Human bocaviruses – role in human acute gastroenteritis

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Human bocaviruses (HBoVs) are a recently discovered class of viruses associated with respiratory and enteric samples. Four genotypes have been reported and all have been detected in faeces. An association with acute gastroenteritis (AGE) has been reported for HBoV2 but more case-control studies are required to clarify their significance. HBoV1 and 3 are not statistically associated with AGE. The role of HBoV4 is unknown.

Human bocavirus (HBoV) was first discovered in nasopharyngeal samples in 2005 by Allander *et al.*[1] using non-specific amplification followed by cloning of the products for sequence analysis. A year later and using a similar technique to screen faeces from a cluster of idiopathic cases enrolled in a prospective case control study into acute gastroenteritis (AGE) in children attending a hospital, we discovered two additional genotypes, HBoV2 and HBoV3[2]. Coincidentally, Kapoor *et al.* simultaneously detected HBoV2 in faeces from children in Pakistan using similar non-specific molecular techniques[3] and the presence of a fourth genotype a year later[4].

Human bocaviruses are members of the *Parvoviridae* family (Figure 1), non-enveloped, primarily single-stranded DNA viruses with a genome of approximately 5.5 kb nucleotides encoding four recognised open reading frames. NS1 and NP1 are non-structural proteins. The two capsid proteins VP1 and VP2 are encoded from overlapping open reading frames and have identical C-termini, with VP2 ~20% shorter than VP1 because it is missing the N-terminus of VP1[2]. VP2 is considered the major capsid protein because it can self-assemble to produce non-infectious Virus Like Particles (VLPs) which resemble the infectious virus (Figure 2) in appearance. Recombination between the viruses is evident, typically at the NP1/VP1 gene boundary, with HBoV3 being a recombinant of HBoV1 (5' region of the genome) and HBoV2 (3' region of the genome). HBoV4 similarly appears to be a recombinant of HBoV2 (5' region of the genome) and an as yet undetected genotype (3' region of the genome). Whole genome diversity between the four genotypes ranges from 11.0% to 24.0%, and to date the intra-genotype diversity generally appears quite low, but this more likely reflects what has been detected to date, rather than actual strain diversity.

The role of these viruses in human disease is, however, confusing. While HBoV1 and HBoV2 were first reported in respiratory and enteric samples respectively, both have been found in both types of samples, with reported prevalence rates of 0.3–33.0% and 2.0% in respiratory specimens and 0–13.0% and 0.4–21.0% in enteric samples, respectively[5]. However, these studies are primarily based on retrospective, uncontrolled screening of diagnostic samples. We investigated the prevalence of HBoVs in samples from the above prospective case control study of AGE, collected during from late 2000 to 2004. In our initial report, based on samples from 186 age-matched cases and controls collected during 2001, we were only able to demonstrate an association between the presence of HBoV2 and AGE when the additional case samples collected 48 hours after enrolment were included (prevalence 11.8%, p=0.007 (McNemar's Test), odds risk ratio = 2.6 (95% CI = 1.2–5.7)[2]. However, this lack of statistical association was also demonstrated for norovirus-2 and adenovirus, viruses which are known to cause AGE. Including data from enrolled cases and controls from the whole study weakened the association further, as the prevalence was lower in other years compared to 2001. No statistical association with AGE was found for HBoV1 (prevalence 8.6% of cases) and HBoV3 (prevalence 2.6% of cases).

While statistical association with AGE for HBoV2 will require further study, some observations can be made.

In our study, we detected a variable prevalence, from a cluster of cases in early 2001 (late summer), and average of 13.7% for the whole of 2001, to 5.0% on average in other years. Further, we detected HBoV2 more commonly in samples taken at least 48 hours after onset of symptoms. Since we know little about the shedding of HBoV2 after infection, it is possible that shedding is delayed and therefore the statistical association with infection when second case samples collected within three days of onset are included, is real.

Proving pathogenesis in AGE is problematic due to the high co-detection of other viruses, 20.0% of cases and 5.0% of controls. While a role as an opportunistic pathogen or "helper virus" cannot be ruled out, HBoV2 infections often occur in late summer when the incidence of other enteric pathogens is low.

Our study used as an enrolment criteria that controls had no history of AGE for the previous seven days. More recent reports of long-term shedding of enteric viruses infers that this time limit is too short and some patients enrolled as controls could still be shedding from a previous infection.

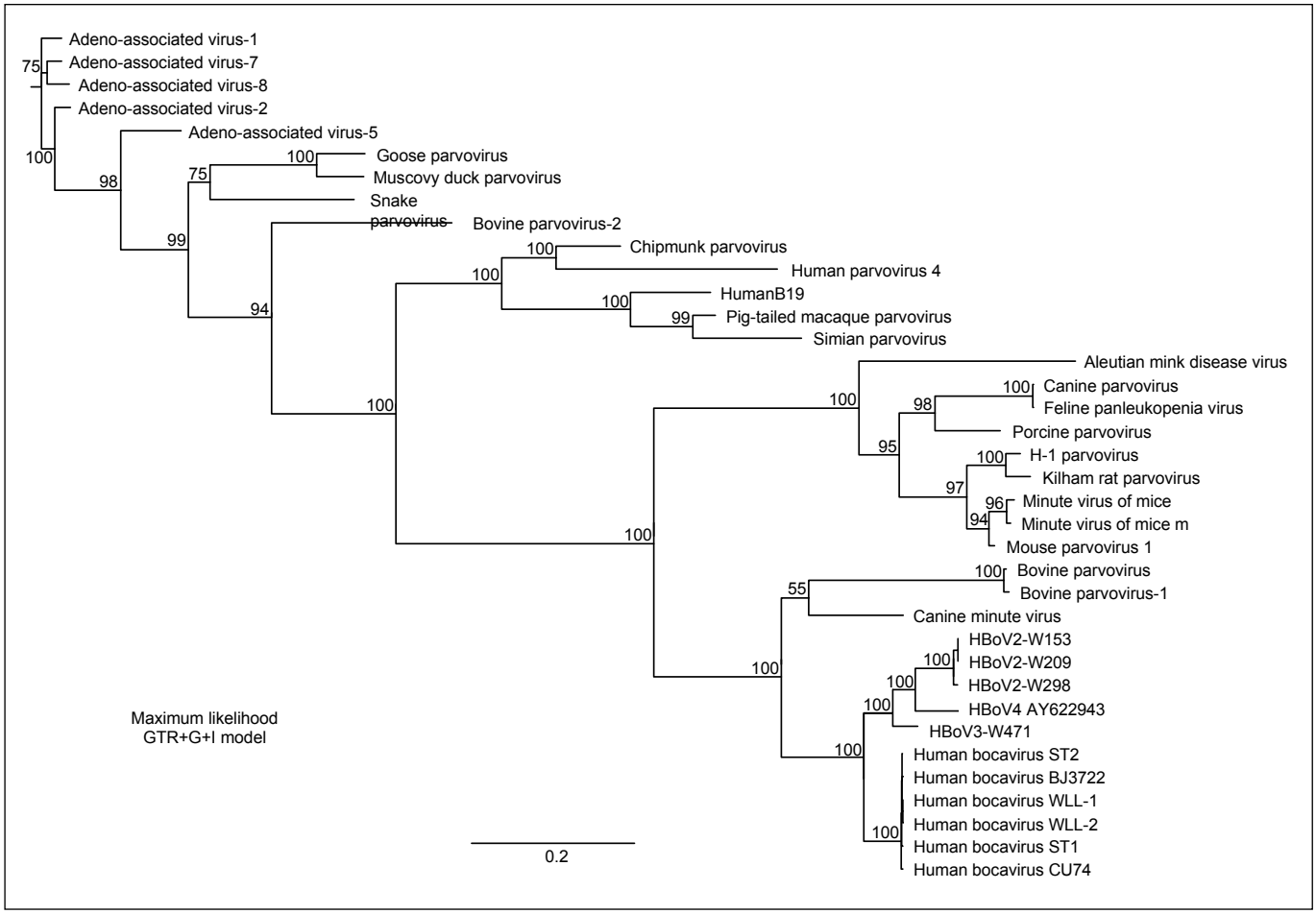

Figure 1. Phylogenetic analysis of parvoviruses: Maximum likelihood dendrogram (bootstrapped, 1,000 replicates) based on full-length parvovirus genome sequence (nucleotide) alignments. 100–200 nucleotides are absent from each termini of the HBoV sequences.

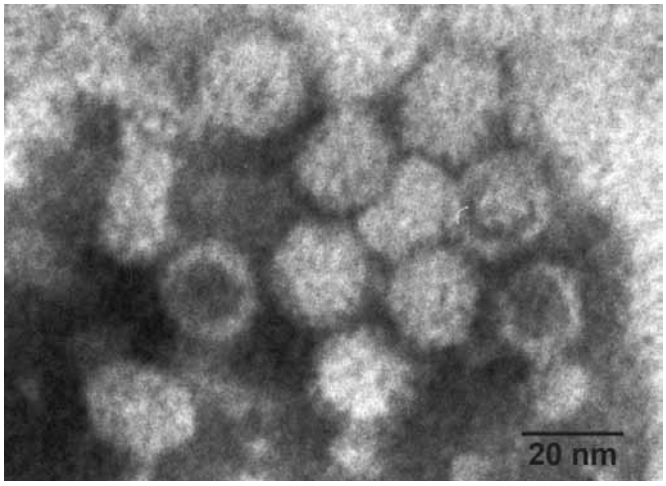

Figure 2. HBoV2 particles in human faeces.

Developing broadly reactive assays to detect a newly discovered virus is problematic as the genetic diversity that needs to be encompassed is unknown. It is, thus, probable that some infections were undetected despite retesting samples with more broadly reactive assays as we became aware of increased genetic diversity. Further, we either sequence confirmed all positives, or confirmed the positive result with an additional PCR assay targeting a second region. We know that a number of positive results could not be so confirmed, likely resulting in under-reporting of prevalence. This inability to confirm some positives is also evidence of genetic diversity greater than what we have so far detected.

In summary, elucidating the role played by viruses in disease, especially AGE, is complex. To date, HBoV1 and HBoV3 appear to not be associated with AGE and HBoV4 not really studied enough to be definitive. HBoV2 may be associated but more case control studies need to be performed. Retrospective uncontrolled screening surveys give an indication of prevalence, but can't assess disease severity, but few case control studies with strict enrolment criteria are reported. Much more needs to be understood about human HBoVs before their role in human disease can be fully assessed and understood.

References

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Rod's biography appears on page 52.