by laboratory contamination with Asian genotype I.

Possible introduction and detection of Asian DENV-4 strains in Brazil should not be ignored because the possibility of multiple introduction events in the country resulting from intense transit of people and commercial activities across Brazil from the Caribbean and Asian regions poses a real risk. However, at this time, only genotype II has been isolated and genetically characterized (*1*). The previously published articles lack strong and reliable scientific evidence.

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Novel Hepatitis E Virus Genotype in Norway Rats, Germany

To the Editor: We read with interest the article by Johne et al. about 2 novel hepatitis E virus (HEV) isolates in Norway rats in Germany (*1*). Some points in the report deserve comment.

First, because of degeneracy of the genetic code, HEV amino acid sequences are more conserved than nucleotide sequences. For instance, although the open reading frame 2 of the avian HEV isolate (GenBank accession no. AY535004) has only 65% nt sequence homology to that of the swine HEV isolate swGX32 (GenBank accession no. EU366959), their amino acid sequences shared >90% identity. However, the table in (*1*) indicated the amino acid sequence homologies between the novel and previous HEV isolates were similar to (some even lower than) the nucleotide sequence homologies. These low sequence identities of the capsid proteins between the novel and previous HEVs may explain why no HEV antibody–positive rat was found in the initial serologic screening with a commercial genotype 1–based ELISA. Furthermore, we wonder how the novel antigen in the hepatocytes could react with the anti-HEV serum in the immunohistochemical staining.

Second, the authors stated they determined the entire virus genome by using a previously described method (*2*). The primers in that method were designed to amplify a genotype 3 HEV isolate with low (55.7%) sequence homology to the 2 novel HEV isolates and therefore cannot amplify their sequences. We ask the authors to list the new primer sequences they used, which will help determine the full viral genome if this virus is found in other regions or animal species.

Suggesting the rabbit HEV sequences may be representative genotype 3 isolates is not yet appropriate because not enough research has yet determined whether rabbit HEV infects other species. Therefore, the rabbit HEV sequence FJ906895 should not be listed as representative genotype 3 isolate as in Figure 1 in (*1*). Also, the swine isolate DQ450072 should not be listed as a representative genotype 4 isolate; a recent report indicated it was a recombinant produced between genotypes 3 and 4 isolates (*3*).

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In Response: The major objective of our study was determination and initial characterization of the entire nucleotide sequence of a novel hepatitis E virus (HEV) from Norway rats. We demonstrated high levels of nucleotide and amino acid sequence divergence between HEV strains from the novel rat and other mammalian and avian HEV strains. In line with our data, nucleotide (and amino acid) sequence identities of 50%–53% (42%–49%), 51%–57% (42%–55%), and 45%–46% (20%–29%) were reported for open reading frame (ORF) 1, ORF-2, and ORF-3, respectively, between rat, human, and avian HEV strains (*1*). Of course, the genetic code is degenerated; however, no strict relationship exists between divergence in nucleotide and corresponding amino acid sequences, e.g., because of natural selection processes (*2*). We could not reproduce the high level (>90%) of amino acid sequence identity between the capsid protein (CP) of avian HEV (GenBank accession no. AY535004) and the unpublished GenBank entry swGX32 (accession no. EU366959) claimed by Zhang et al (*3*).

The low level of amino acid sequence identity between rat and human HEV strains might explain the lack of reactivity of transudates from 6 investigated rats in the genotype 1–based ELISA. Consistent with this assumption, a rat hyperimmune serum specimen, raised against a truncated recombinant rat HEV CP derivative, reacted strongly with the homologous antigen but weakly with genotype 3 HEV antigen (P. Dremsek and R.G. Ulrich, unpub. data). Nevertheless, conserved and cross-reactive epitopes have been identified in the CP of HEV (*4*) and can be expected in the antigenic protruding domain of rat HEV CP (*5*). Therefore, some cross-reacting antibodies might exist that would explain detection of rat HEV by the human anti-HEV serum used in immunohistochemical staining.

For sequencing, novel primers were designed (Table). The recombinant nature of strain DQ450072 was not known at time of analysis. Nevertheless, that this sequence clusters near, but not within, the genotype 4 branch is consistent with the reported recombination event.

Virus taxonomy has to "categorize the multitude of known viruses into a single classification scheme that reflects their evolutionary relationships" (*6*). Because the evolutionary relationships could not be determined without sequence analyses, we could not follow the suggestion of Zhang et al. to use other than genetic information for genotype classification (*3*). Future classification of HEV strains would profit from definition of solid criteria and distinct thresholds for definition of genotypes.

Table. Primers used in study (*1*) of amplification of complete genome sequence of rat HEV from rat sample 63, Germany*

Designation	Binding position	Sequence† (5′ → 3′)
rHEV-RACEn‡	307–284	GTGCTCATTAATAGATCGAGGGTG
rHEV-RACE‡	336–313	GGAAGAAAACATCTGTGAATGACA
HEV-100s	78–102	CGGCCAATTCCGCCYTGGSRAATGC
rHEV-900as	905–881	TATGCCCGCCCGCACTAAACAAACT
HEV-800s	776–801	GTGCGGGCCATTGGCTGYCAYTTTGT
rHEV3300as	3099–3074	AGCCGCCATTCTGTTGGCTCCAGATT
rat5-s	2921–2943	CGCCGGGTTGTGATKGAYGAGGC
rHEV-td1-as	4062–4037	GAAATGCCCTGCCCGACCTTGCCATG
HEV-cs	3977–3999	TCGCGCATCACMTTYTTCCARAA
HEV-cas	4446–4424	GCCATGTTCCAGACDGTRTTCCA
Rat HEV-inv-s	4301–4322	GGGGCRCCYGAGTGGATGTGGA
63-5400as	5449–5428	CTCAGTCGCCATGATATGCGTA
#8-ORF2-s	5399–5421	CCCTTACTGCCTYTKCAGGAYGG
#8-ORF2-as	5604–5582	GTGGAAGTGATGGAATTCATRTC
63-5500s§	5555–5576	CAATCCACAACAGTCCCCACGT

*HEV, hepatitis E virus; RACE, rapid amplification of cDNA ends; ORF, open reading frame.
†D = A + G + T; K = G + T ; M = A + C; R = A + G; S = G + C; Y = C + T.
‡Primers were used for 5′-RACE.
§Primer was used for 3′-RACE.

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