

studies investigating the possibility of vertical transmission by ABV-infected pairs, especially to minimize the risk for such transmission to endangered species with restricted breeding opportunities.

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Novel Astroviruses in Children, Egypt

To the Editor: Human astroviruses (HAstVs) are a common cause of gastroenteritis in children, the elderly, and immunocompromised persons (1). Up to 10% of acute viral gastroenteritis in children and 0.5%–15% of diarrheal outbreaks are attributed to astroviruses (2). Until 2008, eight classical astrovirus serotypes were known to cause human disease; in Egypt, HAstV-1 is the most frequent astrovirus serotype detected (3). Recently, 5 novel astroviruses have been discovered in human fecal samples from patients with diarrhea or acute flaccid paralysis (4–7). Because the prevalence of these viruses in the Middle East is unknown, we screened fecal samples from children with diarrhea residing in Egypt to ascertain the prevalence and diversity of these novel astroviruses.

Fecal samples were collected from a cohort of 364 symptomatic children <5 years of age who had diarrhea and were seeking medical care at Abu Homos Hospital in the Nile Delta of Egypt from September 2006 through September 2007. RNA was extracted from 10% fecal suspensions and reverse transcription PCR for astrovirus was performed as previously described (4–6). Astrovirus consensus primer pair SF0073/SF0076 amplified an ≈409-bp region of open reading frame (ORF) 1b, encoding the RNA polymerase gene. PCR-positive samples were then tested by using primer sets Mon269–Mon270 (8) and SF0053–SF0061, amplifying either a 449-bp or a 402-bp product of the ORF2 capsid gene from classical HAstVs (serotypes I–VIII), or astrovirus MLB1, respectively. DNA sequences of PCR products were determined by using Big Dye Terminator Cycle technology (Applied Biosystems, Foster City, CA, USA). Nucleotide sequences were compared with sequences obtained from GenBank. Phylogenies

were constructed with the MEGA4 software (www.megasoftware.net) by using the neighbor-joining method and a p-distance algorithm. Bootstrap resampling was performed by using 2,000 replicates to demonstrate robustness of grouping. The nucleotide sequences determined in this study were assigned GenBank accession nos. HQ674630–HQ674650.

Consensus astrovirus reverse transcription PCR results were posi-

tive in 23 (6.3%) of 364 fecal samples. Five common serotypes of classical HAsV were identified constituting 16 (70%) of 23 positive samples; HAsV type I was most prevalent ($n = 9$). Alignment of the partial amino acid sequences of the ORF2 capsid region indicates that Egyptian HAsV type I strains share 99%–100% identity (Figure, panel A).

Five of the 7 remaining positive samples were most closely related to

MLB1 on the basis of the partial ORF2 sequence analysis. Egypt MLB1 samples shared $\approx 99\%$ -nt identity with each other, and all grouped in 1 phylogenetic cluster (Figure, panel B) along with a recently identified MLB1 strain (GenBank accession no. HM450380 [9]) isolated from a human fecal sample in Hong Kong. The Egypt MLB1 strains shared $\approx 97\%$ -nt identity with the prototype MLB1 Australia strain but were more divergent from an isolate recently described in the United States (92% nt identity, GenBank accession no. FJ222451). However, all of the observed nucleotide differences represented silent mutations between Egypt MLB1 and Australia and US isolates; comparison of partial capsid protein sequences indicated no amino acid changes.

The 2 remaining HAsV-positive samples were phylogenetically most similar to astrovirus VA2 (VA2) (Figure, panel B). On the basis of the sequence of the amplicon from the ORF1b region, the Egyptian VA2 isolates shared 96.1%–100% aa identity to previously described VA2 and astrovirus human/mink/ovine genomes in GenBank.

Our study describes the detection of the recently identified viruses MLB1 and VA2 in a cohort of symptomatic children with diarrhea residing in Egypt. This study expands the geographic range of these viruses to include northern Africa. The consensus primers used in our study were able to detect a higher percentage of positive HAsV serotypes I–VIII than the Mon340/Astman-2 primers (10) (data not shown), a finding that encourages use of these primers to screen humans with gastroenteritis for astroviruses. Increased understanding of the genetic diversity within viral families infecting humans will assist in future studies of their pathogenicity and the design of specific diagnostic assays. Further epidemiologic studies, including clinical cases and demographically matched healthy con-

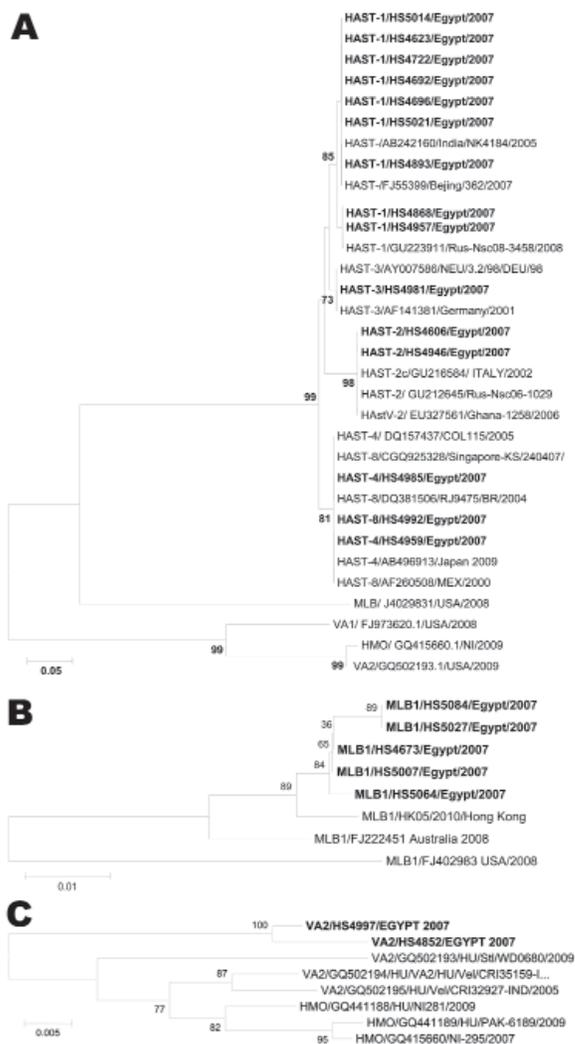


Figure. A) Phylogenetic analysis of the partial amino acid sequences of the open reading frame (ORF) 2 capsid region of human astrovirus (HAsV) types I–VIII (using Mon primer) with other sequences from GenBank. B, C) Phylogenetic trees based on partial nucleotide sequences of MLB1 ORF2 (B) and ORF1b (VA2) (C). Egyptian isolates are shown in **boldface**. Sequence alignment was performed by using ClustalW in the BioEdit software package (www.clustal.org). Dendrograms were constructed by using the neighbor-joining method as in MEGA4 (www.megasoftware.net). Values on the branches represent the bootstrap values from 2,000 replicates. Scale bar in A indicates amino acid substitutions per site; scale bars in B and C indicate nucleotide substitutions per site.

trols, are required to better define their pathogenic potential.

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Humans as Source of *Mycobacterium tuberculosis* Infection in Cattle, Spain

To the Editor: *Mycobacterium tuberculosis* is the main causative agent of tuberculosis in humans. However, little attention has been paid to its transmission from humans to animals. We report *M. tuberculosis* infections in 3 cattle farms in Spain. The epidemiologic investigation traced humans as the source of infection, with 1 of the strains showing multidrug resistance.

Recent studies have reported isolation of *M. tuberculosis* in cattle with prevalences of 4.7%–30.8% in African and Asian countries (1–3). In cattle, this infection occurs in countries with the highest incidence of human tuberculosis in the world. In Europe, only 14 cases of *M. tuberculosis* infection have been described in 3 eastern countries since implementation of eradication programs (4,5). The only reported cases of *M. tuberculosis* in cattle in western Europe were described in Great Britain and date back to the 1950s (6).

During 2007–2009, three cases of tuberculosis caused by *M. tuberculosis* were detected in 3 unrelated cattle farms, 2 of them free of tuberculosis (farms 1 and 2). As part of the surveillance system of bovine tuberculosis, a pool of tissue samples from each cow (respiratory lymph nodes and lung) were homogenized with sterile distilled water, and culture was carried out by the BACTEC mycobacteria growth indicator tube 960 system (Beckton Dickinson, Madrid, Spain). Members of the *M. tuberculosis* complex were identified and genotyped by direct variable repeat spacer oligonucleotide typing and mycobacterial interspersed repetitive unit–variable number tandem repeat (MIRU-VNTR) typing (7).

The 3 *M. tuberculosis*–infected animals were <9 months of age (Table). As described (6), the possibility of infection in young animals could be more probable than infection in older cows.

M. tuberculosis–infected animals from farms 1 and 3 were detected by the intradermal tuberculin test (Table). The animal without immunologic response (farm 2) was detected because an *M. bovis* infection was confirmed in the herd, and all animals were slaughtered. Confirmation of infection by culture without immunologic response is rare, although the high sensitivity of the mycobacteria growth indicator tube system could detect a low bacterial load in the initial stages