Human Gyrovirus in Healthy Blood Donors, France

To the Editor: Gyroviruses (GyVs) are naked, single-stranded DNA viruses that were described in chickens in 1979 (*1*). Chicken anemia virus (CAV), initially the sole member of the genus *Gyrovirus* (family *Circoviridae*), possesses a genome of ≈2.3 kb, containing 3 major partially overlapping open reading frames, viral proteins [VP] 1–3, and a short untranslated region (*1*). For >30 years, this virus, which was responsible for severe anemia and increased death rates in young chickens, was considered to have an extremely low genetic diversity and to be specific to this animal host. In 2011, however, sequence-independent molecular protocols enabled the characterization of highly divergent, GyV-related sequences in human and chicken biological samples. Human GyV 1 (HGyV1), avian GyV 2, and GyV3 sequences were identified from human skin, chicken blood, and human feces, respectively (*2*–*4*). These genomes harbor a genetic organization similar to CAV, despite a high genetic divergence (49%–65%).

One study described the detection of GyVs in HIV-positive patients and kidney transplant recipients (0.7% and 6%, respectively) (*5*), but these viruses had not been identified in blood samples from healthy persons. We investigated the presence of HGyV DNA in 352 blood samples from healthy blood donors in France (mean age 39 years; 185 men; M:F ratio 1:1.11).

Plasma samples were prepared as described (*6*), and 1-mL aliquots were used for nucleic acids extraction (MagNA pure LC; Roche Diagnostics, Meylan, France). HGyV DNA was detected by using 2 systems in separate real-time TaqMan amplification assays (StepOne Plus; Applied Biosystems, Courtaboeuf, France). The first detection assay (VP1 gene) was described previously (HGyV-rtFP/HGyV-rtRP primers, HGyV-rtP probe, 72 nt) (*5*). The second assay was designed following the analysis of available HGyV sequences (Figure): sense primer HGyVsPBs 5′-GCTAAGACTGTRACATGGC-3′, reverse primer HGyVsPBr 5′-CTCCGGGAATAGCGTCTTC-3′, probe HGyVsPBp 5′-FAM-TGGCACTGGAGACACAGACTGCG-TAMRA-3′. This assay targets the VP2 gene of the viral genome, with an expected length of 118–115 bp, depending on the reference sequence considered.

Amplification reactions were performed by using 10% of extracted material with the TaqMan Fast Universal PCR Kit (Applied Biosystems) in a final volume of 20 μL. Cycling conditions for both assays were 95°C for 20 s, followed by 50 cycles of 95°C for 1 s and 60°C for 20 s. The sensitivity of TaqMan assays was estimated to be 10 copies of HGyV DNA by using dilutions of a synthetic template. Each amplification product was subjected to additional agarose gel electrophoresis to help eliminate potential false-negative real-time PCR results.

Among the 352 plasma samples tested, 3 (0.85%) resulted in a positive signal by using our in-house real-time detection assay; no positive signal was identified by using the other system tested. When the tests were repeated, identical results were obtained. No additional amplicons were identifiable after agarose gel analysis. HGyV DNA titers in the 3 positive samples were low (<500 copies/mL plasma). Positive blood samples originated from 1 woman (age 44) and 2 men (ages 29 and 39). No biological or serologic marker evaluated for routine blood donor screening in France was associated with these donations.

Partial HGyV sequences obtained were cloned and sequenced (7 clones each). All sequences characterized clustered with HGyV1 sequences, exhibiting either 100% nucleotide identity or 1 point mutation (corresponding to a nonsynonymous substitution, ala →thr) (Figure). No intragenetic diversity was identified.

Our results demonstrate that recently discovered HGyVs are detectable in blood of healthy persons. The low prevalence (0.85%) suggests, however, that the virus is infrequently found in the general population in France. A study from Italy of HGyVs in blood from healthy donors did not detect such viruses (*5*). This result may be linked to the small number of samples tested (n = 50) or to the use of a nonoptimized detection system designed on the basis of HGyV1 sequences only; moreover, the possibility that the VP1 and VP2 amplified

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9B1        acatggcAAGACTACGAAGAAGACGACCTCGCGGACGCTTTGGCTTCTACCACAGAGGACGATGGCACTGGAGACACAGACTGCGACgaagacg
9F2        acatggcAAGACTACGAAGAAGACGACCTCGCGGACGCTTTGGCTTCTACCACAGAGGACGATGGCACTGGAGACACAGACTGCGACgaagacg
13F1       acatggcAAGACTACGAAGAAGACGACCTCACGGACGCTTTGGCTTCTACCACAGAGGACGATGGCACTGGAGACACAGACTGCGACgaagacg
HGyV1-915  acatggcAAGACTACGAAGAAGACGACCTCGCGGACGCTTTGGCTTCTACCACAGAGGACGATGGCACTGGAGACACAGACTGCGACgaagacg
HGyV1-CL33 acatggcAAGACTACGAAGAAGACGACCTCGCGGACGCTTTGGCTTCTACCACAGAGGACGATGGCACTGGAGACACAGACTGCGACgaagacg
AGV2       acatggcAAGATTACGACGACGACGACCTCGTGGACGCTTTGGCTTCTACCACAGAGGACGATGGCACTGGAGACACAGACTGCGACgaagacg
GyV3       acatggcTAGA---CGATTTCGCCGGCACAGAGGAAAGTTCGGATACTACAGACGGGGAAGATGGCACTGGAGACACAGACTGCGACgaagacg
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Figure. Alignment of partial sequences of human gyroviruses (HGyVs) from healthy blood donors, France. Point mutation corresponding to a nonsynonymous substitution is in **boldface** (13F1 isolate). Reference sequences and GenBank accession nos.: HGyV1-915, FR823283; HGyV1-CL33, JQ308212; avian gyrovirus (AGV) 2, JQ690763; gyrovirus (GyV) 3, JQ308210). Bar above sequences indicates location of the HGyVsPBp probe; lowercase letters indicate 5′/3′ ends of HGyVsPBs/HGyVsPBr real-time primers; asterisks (*) indicate conserved positions.

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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DOI: http://dx.doi.org/10.3201/eid1906.130228

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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 (*1*). 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 the VSP-I island in N16961, including VC0175, VC0178, VC0180, VC0181, and VC0183, were detected by PCR (*8*) 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