Electron Microscopy Observation of Human Bocavirus (HBoV) in Nasopharyngeal Samples from HBoV-Infected Children\textsuperscript{\textcopyright}

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The newly identified human bocavirus is frequently detected by molecular techniques in respiratory samples from children with respiratory tract infections, but virions have not been observed so far. We report the electron microscopy observation of viral particles in nasopharyngeal aspirates previously found to be positive for human bocavirus DNA. The virions presented the expected structural characteristics of a Parvoviridae family member.

Human bocavirus (HBoV) is a newly identified viral pathogen in humans (1). The virus has been discovered by means of molecular methods. DNase sequence-independent single-primer amplification was used as a screening method to identify new viral sequences in nasopharyngeal aspirates from children with respiratory tract infections (1). Phylogenetic analyses showed that these sequences were closely related to those of the canine minute virus and the bovine parvovirus, two members of the Bocavirus genus within the Parvovirinae subfamily. The complete sequence of the virus confirmed its close relationship with the two known members of the Bocavirus genus (1). HBoV has been subsequently iden-

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tified worldwide in clinical specimens using PCR (2, 4, 6–8). Thus, the discovery of HBoV results from progress in nucleic acid technologies. To date, the virus has not been propagated in cell culture, and to our knowledge, the observation of viral particles has not been reported. To investigate the presence of viral particles in respiratory specimens, we have performed electron microscopy (EM) on samples found to be positive for HBoV DNA by PCR.

Nasopharyngeal aspirates were tested by a quantitative real-time PCR assay as previously described (4). Given the low sensitivity of EM to detect viral particles in clinical samples (3), only samples showing a high level of HBoV DNA were chosen for EM testing. Ten samples with a viral load greater than 10⁶ HBoV DNA copies per ml were selected, and five samples negative for HBoV DNA served as controls. These 15 samples were found negative by cell culture using MRC-5 cell monolayers; they were negative for respiratory syncytial virus, influenza virus types A and B, parainfluenza virus types 1, 2, and 3, and adenovirus by direct immunofluorescence assays and for human metapneumovirus by reverse transcriptase PCR.

Nasopharyngeal aspirates were obtained with a disposable catheter connected to a mucus extractor. A volume of 500 μl was clarified by centrifugation through a 0.22-μm spin filter (Ultrafree-MC; Millipore, Billerica, MA) at 5,000 × g for 30 min. The filtrate was then concentrated for viral particles with a 3-kDa-exclusion-size centrifugal-filter device (Ultracell YM-3; Millipore, Billerica, MA) by centrifugation at 14,000 g for 15 min. The filtrate was then concentrated for viral particles with a 3-kDa-exclusion-size centrifugal-filter device (Ultracell YM-3; Millipore, Billerica, MA) at 5,000 × g until a required retentate volume of 25 μl was achieved. A 300-mesh Formvar-covered grid (Agar Scientific, Stansted, United Kingdom) was coated with a 10-μl volume of sample concentrate at room temperature for 20 min. Liquid was drained off with filter paper until a thin film remained on the substrate without drying. Then, the grids were rinsed by dropping water on the surface, and excess fluid was removed with filter paper. For negative staining, a drop of 4% uranyl acetate (Electron Microscopy Science, Hatfield, PA) was placed on the grid and then drained off with filter paper and left to air dry. The grids were examined at a magnification of ×60,000 to ×150,000 on an EM apparatus (H7100; Hitachi, Tokyo, Japan). The accuracy of the EM was previously checked by using a 2,160-lines/mm line grating as a magnification calibration device (TAAB Laboratories Equipment, Aldermaston, United Kingdom). Particles evocative of parvovirus capsids were observed (Fig. 1). These particles were hexagonally shaped, and their centers showed heavier staining (Fig. 1). The mean (standard deviation) size, calculated by measuring 45 particles across the apices at the ×150,000 magnification, was 25 (4) nm. These observations are in agreement with the expected characteristics of parvovirus capsids (5). The parvovirus-like particles were observed in all the samples positive for HBoV by PCR and not in the HBoV-negative samples.

The presence of parvovirus-like particles in clinical samples found positive for HBoV DNA by PCR confirms that HBoV presents the structural characteristics of the Parvoviridae family members. Our observations also suggest that virions are shed in respiratory secretions of infected subjects and corroborate molecular results. EM, which is a time-consuming method with low sensitivity for detection of viruses in clinical specimens, is no longer used for routine diagnosis of viral infections but remains a powerful tool for studying newly identified viruses (3). Characterization of HBoV particles by EM should be of interest for subsequent studies on the structure and pathogenesis of this virus.

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