bred outdoors in malaria-endemic areas is not uncommon. However, with the exception of *P. knowlesi*, it is my understanding that malarial organisms found in cynomolgus monkeys do not pose a major zoonotic concern (although this can always change). Furthermore, it is my understanding that *P. vivax* does not infect macaques, including cynomolgus monkeys.

Other malarial parasites of cynomolgus monkeys, apart from *P. knowlesi*, may include *P. cynomologi*, *P. inui*, *P. fieldi*, and *P. coatneyi*. A recent publication reported that in wild-source cynomolgus monkeys in Malaysia, >90% of the animals tested were positive for ≥1 *Plasmodium* species. Furthermore, >80% of samples from these animals were positive by specific PCR for ≥1 of these organisms (*2*).

Using PCR for *Plasmodium* spp. identification, I have tested newly imported research cynomolgus monkeys from various breeding centers in China. I can confirm that some animals have subclinical malarial infections.

Except for the report by Li et al. (*1*), I am unaware of other reports of *P. vivax* in cynomolgus monkeys. It would be interesting to confirm the presence of this organism by using PCR primers specific for *Plasmodium* spp. My questions to the authors relate to the test method used in their study. Was an ELISA for detecting *P. vivax* antibodies the only diagnostic method used to identify this parasite? It may be useful to re-address the specificity of this test in differentiating various *Plasmodium* spp. Until these issues are clearly addressed, their reported results are not reliable.

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DOI: http://dx.doi.org/10.3201/eid1710.110719

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Dengue Virus Serotype 4, Roraima State, Brazil

To the Editor: Temporão et al. recently reported the detection and characterization of dengue virus serotype 4 (DENV-4) in Boa Vista, Roraima, Brazil (*1*). To date, 4 subtypes of DENV-4 have been recognized: genotype I, which comprises Asian strains (e.g., Thailand-1978-U18441); genotype II, which has been detected since the early 1980s in the Americas (e.g., Brazil-1982-U18425); genotype III, which comprises recently emerged Thai strains (GenBank accession no. AY618989); and genotype IV, which comprises sylvatic strains (GenBank accession no. EF457906) (*2*).

Temporão et al. conducted phylogenetic analysis of envelope gene sequences and concluded that 3 samples of DENV from Roraima in 2010 were DENV-4, genotype I (*1*). Unfortunately, the authors mistakenly labeled Asian strains (Thailand-1978 and -1985) as genotype II, and American strains (e.g., Brazil-1982) as genotype I. Those DENV-4 strains isolated in Roraima in 2010 in fact belong to genotype II (*2*,*3*). We had previously analyzed 2 samples isolated from Roraima in 2010 by using C/prM nucleotide sequencing and maximum-likelihood phylogenetic reconstruction. Our results, presented at the XXI National Meeting of Brazilian Society for Virology in October 2010, show that both isolates are indeed genotype II (*3*). Nucleotide sequences are available in GenBank under accession nos. HQ822125 and HQ822126.

Temporão et al. also concluded that because only genotype II (reported as genotype I) was identified in their samples, "[it] excludes the possibility that Asian genotypes previously circulated in Brazil." Beyond its obviously flawed logic, we believe that this statement lacks scientific support; DENV-4 genotype I, closely related to Chinese and Philippine strains, has in fact been shown to occur in the city of Manaus, ≈800 km south of Boa Vista, as reported in 2 recent articles (*4*,*5*). Circulation of DENV-4 genotype I in northern Brazil, probably related to increasingly intense trade with Asian countries, may be sporadic and geographically limited as yet (*5*), but ignoring this evidence can hardly be helpful for dengue surveillance and control.

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DOI: http://dx.doi.org/10.3201/eid1710.110776

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In Response: With regard to their comment on our letter, Dengue Virus Serotype 4, Roraima State, Brazil (*1*), Amézaga Acosta et al. correctly refer to an erroneous correlation between genotype I and American strains and between genotype II and Asian strains (*2*). That was a typographic error in the text and figure. Our Figure shows the correct association.

Amézaga Acosta et al. also suggested a scientific inconsistency, that our results exclude circulation of previous Asian strains in Brazil, and argue that Figueiredo et al. (*3*) published a letter on the introduction of DENV-4 in Manaus. However, Amézaga Acosta et al. probably do not know that the article by Figueiredo et al. has been contested by the Brazilian Ministry of Health for failing to demonstrate any irrefutable scientific result, including the virus isolation. Our statement was logically based on strong epidemiologic surveillance, virus isolation, serologic evidence (hemagglutination inhibition assay and immunoglobulin ELISA), and clinical aspects.

Regarding circulation of 2 genotypes in Brazil, when the article was written, epidemiologic and molecular evidence supported the hypothesis of circulation of only the American genotype (II) in northern Brazil and not the Asian (I) and American (II) genotypes at the same time (*1,2,4*). More specifically to the Manaus finding, no virus was isolated and no strong serologic evidence (in the lack of virus isolation) was provided, and the Ministry of Health considers this article a mistake, probably caused

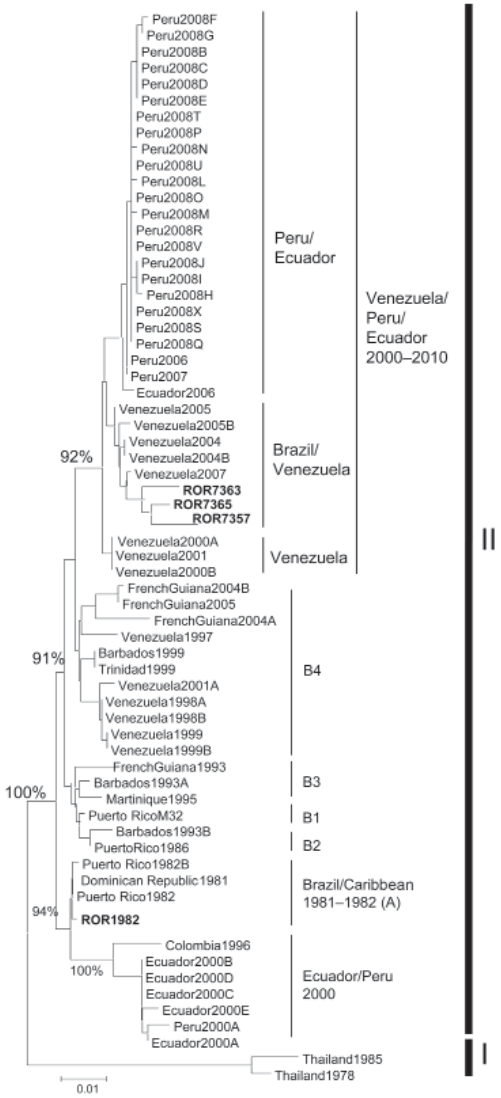

Figure. Phylogenetic tree demonstrating the 2 clusters of dengue virus serotype 4 correctly associated with corresponding genotypes: genotype II, American strains, including strains isolated in Roraima in 2010; and genotype I, 2 Asian strains. **Boldface** indicates strains isolated in Roraima. Scale bar indicates 1% nucleotide sequence divergence.

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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DOI: http://dx.doi.org/10.3201/eid1710.110927

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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*).

This work was supported by the Professional Research Foundation for Advanced Talents of Jiangsu University under grant no.10JDG059 and a grant from the National Natural Science Foundation of China no. 31070132.

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DOI: http://dx.doi.org/10.3201/eid1710.101399

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