# Human Bocavirus: Multisystem Detection Raises Questions about Infection

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**(See the article by Lau et al., on pages 986–93.)**

In this issue of the *Journal*, Lau et al. report an intriguing new aspect of the human bocavirus (HBoV) story—that is, the presence of the virus in the gastrointestinal tract [1]. HBoV was first described by Allander et al. [2] in 3% of nasopharyngeal aspirates (NPAs), the result of intensive investigation of 2 chronologically distinct NPA pools by use of a random polymerase chain reaction (PCR)–based cloning and sequencing approach that also yielded the KI polyomavirus. It was noted at the time that HBoV may, like its namesakes bovine parvovirus and minute virus of canines, have an association with enteritis that could be addressed by testing human feces for HBoV [2]. Since then, clinical indications of gastrointestinal involvement have been frequently described, without testing, until 2007 [3–8]. At long last, a report from Lau et al. and 2 smaller studies have addressed Allander et al.'s foresight. Maggi et al. [9] first described a young child presenting with bronchiolitis from whom HBoV DNA was also detected in

feces. Vicente et al. [10] detected HBoV in 9% of 527 fecal samples from children with gastroenteritis and in 8% of 520 NPAs from children with suspected acute respiratory tract infection (ARTI). Fecal HBoV detection was accompanied by coincident detection of an intestinal pathogen in 58% of cases and also occurred in 2 children with HBoV-positive NPAs. In the largest study to date, Lau et al. now report HBoV DNA in 7% of 1200 NPAs from children presenting with suspected ARTI (10% of whom had additional symptoms of gastroenteritis) and in 2% of 1435 fecal samples from children with gastroenteritis.

However, principal detection of HBoV in respiratory secretions had already bestowed upon it the colloquial title of a "respiratory virus." Recent data from the burgeoning area of respiratory virus discovery are invigorating the old question of how one determines whether a virus detected in the respiratory tract causes respiratory disease. A similar question may have prompted Robert Koch, in 1890, to postulate ways to associate infection with disease [11]. Koch's postulates suggested that a true and nonfortuitous pathogen isolated and purified from a diseased host would induce disease anew upon infection of a healthy body—a moot point for most recently identified respiratory viruses, which have not been cultured. Further-

more, if the putative pathogen can cause severe disease, then, by today's standards, it would be ethically unconscionable to reproduce that state in humans by intentional infection. But Koch's likely intention was to use his postulates to prompt topical discussion about the best way to implement contemporary methods to identify and define associations [12]. The latest findings on HBoV should greatly increase the frequency of such discussions.

Contemporary virus-detection methods identify nucleic acid rather than infectious virus, so we more accurately describe detection, not infection. These methods have proven to be extremely successful at finding and characterizing viruses that are fastidious or do not grow in standard culture, that have been identified by molecular means, that are endemic and globally distributed (not emerging), and for which infection cannot be discriminated on the basis of clinical features alone. We call them "newly identified viruses" (NIVs). The recent discovery of 7 respiratory NIVs from 5 virus families over 6 years suggests that culture has reached the limit of its usefulness for identifying new respiratory viruses. PCR-based methods are currently unmatched for identifying and studying human respiratory NIVs, which include human metapneumovirus (HMPV), human coronaviruses (HCoVs) NL63 and

HKU1, HBoV, polyomaviruses KI [13] and WU [14], and a new human rhinovirus (HRV), HRV-QPM [15].

HBoV has, to date, been described in 0%–19% of respiratory tract specimens (averaging 7%), reflecting an equally broad variation in study populations [16, 17]; the highest values generally occur in studies of hospitalized young children, among specimens collected during months with a high frequency of infectious respiratory tract diseases. The lowest values occur in adults. Studies from our laboratory [18, 19] have detected HBoV DNA in 6% and 5% of specimen extracts from Australia, coinciding with other viral nucleic acids in 56% and 67% of detections, usually from viruses with an overlapping peak season or affected age group. A high frequency of codetection is a feature of HBoV studies that is shared by other respiratory NIVs [13–15]. The original description of HBoV reported a codetection frequency of 18%, but subsequent studies have described rates as high as 90% (averaging 42%) [17, 20]; higher values occur when more viral targets are sought by use of reliable PCR assays rather than non-PCR methods. Lau et al. reported codetection rates of 33% in respiratory material and 56% in fecal extracts, also noting that HBoV DNA was shed from both sites for more than a month in immunosuppressed individuals. Others have identified HBoV in acute- and convalescent-phase serum samples, strengthening the case for systemic and, perhaps, persistent infection [17, 20]. HRV and HMPV alone accounted for all of the codetections (20% of HBoV detections) among NPAs in the Hong Kong study, exemplifying the importance of their inclusion in studies of respiratory viruses. Codetections also occur among the other respiratory NIVs, indicating that these viruses do not completely account for the respiratory specimens previously lacking a laboratory diagnosis; rather, they provide a fractional contribution. Accumulating data from respiratory studies using comprehensive PCR panels indicate that reports of multiple detections are increasing, and their occurrence may eventually become the norm rather than the exception. If detection represents active infection, then one can speculatively foresee a time when we find that the microbial mix, and perhaps the order of infection, is what tips the balance from benign infection to morbid state. Regardless, today's thinking, for the most part, associates acute respiratory tract disease with infection by a single virus; for example, respiratory syncytial virus is often described as the most common cause of bronchiolitis and pneumonia among young children [21]. The relatively extreme rates of codetection and a second, anatomically distinct site of discovery understandably cloud the link between HBoV detection and disease causation. However, there is precedent suggesting that suspected coinfection is associated with more serious clinical outcomes [22, 23].

Studies associating serious outcomes with HBoV detection are relatively common, although too often they do not use PCR for all likely respiratory pathogens [24–27]. Clinical features in HBoV-positive patients cannot be differentiated from those attributed to infection with other respiratory viruses and frequently include cough, fever, bronchitis, and bronchiolitis. Acute expiratory wheezing and pneumonia feature prominently [16, 28–30]. Now, acute gastroenteritis can be added to the list.

But what do we know of the presence of HBoV in generally healthy young individuals? A small number of studies of HBoV have included an asymptomatic or differently symptomatic control population, but often without precise age matching, an important requirement when the highest frequency of infection occurs across a very narrow age range [4, 9, 17, 29]. Furthermore, the type of specimen sampled between groups often differs—for example, NPAs may be obtained from symptomatic individuals but nasal washes are collected from control subjects, which may affect detection rates [4, 17]. Once again, major families of respiratory viruses can be overlooked during testing, usually the HRVs and HCoVs [4, 29]. In a comprehensive controlled study of nasopharyngeal swabs that compared hospitalized patients with pneumonia to outpatients with influenza-like illness, an association between pneumonia and HBoV infection was demonstrated [20]. Controlled studies report no or few HBoV detections in asymptomatic populations but, when detected, clinical follow-up is not described. We should be cautious of overinterpreting these positive results; detection of HBoV without significant symptoms does not alone assign an insignificant role to HBoV in disease. It is quite possible that infection causes transient mild illness that goes undetected either because the criteria for asymptomatic cases are biased against such disease or because symptoms develop after sample collection.

Quantification of HBoV by real-time PCR has often been used to determine whether HBoV loads are elevated in NPAs from children with more serious clinical features [3, 17]. Some have found no correlation, exemplifying the near-impossible task of using PCR to accurately quantify viruses sourced from the respiratory tract [31]. Nonetheless, studies agree that low to moderate HBoV loads ($< 1 \times 10^4$ copies/mL) are commonplace [7, 16, 31, 32]. To date, 2 genotypes comprising subtle but globally conserved nucleotide variations have been reported, based on the branching patterns of phylogenetic trees created from capsid gene sequence alignments [4, 33]. These genotypes share >98% amino acid identity, suggesting the existence of a single HBoV lineage; this has been confirmed by Lau et al., who sequenced 12 complete genomes from each anatomical site and found little difference.

The discovery of HBoV in feces is intriguing, but we should not jump off the respiratory bandwagon and onto that gastrointestinal one just yet, because the codetection factor travels on both. These new findings raise many new questions,

some of which include: What is the immune response doing, and could an immune response triggered by ARTI induce gastroenteritis-like clinical features (and vice versa)? Should we look for so-called respiratory viruses systemwide? Is HBoV a gastrointestinal pathogen? What is the method of transmission? Is HBoV frequently detected in both systems simultaneously? Can our PCR assay menus cope with more viruses? Are we doing enough to address the role played by respiratory virus coinfection in morbidity? After more than half a century of concerted study, the most widespread and frequent causes of human infection, the respiratory viruses, are proving to be as confounding as ever, raising questions faster than we can answer them.

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