-35.

95 Kainulainen L, Waris M, Soderlund-Venermo M, Allander T, Hedman K, Ruuskanen O. Hepatitis and human bocavirus primary infection in a child with T-cell deficiency. *J Clin Microbiol* 2008; **46**: 4104-5.

96 de Vries JJ, Bredius RG, van Rheenen PF, et al. Human bocavirus in an immunocompromised child presenting with severe diarrhea. *J Clin Microbiol* 2009; **47**: 1241-3.

97 Tozer SJ, Lambert SB, Whiley DM, et al. Detection of human bocavirus in respiratory, fecal, and blood samples by real-time PCR. *J Med Virol* 2009; **81**: 488-93.

98 Jartti T, Kuusipalo H, Vuorinen T, et al. Allergic sensitization is associated with rhinovirus-, but not other virus-, induced wheezing in children. *Pediatric Allergy and Immunology* 2010; **21**: 1008-14.

99 Jartti T, Soderlund-Venermo M, Allander T, Vuorinen T, Hedman K, Ruuskanen O. No efficacy of prednisolone in acute wheezing associated with human bocavirus infection. *Pediatr Infect Dis J* 2011; **30**: 521-3.

Appendix

Major studies on human bocavirus respiratory tract infections in children using qPCR, published May 23. 2011 to April 30. 2018 (see Jartti et al 2011 for an overview of earlier publications). References are listed below in supplementary.

1st author	Study site	Study year	n	Age (years)	Symptoms	HBoV DNA +, n (%)	Copy number (copies/ml)	HBoV1 Serodiagnosis n (%)	Viruses tested	Total viral co-detections %	HBoV co-detections with other viruses, n (%)	Total viral detections %
Nunes[1]	South-Africa	2000 – 2002	943 ‖	<2	LRTI	125 (13·3)	NA	ND	16	28·7 *	93 (74·4)	54
Nascimento-Carvalho[2]	Brazil	2003 – 2005	268 ‖	<5	CAP	62 (23)	26 > 10^4, 36 (low load)	21 (8) ††	17 †	NA	NA	NA
Nokso-Koivisto[3]	USA	2003 – 2007	707 ‖	6 – 35 months	URTI and AOM	HBoV1: 172 (24)	2·7 x10^10	ND	15	44·1 *	128 (74·4) *	77
Pettigrew[4]	USA	2003 – 2007	640 ‖	6 months – 3 years	AOM	157 (25)	3·23x10^3 – 1·73x10^12	ND	3	21·5 *	NA	51·0
Pogka[5]	Greece	2005 – 2008	1272	≤ 18	ILI	81 (6·4) *	LOD: 15	ND	16	21·3 *	48 (59·3) *	64
Honkinen[6]	Finland	2006 – 2007	76	6 months – 15 years	CAP	14 (18)	NA	ND	18	41·8 *	11 (79)	72
Deng[7]	China	2006 – 2009	186	23·4 months (mean)	LRTI	31 (16·7) *	< 500 – 1x10^9	ND	11	NA	19 (61·3)	67·7 *
Rodrigues da Silva[8]	Brazil	2007	260	<3	LRTI	27 (10·4)	NA	ND	13	65	25 (92·6)	85

Ursic[9]	Slovenia	2007 – 2009	891 ‖	≤ 17	ARTI	HBoV1: 164 (18·4)	2·0x10^3 – 9·1x10^10	ND	11	24·7	98 (59·8)	75
Chonmaitree[10]	USA	2008 - 2013	394 ‖	≤ 1	URTI	3 (0·8) *	2·85x10^5 (median viral load among all viruses)	ND	13	39·8 *	NA	76
Akinloye[11]	Nigeria	2009	246	children	ARTI	6 (2·4) *	NA	ND	12	16	5 (83·3)	77
Hao[12]	China	2009 – 2011	846	≤ 16	RTI	HBoV1: 112 (13·2)	NA	ND	8	72·2 ¶	67 (59·8)	NA
Liu[13]	China	2009 – 2012	4242	≤ 14	ARTI	125 (2·9) *	NA	ND	17 †	21·3 †	57 (45·6) †	55·7 †
Chen[14]	China	2009 – 2012	7626	< 14	LRTI	502 (6·6)	NA	ND	10 †	11·1 *, †	146 (29·1) †	45·8 †
Zhao[15]	China	2009 – 2012	554	< 5	LRTI	HBoV1: 39 (7·0)	1·4x10^3 - 5·0x10^9	ND	19	13·7 *	19 (48·7)	61·2 *
Feng[16]	China	2009 - 2013	5298	<6 months	ALRI	126 (2·4) *	NA	‡‡	7	23·1 *	413 (75) ‡	48·5 *
			3333	6 – 11 months	ALRI	110 (3·3) *	NA	‡‡	7	25·3 *		50·4 *
			3337	12 – 23 months	ALRI	137 (4·1) *	NA	‡‡	7	23·5 *		48·8 *
			5159	2 – 4	ALRI	116 (2·2) *	NA	‡‡	7	17·7 *		42·8 *
			2618	5 – 9	ALRI	29 (1·1) *	NA	‡‡	7	14·7 *		30·4 *

			892	10 – 14	ALRI	7 (0·8) *	NA	‡‡	7	17·2 *		23·4 *
Zhou[17]	China	2009 – 2013	1229	1 – 203 months	RTI	HBoV1: 127 (10·3) *	<500 – 3·8x10^11	ND	15	40·8 *	61 (48)	53·1
Cui[18]	China	2010 - 2011	1074	< 16	ARTI	78 (7·3) *	NA	ND	21	46·9 *	54 (69·2) *	82·3
Kaida[19]	Japan	2010 – 2011	1044	<6	ARTI	HBoV1: 176 (16·9) *	NA	ND	19	43·5	152 (86·4)	85·3
Xu[20]	China	2010 – 2011	1686	≤ 15	ARTI	HBoV1: 52 (3·08)	NA	ND	12	8·8 *,‡	16 (30·8)	41·6
Proenca-Modena[21]	Brazil	2010 – 2012	172	1 – 13	Chronic adeno-tonsillar disease	53 (31·1)	NA	ND	9	62·2	NA	87
Karadag-Oncel[22]	Turkey	2011 – 2012	200	<18	ILI	3 (1·5) *	NA	ND	NA	0·9	1 (33·3)	51
Ju[23]	China	2011 – 2013	461	≤ 4	ILI	13 (2·8) *	NA	ND	14	11·2 *	11 (84·6) *	57·92
			135	5 – 14	ILI	1 (0·7) *	NA	ND	14	7·6 *	1 (100)	48·96
Kenmoe[24]	Cameroon	2011 - 2013	347	≤ 15	SARI	37 (10·6)	NA	ND	17	29·5	24 (64·9) *	65·4
Rhedin[25]	Sweden	2011 – 2014	121	≤ 5	CAP	14 (12)	NA	ND	15	38·8 *	NA	81
			240	≤ 5	Control	50 (21)	NA	ND	15	37·3 *	NA	56

Zar[26]	South-Africa	2012 – 2014	284	3 – 9 months	Pneumonia	37 (13)	NA	ND	33 †	NA	NA	97 †
			418	2 – 8 months	Control	32 (7·8) *	NA	ND	33 †	NA	NA	97 †
Obuchi[27]	Japan	2013 – 2014	104 **	< 12	ARTI	HBoV1: 21 (20·2) *	1x10^3 – 4·3x10^8 copies/swab	ND	21	9·1 *	7 (33)	85
Jiang[28]	China	2013 – 2014	7393	6 – 24 months	LRTI	654 (8·85)	< 10^3 – 3·97 × 10^9	ND	9	NA	321 (49)	NA
Aktürk[29]	Turkey	2013 – 2014	178	2 – 16	URTI / LRTI	8 (4·5) *	NA	ND	21	2·9 *	3 (37·5) *	78·6
Finianos[30]	Lebanon	2013 – 2014	236	≤ 16	ARTI	36 (15)	NA	ND	17	37	28 (78)	70
Goktas[31]	Turkey	2014 – 2015	309	0 – 15	ARTI	35 (11)	NA	ND	22 †	27·2 †	(64·8) †, ‡	75·1 †

* Percentage re-calculated, † including bacteria, ‡ including all subgroups, § from 760 children, ¶ of all subjects (not virus positive), ‖ number of specimens, ** number of specimens negative for influenza by a rapid test kit, †† different number of serum samples than reported in n number, ‡‡ serodiagnosis done but data not shown in the publication, ALRI = acute lower respiratory infection, AOM = acute otitis media, ARTI = acute respiratory tract infection, ILI = influenza like illness, LOD = limit of detection, LRTI = lower respiratory tract infection, NA = not available, ND = not done, RTI = respiratory tract infection, SRTI = severe respiratory tract infection, URTI = upper respiratory tract infection, SARI = severe acute respiratory infection

Major studies on human bocavirus detections in gastrointestinal illness, published May 23. 2011 to April 30. 2018 (see Jartti et al 2011 for an overview of earlier publications). References are listed below in supplementary.

1st author	Study site	Study year	n	Age (years)	Symptoms	HBoV	Total HBoV DNA + n (%) and n (%) of HBoV1-4/ HBoV	HBoV qPCR reported	Copy number (copies/ml)	HBoV Serodiagnosis n (%)	Viruses tested	Total viral co-detections %	HBoV co-detections with other viruses, n (%)	Total viral detections %
Levican[48]	Chile	1985 – 1986, 1997 – 2004, 2009 – 2010	462	≤ 5	AGE	HBoV	89 (19·3)	No	NA	ND	1	NA	NA	NA
						HBoV1	65 (73·0) *							
						HBoV2	18 (20·2) *							
						HBoV3	6 (6·7) *							
Teixeira de Sousa[49]	Brazil	1994 – 1996, 1998 – 2004	762	< 5	AGE	HBoV	44 (5·8)	No	NA	ND	6 §	NA	14 (31·8)	NA
						HBoV1	11 (91·7) *,‖							
						HBoV3	1 (8·3) *,‖							
Proenca-Modena[50]	Paraguay	2004	349	< 5	AGE (non-bacterial)	HBoV1	37 (10·6)	Yes	1·88×10^4 (median)	ND	3 ‡	NA	15 (40·5)	NA
Mitui[51]	Turkey	2004 – 2005	150	< 5	Acute diarrhea	HBoV	13 (8·7)	No	NA	ND	5	20·7	10 (76·9) *	58
						HBoV1	1 (7·7)							
						HBoV2A	7 (53·8)							
						HBoV3	4 (30·8)							

						HBoV4	1 (7·7)							
	Bangladesh	2005 – 2006	138	< 5	Acute diarrhea	HBoV	87 (63·0)	No	NA	ND	5	76·4	81 (91·0) *	89·1
						HBoV1	29 (38·7) ‖							
						HBoV2A	36 (48·0) ‖							
						HBoV3	8 (10·7) ‖							
						HBoV4	2 (2·7) ‖							
Wang[52]	China	2006 – 2007	366	≤ 13	AGE	HBoV	44 (12·0)	No	NA	ND	6 §	NA	35 (79·5) *	NA
						HBoV1	9 (20·5) *						9 (100) *	
						HBoV2	33 (75) *						24 (72·7) *	
						HBoV3	2 (4·5) *						2 (100) *	
Cashman[53]	Ireland	2006 – 2008	155	All ages	Non-bacterial GE	HBoV	12 (7·7)	No	NA	ND	4	NA	12 (100)	100
						HBoV1	2 (16·7)*, ‖					100		
						HBoV2	4 (33·3)*, ‖					100		
						HBoV3	2 (16·7)*, ‖					100		
						Recombinations	4 (33·3) *, ‖					100		
Jin[54]	China	2006 – 2008	632	< 5	AGE	HBoV	162 (25·6) *	Yes	NA	ND	5	NA	NA	58·2
						HBoV1	27 (16·7) *		NA				20 (74·1) *	
						HBoV2	129 (79·6) *		42·3 (mean)				94 (72·9)	
						HBoV3	6 (3·7) *		NA				6 (100)	
			162	< 5	Control	HBoV	24 (14·8) *	Yes	NA	ND	5	NA	NA	19·1 *

						HBoV1	4 (16·7) *		NA					
						HBoV2	20 (83·3) *		54·3 (mean)					
Risku[55]	Finland	2006 – 2008	878	< 15	AGE	HBoV	85 (9·7)	No	NA	ND	7 §	NA	69 (81·2) *	NA
						HBoV1	49 (57·0) *						42 (85·7)	
						HBoV2	29 (33·7) *						21 (72·4)	
						HBoV3	8 (9·3) *						7 (87·5)	
			112	< 15	Control	HBoV	6 (5·4)	No	NA	ND	7 §	NA	0 (0)	NA
						HBoV1	2 (33·3) *							
						HBoV2	2 (33·3) *							
						HBoV3	2 (33·3) *							
Chhabra[56]	USA	2008 – 2009	782	< 5	AGE	HBoV	11 (1·4)	Yes	NA	ND	8	13·1	7 (21·2) *, †	40·8 †
			499	< 5	Control	HBoV	12 (2·4)	Yes	NA	ND	8	1·3		
Medici[57]	Italy	2008 – 2009	712	< 154 months	AGE	HBoV	23 (3·2)	No	NA	ND	3 ‡	1·8 *	1 (4·3) *	7·7 *
						HBoV1	5 (21·7)							
						HBoV2	10 (43·5)							
						HBoV3	8 (34·8)							
Rimoldi[58]	Italy	2008 – 2009	154	≤ 252 months	AGE	HBoV	21 (13·6)	Yes	NA	ND	12 ‡‡	13·1 *, ‡‡	7 (33·3) *	64·3
Romani[59]	Iran	2008 –	227	< 18	AGE	HBoV	24 (10·57)	No	NA	ND	1	NA	NA	NA
			67	> 18	AGE	HBoV	3 (4·48)	No	NA	ND	1	NA	NA	NA

		2010				HBoV1	3 (17·6) *, ‖							
						HBoV2	13 (76·5) *, ‖							
						HBoV3	1 (5·9) *, ‖							
Alam[60]	Pakistan	2009	365	< 5	AGE	HBoV	47 (13)	Yes	NA	ND	2	12·9 *	46 (98)	97·8 *
						HBoV1	26 (94) ‖							
						HBoV2	1 (3) ‖							
						HBoV3	1 (3) ‖							
Khamrin[61]	Japan	2009 – 2010	177	< 5	AGE	HBoV	11 (6·2)	No	NA	ND	12	NA	9 (81·8)	NA
						HBoV1	7 (63·6) *							
						HBoV2A	4 (36·4) *							
Lasure[62]	India	2009 – 2011	418	≤ 5	AGE	HBoV	24 (5·7)	No	NA	ND	6	NA	5 (21)	NA
						HBoV1	15 (62)							
						HBoV2	4 (17)							
						HBoV3	2 (8)							
						HBoV4	3 (12)							
Paloniemi[63]	Finland	2009 – 2011	172	Children	AGE	HboV	14 (8·1) *	Yes	NA	10 (33·3) *, †, **	1	NA	12 (85·7) *	NA
						HBoV1	3 (21·4) *						2 (66·7) *	
						HBoV2	10 (71·4) *						9 (90·0) *	
						HBoV3	1 (7·1) *						1 (100) *	

			238	Children	AGE+ARTI	HBoV	37 (15·5) *	Yes	NA	10 (33·3) *, †, **	1	NA	7 (18·9) *	NA
						HBoV1	22 (59·5) *						NA	
						HBoV2	13 (35·1) *						5 (38·5) *	
						HBoV3	2 (5·4) *						2 (100) *	
Babkin[64]	Russia	2010 – 2011	1781	≤ 3	AGE	HBoV	34 (1·9)	No	NA	ND	4	11·5 *	15 (44·1) *	39·7
						HBoV1	11 (32·4) *						5 (45·5) *	
						HBoV2	23 (67·6) *						10 (43·5) *	
Monavari[65]	Iran	2010 – 2011	200	1-5	AGE	HBoV	16 (8)	Yes	NA	ND	6 ††	NA	0 (0)	NA
Thongprachum[66]	Japan	2010 – 2012	751	≤ 15	AGE	HBoV	48 (6·4)	No	NA	ND	14	26·7 *	38 (79·2) *	70·3
Tymentsev[67]	Russia	2010 – 2012	5250	≤ 5	AGE (hospitalized)	HBoV	62 (1·2)	No	NA	ND	11 ††	NA	28 (45·2) *	NA
						HBoV1	24 (38·7)						13 (54·2) *	
						HBoV2	35 (56·5)						14 (40) *	
						HBoV3	1 (1·6)						0 (0)	
						HBoV4	2 (3·2)						1 (50) *	
			252	≤ 5	Control	HBoV	1 (0·3)	No	NA	ND	11 ††	NA	NA	NA
Zhang[68]	China	2010 – 2012	1128	< 14	Diarrhea	HBoV1	17 (1·5)	Yes	NA	ND	6	11·1 *	4 (23·5)	32·8
Khamrin[69]	Thailand	2011	222	< 5	Diarrhoea	HBoV	17 (7·7)	No	NA	ND	12 §	NA	10 (58·8)	NA

						HBoV1	11 (64·7)						
						HBoV2A	3 (17·6) *						
						HBoV3	2 (11·8) *						
						HBoV4	1 (5·9) *						
Rovida[70]	Italy	2011 – 2012	689	≤ 96	GI symptoms	HBoV	17 (2·5) *	No	NA	ND	12	22·3 *	12 (70·6) *	35·7
Soares Campos[71]	Brazil	2012	105	≤ 5	AGE	HBoV	44 (42)	No	NA	ND	4	18·1	12 (27)	68·6 *
						HBoV1	3 (30) ‖						
						HBoV2A	7 (70) ‖						
Tang[72]	Taiwan	2012 – 2013	110	≤ 18	AGE	HBoV	4 (3·5)	No	NA	ND	1	NA	NA	NA
Zhou[73]	China	2012 – 2013	346	< 6	AGE	HBoV	60 (17·34)	No	NA	ND	6	NA	26 (43·33)	35·3
						HBoV1	9 (56·3)*, ‖						
						HBoV2	7 (43·8)*, ‖						
La Rosa[74]	Albania	2013 – 2015	142	Children	AGE	HBoV	13 (9·2) *	No	NA	ND	3 §	NA	13 (100)	NA
						HBoV1	12 (92·3) *						
						HBoV2	1 (7·7)*						
Lee[75]	South Korea	2015	155	< 6	GE symptoms	HBoV1	10 (6·5)	No	NA	ND	4 §	NA	3 (30) *	NA
Nawaz[76]	U.K	NA	2256	all ages	GE	HBoV	149 (6·6)	Yes	4·56x10^3 –	ND	1	NA	88 (59·1)	NA

						4·56x10^4					
			HBoV1	6 (12·0) *							
			HBoV2	34 (68·0) *							
			HBoV3	10 (20·0) *							
2124	all ages	Control	HBoV	175 (8·2)	Yes	4·56x10^3 – 4·56x10^4	ND	1	NA	87 (49·7)	NA
			HBoV1	25 (42·4) *							
			HBoV2	20 (33·9) *							
			HBoV3	14 (23·7) *							

* Percentages re-calculated, † in the whole study population, ‡ previously tested negative for certain pathogens, § Samples previously tested for certain pathogens, ¶, AGE = acute gastroenteritis, || percentages calculated out of total typed HBoV (not all HBoVs typed), ** different number of serum samples than reported in n number, †† only co-infections with other viruses tested, ‡‡ including bacteria, ARTI = acute respiratory tract infection, GE = gastroenteritis, GI = gastrointestinal, NA = not available, ND = not done

Major studies on human bocavirus respiratory tract infections in adults, published May 23. 2011 to April 30. 2018 (see Jartti et al 2011 for an overview of earlier publications). References are listed below in supplementary.

1st author	Study site	Study year	n	Age (years)	Diagnosis	HBoV DNA, n (%)	Viruses tested	Copy number	qPCR reported	HBoV-1 Serodiagnosis n (%)	Total viral co-detections %	HBoV co-detections with other viruses, n (%)	Total viral detections (%)
Aronen[32]	Finland	2007 – 2009	438	65 – 100	Respiratory symptoms	HBoV1: 2 (0·5) *, §	16	NA	No	No acute HBoV1 - 4 infection in 396 episodes	13·8 *	NA	37
			200	65 – 100	Respiratory symptoms with dyspnea	HBoV1: 1 (0·6) *, §	16	NA	No		15·8 *	NA	38
			238	65 – 100	Respiratory symptoms without dyspnea	HBoV1: 1 (0·5) *, §	16	NA	No		11·4 *	NA	37
			291	65 – 100	No respiratory symptoms	HBoV1: 0 (0) *, §	16	NA	No		20·9 *	NA	23
Guido[33]	Italy	2008 – 2009	22	15 – 64	ILI	HBoV1: 10 (45·5) *	4	NA	No	ND	26·7 *, ‡	(49·1) ‡	67·2 ‡
			42	>64	ILI	HBoV1: 14 (33·3) *	4	NA	No	ND			
Memish[34]	Saudi Arabia	2009	172	41 (mean)	Healthcare workers	0 (0)	18	NA	No	ND	NA	0 (0)	12·8
Paño-Pardo[35]	Spain	2009	45	Pregnant	ILI	1 (2·2) *	12	NA	No	ND	2·2 *	0 (0)	66·58

Liu[36]	China	2009 - 2010	1014	≥ 18	ARTI	4 (0·4)	16 †, ¶	NA	Yes	ND	NA	1 (25)	NA
Lu[37]	China	2009 – 2010	266	14 – 25	URTI	27 (10·15)	11	NA	No	ND	17·5 *	12 (44·4) *	42·86
			303	26 – 65	URTI	21 (6·93)	11	NA	No	ND	7·2 *	6 (28·6) *	36·63
			27	≥ 66	URTI	1 (3·70)	11	NA	No	ND	0	0 (0)	22·22
Pilorge[38]	France	2009 – 2010	78	Pregnant	ILI	1 (1·3) *	16	NA	Yes	ND	0	0 (0)	65
Dia[39]	Senegal	2009 - 2011	232	≥50	ILI	1 (0·4) *	16	NA	Yes	ND	11·4 *	NA	56·9
Lu[40]	China	2009 - 2011	981 ‖	14 – 91	URTI	2 (0·2)	15	NA	No	ND	NA	NA	41·1 *
Feng[16]	China	2009 – 2013	2629	15 – 49	ALRI	9 (0·3) *	7	NA	Yes	ND	7·1 *	(75) ‡	19·8 *
			1790	50 – 64	ALRI	6 (0·3) *	7	NA	Yes	ND	7·8 *		16·4 *
			3313	≥ 65	ALRI	11 (0·3) *	7	NA	Yes	ND	5·8 *		14·7 *
Serin[41]	Turkey	2010	50	≥ 18	CAP	0 (0)	26	NA	No	ND	10 *	0 (0)	36
Ghietto[42]	Argentina	2010	19	≥ 16	LRTI	HBoV1: 5 (26·3) *	8 ¶	NA	No	ND	NA	0 (0)	NA
Xu[20]	China	2010 – 2011	1774	>15	ARTI	HBoV1: 6 (0·37)	12	NA	Yes	ND	8·8 *, ‡	4 (60)	38·7
Koul[43]	India	2011 – 2012	233	40 – 100	AECOPD	1 (0·4)	18	NA	Yes	ND	8·7 *	0 (0)	19·7

Noh[44]	South-Korea	2011 – 2012	1983	≥18	ILI	0 (0)	17	NA	No	ND	5·6	0 (0)	52·1
Ju[23]	China	2011 – 2013	135	15 – 24	ILI	1 (0·7)*	14	NA	Yes	ND	12·2 *	1 (100)	36·30
			167	25 – 59	ILI	1 (0·60)	14	NA	Yes	ND	13·1 *	0 (0)	36·53
			42	≥ 60	ILI	1 (2·4)*	14	NA	Yes	ND	21·4 *	1 (100)	33·33
Remolina[45]	Colombia	2012	91	≥ 18	SRTI	26 (28·6)	19	NA	No	ND	41·3	15 (57·7) *	69·2
Ye[46]	China	2012 – 2015	967	> 60	ARTI	0 (0)	17	NA	No	ND	9·8 *	0 (0)	31·64
Dai[47]	China	2014	81	71±10	AECOPD	6 (7·4)	18	NA	No	ND	31·6 *	NA	38·3 *
Goktas[31]	Turkey	2014 – 2015	536	> 15	ARTI	56 (10·4) *	22 †	NA	Yes	ND	34·5 †	(64·8) †, ‡	70·9 †

* Percentages re-calculated, † Including bacteria, ‡ in all subgroups, § not all samples tested for HBoV, ¶ Only bocavirus positive specimens tested, || number of specimens, AECOPD = acute exacerbtions of chronic obstructive pulmonary disease, ALRI = acute lower respiratory infection, ARTI = acute respiratory tract infection, CAP = community acquired pneumonia, COPD = chronic obstructive pulmonary disease, ILI = influenza like illness, LRTI = lower respiratory tract infection, NA = not available, ND = not done, SRTI = severe respiratory tract infection, URTI = upper respiratory tract infection

List of references in the Appendix

1 Nunes MC, Kuschner Z, Rabede Z, et al. Clinical Epidemiology of Bocavirus, Rhinovirus, Two Polyomaviruses and Four Coronaviruses in HIV-infected and HIV-uninfected South-African children. *PloS One* 2014; **9:** e86448.

2 Nascimento-Carvalho CM, Cardoso MR, Meriluoto M, et al. Human bocavirus infection diagnosed serologically among children admitted to hospital with community-acquired pneumonia in a tropical region. *J Med Virol* 2012; **84:** 253 – 8.

3 Nokso-Koivisto J, Pyles RB, Miller AL, Jennings K, Loeffelholz M, Chonmaitree T. Role of Human Bocavirus in Upper Respiratory Tract Infections and Acute Otitis Media. *Journal of the Pediatric Infectious Diseases Society* 2014; **3:** 98 – 103.

4 Pettigrew M, Gent J, Pyles R, Miller AL, Nokso-Koivisto J, Chonmaitree T. Viral-bacterial interactions and risk of acute otitis media complicating upper respiratory tract infection. *J Clin Microbiol* 2011; **49:** 3750 – 55.

5 Pogka V, Moutousi A, Kossyvakis A, et al. Genetic variability of human metapneumo- and bocaviruses in children with respiratory tract infections. *Influenza and other respiratory viruses* 2013; **8**: 107 – 15.

6 Honkinen M, Lahti E, Österback R, Ruuskanen O, Waris M. Viruses and bacteria in sputum samples of children with community-acquired pneumonia. *Clin Microbial Infect* 2012; **18:** 300 – 7.

7 Deng Y, Gu X, Zhao X, et al. High Viral Load of Human Bocavirus Correlates with Duration of Wheezing in Children with Severe Lower Respiratory Tract Infection. *PLoS One* 2012; **7:** e34353.

8 Rodrigues da Silva E, Pitrez PMC, Arruda E, et al. Severe lower respiratory tract infection in infants and toddlers from a non-affluent population: viral etiology and co-detection as risk factors. *BMC infectious* diseases 2013; **13:** 41.

9 Ursic T, Jevsnik M, Zigon N, et al. Human bocavirus and other respiratory viral infections in a 2-year cohort of hospitalized children. *J Med Virol* 2012; **84**: 99 – 108.

10 Chonmaitree T, Alvarez-Fernandez P, Jennings K, et al. Symptomatic and Asymptomatic Respiratory Viral Infections in the First Year of Life: Association With Acute Otitis Media Development. *Clin Infect Dis* 2015; **60:** 1 – 9.

11 Akinloye O, Rönkkö E, Savolainen-Kopra C, et al. Specific Viruses Detected in Nigerian Children in Association with Acute Respiratory Disease. *Journal of Tropical Medicine* 2011; doi:10.1155/2011/690286

12 Hao R, Ni K, Xia Q, et al. Correlation between nucleotide mutation and viral loads of human bocavirus 1 in hospitalized children with respiratory tract infection. *J Gen Virol* 2013; **94:** 1079 – 85.

13 Liu WK, Liu Q, Chen DH, et al. Epidemiology of Acute Respiratory Infections in Children in Guangzhou: A Three-Year Study. *PLoS One* 2014; **9:** e96674.

14 Chen ZR, Mize M, Wang YQ, et al. Clinical and Epidemiological Profiles of Lower Respiratory Tract Infection in Hospitalized Children due to Human Bocavirus in a Subtropical Area of China. *J Med Virol* 2014; **86:** 2154 – 62.

15 Zhao B, Yu X, Wang C, et al. High Human Bocavirus Viral Load Is Associated with Disease Severity in Children under Five Years of Age. *PLoS One* 2013; **8:** e62318.

16 Feng L, Li Z, Zhao S, et al. Viral Etiologies of Hospitalized Acute Lower Respiratory Infection Patients in China, 2009-2013. *PLoS One* 2014; **9:** e99419.

17 Zhou L, Zheng S, Xiao Q, et al. Single detection of human bocavirus 1 with a high viral load in severe respiratory tract infections in previously healthy children. *BMC infectious diseases* 2014; **14**: 424.
18 Cui B, Zhang D, Pan H, et al. Viral aetiology of acute respiratory infections among children and associated meteorological factors in southern China. *BMC Infectious diseases* 2015; **15:** 124.
19 Kaida A, Kubo H, Takakura K, et al. Associations between Co-Detected Respiratory Viruses in Children with Acute Respiratory Infections. *Jpn J Infect Dis* 2014; **67:** 469 – 75.
20 Xu L, He X, Zhang D, et al. Surveillance and Genome Analysis of Human Bocavirus in Patients with Respiratory Infection in Guangzhou, China. *Plos One* 2012; **7:** e44876.
21 Proenca-Modena JL, Buzatto GP, Paula FE, et al. Respiratory viruses are continuously detected in children with chronic tonsillitis throughout the year. *International journal of pediatric otorhinolaryngology* 2014; **78:** 1655 – 61.
22 Karadag-Oncel E, Ciblak MA, Ozsurekci Y, Badur S, Ceyhan M. Viral Etiology of Influenza-Like Illnesses During the Influenza Season Between December 2011 and April 2012. *J Med Virol* 2014; **86:** 865 – 71.
23 Ju X, Fang Q, Zhang J, Xu A, Liang L, Ke C. Viral etiology of influenza-like illnesses in Huizhou, China, from 2011 to 2013. *Archives of Virology* 2014; **159:** 2003 – 10.
24 Kenmoe S, Tchendjou P, Vernet MA, et al. Viral etiology of severe acute respiratory infections in hospitalized children in Cameroon, 2011–2013. *Influenza and Other Respiratory Viruses* 2016: DOI: 10.1111/irv.12391.
25 Rhedin S, Lindstrand A, Hjelmgren A, et al. Respiratory viruses associated with community-acquired pneumonia in children: matched case-control study. *Thorax* 2015; **70**: 847 – 53.
26 Zar HJ, Barnett W, Stadler A, Gardner-Lubbe S, Myer L, Nicol MP. Aetiology of childhood pneumonia in a well vaccinated South African birth cohort: a nested case-control study of the Drakenstein Child Health Study. *Lancet Respir Med* 2016; **4:** 463 – 72.
27 Obuchi M, Yagi S, Oguri A, Takizawa T, Kimura H, Sata T. Outbreak of Human Bocavirus 1 Infection in Young Children in Toyama, Japan. *Jpn J Infect Dis* 2015; **68:** 259 – 61.
28 Jiang W, Yin F, Zhou W, Yan Y, Ji W. Clinical significance of different virus load of human bocavirus in patients with lower respiratory tract infection. *Sci Rep* 2016; **6:** 20246.
29 Aktürk H, Sütçü M, Badur S, et al. Evaluation of epidemiological and clinical features of influenza and other respiratory viruses. *Turkish Archives of Pediatrics* 2015; **50:** 217 – 25.
30 Finianos M, Issa R, Curran MD, et al. Etiology, seasonality, and clinical characterization of viral respiratory infections among hospitalized children in Beirut, Lebanon. *J Med Virol* 2016; **88**: 1874 – 81.
31 Goktas S, Sirin MC. Prevalence and Seasonal Distribution of Respiratory Viruses During the 2014 - 2015 Season in Istanbul. *Jundishapur J Microbiol* 2016; **9:** e39132.
32 Aronen M, Vuorinen T, Langen H, Hämeenaho M, Viitanen M, Jartti T. Virus Etiology of Airway Illness in Elderly Adults. *J Am Geriatr Soc* 2016; **64:** 1358 – 60.
33 Guido M, Quattrocchi M, Campa A, et al. Human metapneumovirus and human bocavirus associated with respiratory infection in Apulian population. *Virology* 2011; **417:** 64 – 70.
34 Memish ZA, Assiri AM, Alshehri M, Hussain R, Alomar I. The prevalance of respiratory viruses among healthcare workers serving pilgrims in Makkah during the

2009 influenza A (H1N1) pandemic. *Travel Medicine and Infectious Disease* 2012; **10:** 18 – 24.
35 Paño-Pardo JR, Martínez-Sánchez N, Martín-Quirós A, et al. Influenza-like illness in pregnant women during summertime: clinical, epidemiological and microbiological features. *Eur J Microbiol Infect Dis* 2011; **30:** 1497 – 502.
36 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 Infectious Diseases* 2011; **11:** 345.
37 Lu Y, Tong J, Pei F, et al. Viral Aetiology in Adults with Acute Upper Respiratory Tract Infection in Jinan, Northern China. *Clinical and developmental immunology* 2013; doi: 10.1155/2013/869521 38.
38 Pilorge L, Chartier M, Meritet JF, et al. Rhinoviruses as an Underestimated Cause of Influenza-Like Illness in Pregnancy During the 2009–2010 Influenza Pandemic. *J Med Virol* 2013; **85:** 1473 – 77.
39 Dia N, Richard V, Kiori D, et al. Respiratory viruses associated with patients older than 50 years presenting with ILI in Senegal, 2009 to 2011. *BMC Infectious Diseases* 2014; **14:** 189.
40 Lu R, Yu X, Wang W, et al. Characterization of Human Coronavirus Etiology in Chinese Adults with Acute Upper Respiratory Tract Infection by Real-Time RT-PCR Assays. *Plos One* 2012; **7:** e38638.
41 Serin DC, Pullukçu H, Çiçek C, et al. Bacterial and viral etiology in hospitalized community acquired pneumonia with molecular methods and clinical evaluation. *J Infect Dev Ctries* 2014; **8:** 510 – 18.
42 Ghietto LM, Camara A, Camara J, Adamo MP. High frequency of human bocavirus 1 DNA in infants and adults with lower acute respiratory infection. *J Med Microbiol* 2012; **61:** 548 – 51.
43 Koul PA, Mir H, Akram S, Potdar V, Chadha MS. Respiratory viruses in acute exacerbations of chronic obstructive pulmonary disease. *Lung India* 2017; **34:** 29 – 33.
44 Noh JY, Son JY, Cheong HJ, et al. Laboratory Surveillance of Influenza-Like Illness in Seven Teaching Hospitals, South Korea: 2011–2012 Season. *Plos One* 2013; **8:** e64295.
45 Remolina YA, Ulloa MM, Vargas H, et al. Viral Infection in Adults with Severe Acute Respiratory Infection in Colombia. *Plos One* 2015; **10:** e0143152.
46 Ye C, Zhu W, Yu J, et al. Viral pathogens among elderly people with acute respiratory infections in Shanghai, China: Preliminary results from a laboratory-based surveillance, 2012-2015. *J Med Virol* 2017; **89:** doi: 10.1002/jmv.24751.
47 Dai MY, Qiao JP, Xu YH, Fei GH. Respiratory infectious phenotypes in acute exacerbation of COPD: an aid to length of stay and COPD Assessment Test. *International Journal of COPD* 2015; **10**.
48 Levican J, Navas E, Orizola J, Avendaño LF, Gaggero A. Human Bocavirus in Children with Acute Gastroenteritis, Chile, 1985–2010. *Emerging Infectious Diseases* 2013; **19:** 1877 – 80.
49 Teixeira de Sousa T, Souza M, Souza Fiaccadori F, Tavares Borges AM, Sucasas de Costa P, Cardoso DDP. Human bocavirus 1 and 3 infection in children with acute gastroenteritis in Brazil. *Mem Inst Oswaldo Cruz* 2012; **107:** 800 – 4.
50 Proenca-Modena JL, Martinez M, Amarilla AA, et al. Viral load of human bocavirus-1 in stools from children with viral diarrhoea in Paraguay. *Epidemiol Infect* 2013; **141:** 2576 – 80.
51 Mitui MT, Bozdayi G, Ahmed S, Matsumoto T, Nishizono A, Ahmed K. Detection and Molecular Characterization of Diarrhea Causing Viruses in Single and Mixed Infections

in Children: A Comparative Study Between Bangladesh and Turkey. *J Med Virol* 2014; **86:** 1159 – 68.
52 Wang Y, Gonzalez H, Zhou H, et al. Detection of human bocavirus 3 in China. *Eur J Clin Infect Dis* 2011; **30:** 799 – 805.
53 Cashman O, O'Shea H. Detection of human bocaviruses 1, 2 and 3 in Irish children presenting with gastroenteritis. *Arch Virol* 2012; **157:** 1767 – 73.
54 Jin Y, Cheng W, Xu Z, et al. High prevalence of human bocavirus 2 and its role in childhood acute gastroenteritis in China. *J Clin Virol* 2011; **52:** 251 – 53.
55 Risku M, Kätkä M, Lappalainen S, Räsänen S, Vesikari T. Human bocavirus types 1, 2 and 3 in acute gastroenteritis of childhood. *Acta Pediatrica* 2012; **101:** e405 – 10.
56 Chabbra P, Payne DC, Szilagyi PG, et al. Etiology of Viral Gastroenteritis in Children <5 Years of Age in the United States, 2008–2009. *J Infect Dis* 2013; **208:** 790 – 800.
57 Medici MC, Tummolo F, Albonetti V, Abelli LA, Chezzi C, Calderaro A. Molecular Detection and Epidemiology of Astrovirus, Bocavirus, and Sapovirus in Italian Children Admitted to Hospital With Acute Gastroenteritis, 2008–2009. *J Med Virol* 2012; **84:** 643 – 50.
58 Rimoldi SG, Stefani F, Pagani C, et al. Epidemiological and clinical characteristics of pediatric gastroenteritis associated with new viral agents. *Arch Virol* 2011; **156:** 1583 – 89.
59 Romani S, Mohebbi SR, Khanyaghma M, et al. Detection of human Bocavirus 1, 2 and 3 from patients with acute gastroenteritis. *Gastroenterol Hepatol Bed Bench* 2013; **6:** 77 – 81.
60 Alam MM, Khurshid A, Shaukat S, et al. Human Bocavirus in Pakistani Children with Gastroenteritis. *J Med Virol* 2015; **87:** 656 – 63.
61 Khamrin P, Thongprachum A, Shimizu H, et al. Detection of Human Bocavirus 1 and 2 From Children With Acute Gastroenteritis in Japan. *J Med Virol* 2012; **84:** 901 – 5.
62 Lasure N, Gopalkrishna V. Molecular Epidemiology and Clinical Severity of Human Bocavirus (HBoV) 1–4 in Children With Acute Gastroenteritis From Pune, Western India. *J Med Virol* 2017; **89:** 17 – 23.
63 Paloniemi M, Lappalainen S, Salminen M, et al. Human bocaviruses are commonly found in stools of hospitalized children without causal association to acute gastroenteritis. *Eur J Pediatr* 2014; **173:** 1051 – 57.
64 Babkin IV, Tyumentsev AI, Tikunov AY, et al. Molecular Epidemiology and Clinical Severity of Human Bocavirus (HBoV) 1–4 in Children With Acute Gastroenteritis From Pune, Western India. *Infection, Genetics and Evolution* 2013; **14:** 265 – 74.
65 Monavari SH, Noorbakhsh S, Mollaie H, Fazlalipour M, Kiasari BA. Human *Bocavirus* in Iranian children with acute gastroenteritis. *Medical Journal of the Islamic Republic of Iran* 2013; **27:** 127 – 31.
66 Thongprachum A, Khamrin P, Pham NTK, et al. Multiplex RT-PCR for Rapid Detection of Viruses Commonly Causing Diarrhea in Pediatric Patients. *J Med Virol* 2017; **89:** 818 – 24.
67 Tymentsev A, Tikunov A, Zhirakovskaia E, et al. Human bocavirus in hospitalized children with acute gastroenteritis in Russia from 2010 to 2012. *Infection, Genetics and Evolution* 2016; **37:** 143 – 49.
68 Zhang DM, Ma MM, Wen WT, et al. Clinical epidemiology and molecular profiling of human bocavirus in faecal samples from children with diarrhoea in Guangzhou, China. *Epidemiol Infect* 2015; **143:** 2315 – 29.
69 Khamrin P, Malasao R, Chaimongkol N, et al. Circulating of human bocavirus 1, 2, 3, and 4 in pediatric patients with acute gastroenteritis in Thailand. *Infection, Genetics and Evolution* 2012; **12:** 565 – 69.

70 Rovida F, Campanini G, Piralla A, Adzasehoun KMG, Sarasini A, Baldanti F. Molecular detection of gastrointestinal viral infections in hospitalized patients. *Diagnostic Microbiology and Infectious Disease* 2013; **77:** 231 – 35.
71 Soares Campos G, Silva Sampaio ML, Luz Menezes AD, et al. Human Bocavirus in Acute Gastroenteritis in Children in Brazil. *J Med Virol* 2016; **88:** 166 – 70.
72 Tang MB, Chu CM, Chou YC, Kuan JC, Yu CP. Molecular Detection of Human Bocavirus 1 and 2 in Cildren with Acute Gastroenteritis in Taiwan. *Southeast Asian J Trop Med Public Health* 2015; **46:** 1005 – 12.
73 Zhou T, Chen Y, Chen J, et al. **P**revalence and clinical profile of human bocavirus in children with acute gastroenteritis in Chengdu, West China, 2012-2013. *J Med Virol* 2017; doi: [10.1002/jmv.24787].
74 La Rosa G, Della Libera S, Iaconelli M, et al. Human Bocavirus in Children With Acute Gastroenteritis in Albania. *J Med Virol* 2016; **88:** 906 – 10.
75 Lee EJ, Kim HS, Kim HS, et al. Human Bocavirus in Korean Children with Gastroenteritis and Respiratory Tract Infections. *BioMed Research International* 2016; **2016:** 7507895.
76 Nawaz S, Allen DJ, Aladin F, Gallimore C, Iturriza-Gomara M. Human Bocaviruses Are Not Significantly Associated with Gastroenteritis: Results of Retesting Archive DNA from a Case Control Study in the UK. *Plos One* 2012; **7:**