regions would be conserved differently must be considered.

It is probable that subsequent sequences of HGyVs remain to be identified in human blood. A recent study reported the characterization of a highly divergent GyV sequence (GyV4) in human fecal samples and chicken meat (7); as with avian GyV2 and GyV3, further research is needed to determine whether this variant replicates in the human body or is solely ingested in food and passively excreted. A better knowledge of the genetic diversity of these newly discovered viruses will enable development of improved molecular detection systems and their subsequent use in epidemiologic studies involving diverse human cohorts.

The potential clinical importance of HGyVs remains to be clarified. Although infection with CAV in birds is frequently associated with clinical signs and disease, the presence of HGyVs in immunocompromised or immunocompetent humans does not appear to be correlated with visible symptoms. Further studies of the natural history and distribution of HGyVs in human hosts are needed.


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Vibrio cholerae O1 Isolate with Novel Genetic Background, Thailand–Myanmar

To the Editor: Vibrio cholerae O1, a causative agent of cholera, was classified into 2 biotypes, classical and El Tor (7). However, accumulating evidence suggests that atypical El Tor V. cholerae, which possesses traits of both classical and El Tor biotypes, has replaced the seventh pandemic prototypic El Tor V. cholerae worldwide in recent years. Cholera outbreaks in Thailand during 2007–2010 were caused by atypical El Tor isolates carrying the classical type cholera toxin gene (2). Epidemiologic surveys in a Thailand–Myanmar border area during 2008–2012 yielded more than 500 isolates of V. cholerae O1. We identified an isolate that possessed the typical El Tor type cholera toxin gene (genotype 3) and designated it MS6 (later assigned strain number DMST28216). It does not belong to either the seventh pandemic prototypic biotype identified in 1961 or the group of atypical El Tor strains found during 1991–present (3).

MS6 was isolated from stool samples from a 26-year-old woman (migrant worker) from Myanmar who had been admitted to Mae Sot General Hospital in Tak Province, Thailand, for 3 days with vomiting, watery diarrhea, nausea, fever, and headache. The illness was considered mild to moderate. Acute gastroenteritis was diagnosed on the basis of the symptoms and laboratory results. The key virulence factors of V. cholerae O1 include cholera toxin (CTX), which is responsible for profuse watery diarrhea, and a pilus colonization factor known as toxin-coregulated pilus (TCP). The virulence-related genes (ctxAB and tcpA) and the phage repressor gene (rstR) of MS6 had identical sequences to those of the seventh pandemic prototypic El Tor V. cholerae O1 N16961 strain. The isolate was found to be positive for enteric bacteria in the Voges-Proskauer test and resistant to polymyxin B (50 units). We further investigated 2 gene clusters, Vibrio seventh pandemic island I (VSP-I) and II (VSP-II), associated with the seventh pandemic strains and absent in classical and pre–seventh pandemic strains (4–7). The common genes on VSP-I and VSP-II were detected by PCR in MS6 but were lacking in VSP-II; 26.9 kb of VSP-II was originally found in N16961. Moreover, PCR analysis showed that the isolate did not
possess the VC2346 gene, a specific marker of the seventh pandemic clone (5,9). However, we found a VC2346 homolog with 83.9% sequence identity with VC2346 at the nucleotide level and 97% at the amino acid level in the 624-bp region. The coding region of the homolog is considered to be shorter than VC2346 (684 bp) because it contains the stop codon, TAG. This homolog was identified in environmental, classical, or pre–seventh pandemic strains of V. cholerae O1 (5), including MS6, and in 2740–80 (US Gulf Coast, 1980), 3569–08 (US Gulf Coast, 2008), BX33026 (environmental water in Australia, 1986), RC27 (classical, human isolate in Indonesia, 1991), O395 (classical, human isolate in India, 1965), MAK757 and M66–2 (pre–seventh pandemic, human isolate in Indonesia, 1937), and NCTC 8457 (pre–seventh pandemic, human isolate in Saudi Arabia, 1910), excluding seventh pandemic strains.

This conservation of the homologue of VC2346 in strains isolated over the course of a century and the geographic distribution of the strains suggest a notable biologic function and a specific marker. In addition, we determined the sequences of 15 housekeeping genes which exhibited sequence variations in toxigenic V. cholerae (10). The results indicated that by comparison, MS6 is closely related to the US Gulf clones (Figure). However, 2 genes, malP and pepN, of MS6 are remotely related to them and are novel sequence types, based on results of a BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) search. Antimicrobial susceptibility testing by using the disk diffusion method revealed that MS6 was susceptible to chloramphenicol, ciprofloxacin, gentamicin, sulfamethoxazole/trimethoprim, tetracycline, streptomycin, furazolidone, doxycycline, and norfloxacin, and had intermediate susceptibility to ampicillin and erythromycin, suggesting that MS6 had not been exposed to several antimicrobial drugs. The V. cholerae SXT element, which usually shows code drug-resistance markers, integrates into a specific site of the prfC gene. In MS6, the complete prfC gene was detected. The accession numbers of DDBJ for nucleotide sequences determined in this study are AB699244-AB699265. MS6 possesses unique properties in terms of the ribotype, pulsed-field gel electrophoresis pattern, and multiple locus variable-number tandem-repeat analysis profile, compared with other V. cholerae O1 isolates (2).

This case was probably an episode of sporadic cholera from indigenous V. cholerae O1, such as US Gulf Coast and Australian clones, which are mainly associated with environmental sources. We have been unable to isolate another MS6-like clone, which could have escaped detection because of low prevalence or might exist in a dormant state in a rural area. Nevertheless, the transmission route and its pathogenicity must be of concern for public health.

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References

Crimean-Congo Hemorrhagic Fever
Asia-2 Genotype, Pakistan

To the Editor: Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne zoonotic disease caused by a member of the virus family Bunyaviridae, genus Nairovirus. This virus (CCHFV) has caused illness throughout Asia, Europe, Africa, and the Middle East (1). CCHFVs are clustered among 7 genotypes (Asia-1, Asia-2, Euro-1, Euro-2, Africa-1, Africa-2, and Africa-3) on the basis of genetic variation in the small segment (2). These genotypes are well conserved among their regions of origin; however, >1 genotype is prevalent in many countries (2). In Pakistan, the first CCHF case was reported in 1976; multiple sporadic cases and outbreaks have occurred in subsequent years (3).

To determine which genotypes were present in Pakistan, we performed molecular analysis of archived serum samples collected during 2008 in Fatima Jinnah General and Chest Hospital, Quetta, Baluchistan, in southwestern Pakistan. Because of limited diagnostic facilities for CCHF in this country, samples collected during 1976–2002 were occasionally sent to laboratories in countries such as South Africa and the United States, where genetic analysis showed that all viruses tested from that location belonged to the Asia-1 genotype (4). However, because of improved molecular diagnostic facilities at the Department of Virology, National Institute of Health, Pakistan, blood samples collected from patients with suspected cases attending in-country hospitals are now examined by the institute for confirmation. Our findings substantiate the presence of Asia-1 and Asia-2 genotypes in Baluchistan.

Thirteen IgM-positive samples collected during 2008 and stored at −70°C were available for study. The samples were processed for amplification of 260 bp of the small segment by using reverse transcription PCR with a previously described protocol (5). The mean age of patients with serology-confirmed CCHF was 31.3 (range 18–40) years; male-to-female IgM positivity ratio was 1:2. Common symptoms were fever, headache, and nosebleeds. Platelet counts ranged from 16,000 to 43,000/μL of blood.

Of the 13 samples, viral RNA was detected in 2 (CCHF-65–2008PAK and CCHF-43–2008PAK); the amplicons were subjected to bidirectional sequencing by using the BigDye Terminator v3.1 cycle sequencing kit (Applied BioSystems, Foster City, CA, USA). Sequences were analyzed with Sequencher (GeneCodes Corp., Ann Arbor, MI, USA) and MEGA v4.0 (http://megasoftware.net/). The 2 viruses were phylogenetically clustered into Asia-1 and Asia-2 genotypes, with 7% nucleotide divergence, although both samples were collected during September–October, 2008.

The closest nucleotide identity (99%–100%) for CCHF-65–2008PAK was found with the previously reported Asia-1 strains from Pakistan, Afghanistan, and Iran; CCHF-43–2008PAK had 96%–97% similarity to viruses from Dubai and Tajikistan (Figure). The sequences reported from United Arab Emirates, Pakistan, Afghanistan, Iran, and Iraq belong to the Asia-1 genotype; the Asia-2 genotype sequences were mostly from China and Central Asian countries such as Uzbekistan, Tajikistan, and Kazakhstan (6). All viruses detected intermittently in Pakistan during 1976–2002 were of the Asia-1 genotype (4). However, the analysis of the 2 samples reported here enhances our knowledge of CCHFV genetic diversity in Pakistan.

The closest phylogenetic positioning of CCHF-43–2008PAK