

Searching for HAdV-52, the putative gastroenteritis-associated human adenovirus serotype in Southern Hungary

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SUMMARY

Human adenovirus (HAdV) serotype 52 has recently been discovered in the United States in samples from human patients with gastroenteritis of unknown etiology and is suspected to be a new human enteric pathogen. The aim of the present pilot study was to investigate whether this virus is circulating in the population of Southern Hungary by screening stool specimens collected from gastroenteritis cases and communal sewage samples in the area of Baranya County. A total of 209 diarrheic stool (124 from children and 85 from adults) and 45 influent sewage samples were screened for HAdV-52 by PCR using a primer pair specific to the gene of 12.5K protein in the E3 genomic region. The novel human adenovirus was not detected in any of the tested samples, suggesting that HAdV-52 was not circulating in the target population and the area during the study period. Since temporal and geographical fluctuations may markedly affect the epidemiology of human enteric pathogens, additional investigations are required to gain more in-depth insights into the ecology of this novel adenovirus.

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Adenoviruses cause a variety of diseases in human, including lesions in the upper and lower respiratory organs, the eye, the urinary tract and the central nervous system, as well as hepatitis and gastroenteritis (Horwitz, 2001).

Virtually all human adenovirus (HAdV) serotypes have been found to be shed through the feces and a variety of serotypes have been detected in diarrheic stools. Thus far, however, only two serotypes, HAdV-40 and HAdV-41

(members of virus species HAdV-F) have been proven as a causative agent of acute gastroenteritis mostly in early childhood (Horwitz, 2001). In 2007, a novel human HAdV serotype, HAdV-52, was described from cases of diarrhea in California, United States.

Based on phylogenetic analysis of homologous genes and the overall gene content of the genome, this virus has been proposed to constitute a new species distinct from other well-characterized species of HAdVs (Jones *et al.*, 2007). Because HAdV-52 shows significant homology to some simian adenoviruses (SAdVs) isolated from Old World monkeys, concerns have arisen whether this new AdV serotype is an artifact, generated by contamination of the primary monkey cell culture used for the isolation of the

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virus. However, a second detection of the virus, in an epidemiologically unrelated case of gastroenteritis, has suggested that HAdV-52 might have already adapted to, and can perhaps be transmitted in, the new host species (Jones *et al.*, 2007). These findings warrant further epidemiological surveys to assess the spread of this novel AdV in various human populations, notably in different geographical areas, and to estimate its role as a human enteric pathogen more precisely.

We set up a pilot study in which a one-step PCR assay was utilized using a primer pair specific to HAdV-52 to test diarrheic stool specimens collected from children and adults.

Nucleic acid extracts from influent sewage samples as surrogate of our epidemiologic survey were also tested.

The PCR primers were designed to target the gene coding for the 12.5K a protein within the E3 genome region (Jones *et al.*, 2007). The nucleotide position of the forward (5' ATG ACA GAT GGT GCG GCC GTG AGA GCT 3') and reverse (5' TAA TCC GGG CGT GGG GCA GAT GCA GAA C 3') primers correspond to the genomic nt 25760 - 25786 and 26029 - 26002, respectively, of serotype HAdV-52.

The choice of target was influenced by the fact that although this gene is present in a variety of non-enteric adenoviruses, it is absent in the members of species HAdV-F (Davison *et al.*, 2003; Jones *et al.*, 2007).

Such selectivity was an important prerequisite in our study for screening sewage samples most of which tested positive for enteric HAdVs. *In silico*, the primers did not show a high ability to cross react with a variety of DNA viruses known to be shed in the human feces, and did not show significant complementarities to other HAdV and SAdV strains known to carry a homolog of the 12.5K gene in their genome. The only exceptions were SAdV-1 and SAdV-7 that are genetically closely related to HAdV-52 (Jones *et al.*, 2007).

The prototype strains of these viruses have been isolated from *Macaca fascicularis* and *Macaca mulatta*, respectively. Lacking a sample from HAdV-52, we utilized SAdV-1 (strain ATCC VR-195) and SAdV-7 (strain ATCC VR-201) for the optimization of the reaction conditions, and also as positive control in our assay.

The DNA from 10-20% suspensions of human stool specimens was extracted by a standard method using proteinase K digestion followed by phenol:chloroform fractionation and then ethanol precipitation.

Subsequently, the DNA was further purified with the guanidine thiocyanate/silica method as described by Boom *et al.* (1990). The nucleic acid from raw sewage samples was extracted directly by the same method as described in detail elsewhere (Meleg *et al.*, 2006, 2008). Five μ l of sample DNA was added to the PCR mixture of 50 μ l final volume which contained 1X final concentration of *Taq* polymerase buffer (75 mM Tris-HCl (pH 8.8 at 25°C), 20 mM $(\text{NH}_4)_2\text{SO}_4$, 0.01% (v/v) Tween 20 and 2 mM MgCl_2 ; Fermentas), 200 μ M each of the four dNTPs (Promega), 1 μ M each of the two primers (Integrated DNA Technologies), and 2.5 U of *Taq* polymerase (Zenon). Cycling conditions included an initial denaturation at 94°C for 5 min, followed by 40 cycles consisting of steps at 94°C for 1 min, 60°C for 2 min, and 72°C for 1 min. The terminal elongation was at 72°C for 5 min. Fifteen μ l PCR products were run in 2% (w/v) agarose gel (Sigma) containing 0.5 μ g/ml ethidium-bromide, and photographed using a UV transillumination.

A total of 124 diarrheic samples, collected from children between 2004 and 2006, and 85 diarrheic stool samples from adults collected in 2006 in Baranya County, Hungary were screened. Both sample sets had previously been tested only for group A rotaviruses by polyacrylamide gel electrophoresis. With the exception of 6 adult stool specimens, all samples tested negative for rotavirus. In addition, 45 sewage samples collected between 2004 and 2006 from the same area as the clinical specimens were also screened for HAdV-52. Both SAdV serotypes employed in our assay optimization procedure gave amplicons of the expected size (270 bp), however no size specific amplicon was obtained with any of the clinical or the environmental specimens. In a few cases, nonspecific bands of size similar to the expected one (~260 to 290 bp) were obtained but the sequence analysis ruled out their origin being from adenoviral DNA (data not shown).

A convincing amount of evidence supports the theory of the supposed co-evolution and co-speciation of AdVs with their vertebrate host, in-

cluding rare examples for crossing the host species barrier (Benkő and Harrach, 2003). In some instances, however, host switching events have been hypothesized to explain the strikingly close genetic relationships between AdV serotypes derived from distantly related host species. Such examples include a feline adenovirus that is virtually indistinguishable from HAdV-1 (from species HAdV-C) based on partial nucleotide sequence of the hexon gene (Lakatos *et al.*, 1999), or the bovine AdV-9 that is closely related to other serotypes of species HAdV-C (Benkő *et al.*, 1990).

A recent seroepidemiological study (Xiang *et al.*, 2006) demonstrated that 1.7% to 18.7% of individuals in cohorts of the African population in the sub-Saharan area have antibodies to selected AdV serotypes of chimpanzee origin, suggesting that bush meat hunting may represent an occupational risk for acquisition of SAdV infection. In contrast, zoo workers in the United States who have frequent contact with chimpanzees and thus are potentially exposed to chimpanzee AdV infection are not at higher risk for infection than the control population, suggesting that regular contact with chimpanzees is not a major mode of transmission of heterologous adenoviruses (Xiang *et al.*, 2006). All these findings seem to be of interest and merit further scrutiny. Seroepidemiological studies with different SAdV serotypes, concerning various contact patterns between humans and monkeys or apes, may shed light on the eventual zoonotic potential and the mode of transmission of heterologous adenoviruses.

It is currently unknown whether HAdV-52 needs to be considered an emerging virus in the human population. At present, information on a total of six human cases is available, five of which came from a single gastroenteritis outbreak. A sixth case was independent from this outbreak (Jones *et al.*, 2007). This indicates that the virus might have been in circulation for a while in parts of the United States. As successful *in vitro* propagation of HAdV-52 has been reported (Jones *et al.*, 2007), retrospective seroepidemiological surveys may be conducted to better explore the distribution of this virus in the general population in various geographic areas and to determine if antibody prevalence has been increasing in a temporal fashion.

The present pilot study disclosed no evidence of HAdV-52 infection in patients treated in hospital with acute gastroenteritis in a small area of Hungary. Because communal sewage may serve as an indicator for viral infections of low prevalence or unrecognized clinical importance of the local population we sought to investigate the presence of this virus in communal waste water samples too.

Unlike conventional HAdVs (including both enteric and non-enteric serotypes) that were frequently detected in these specimens (Meleg *et al.*, manuscript in preparation), no evidence was found for shedding of HAdV-52 into the environment.

In conclusion, the findings of this investigation suggest that HAdV-52 was not circulating in the surveyed area during the study period.

Evaluation of our results requires some caution. Since temporal and geographical fluctuations in the prevalence of enteric pathogens may have a remarkable effect on the outcome of such epidemiologic surveys, additional investigations are required to gain more data on the prevalence of this novel adenovirus in the healthy population, or in mildly or severely affected diarrheic patients.

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