

## **Hepacivirus Infection in Domestic Horses, Brazil, 2011–2013**

**To the Editor:** An estimated  $\approx 150$  million persons (3% of the world population) are chronically infected with hepatitis C virus (HCV). This virus is the prototype of the genus *Hepacivirus* and a major cause of liver cirrhosis and hepatocellular carcinoma around the world. Every year, 3–4 million persons become infected with HCV, and  $\approx 350,000$  die of this infection (1).

Development of an HCV vaccine has been hampered by the difficulty of in vitro cultivation of the agent and by lack of animal models for studies of viral functions and host immune reactions (1,2). The only effective experimental model that can be infected with HCV and in which the course of infection is similar to that in humans is the chimpanzee.

Since the discovery of HCV-like virus in dogs with respiratory disease and nonspecific gastrointestinal disorder in the United States in 2011, tentatively named canine hepacivirus (2), new hepaciviruses have been detected in insectivorous bats (3), Old World monkeys (4), wild rodents (5,6), and domestic horses (6–8). These animals could potentially serve as HCV models, but the accuracy of HCV tissue tropism, pathology, and immunology in natural hosts needs to be demonstrated.

No official nomenclature for these recently described hepaciviruses has been defined by the International Committee of Viral Taxonomy. In this article, we refer to the viruses detected in horses as conventional nonprimate hepaciviruses (NPHVs). The aim of this study was to verify NPHV infection in horses from 8 locations in the eastern Brazilian Amazon.

During January 2011–November 2013, serum samples were collected from 300 equids in 7 cities and 1 district of the State of Pará, Brazil (online

Technical Appendix, <http://wwwnc.cdc.gov/EID/article/20/12/14-0603-Techapp1.pdf>). Samples came from 265 horses (*Equus caballus*), 30 mules (*Equus mulus*), and 5 donkeys (*Equus asinus*). All procedures for obtaining and using the samples were approved by the Ethics Committee on the Use of Animals in Research from the Evandro Chagas Institute (protocol code 0023/2012 CEUA/IEC/CENP/SVS/MS).

Viral RNA was extracted from serum samples by using the TRIzol LS Reagent (Invitrogen, Carlsbad, CA, USA), and a target sequence in the nonstructural 3 protein (NS3) region of the NPHV genome was amplified from the cDNA by using a previously reported nested PCR protocol (8). Second-round product reactions of  $\approx 380$  bp were considered positive.

We used the BigDye Terminator version 3.1 Cycle Sequencing Kit and an ABI 3500 Genetic Analyzer (both from Applied Biosystems, Foster City, CA, USA) for sequencing. To obtain consensus sequences, we used BioEdit software, version 7.0.5.3 (<http://www.mbio.ncsu.edu/BioEdit/BioEdit.html>). To phylogenetically analyze the virus sequences based on an NS3 partial nucleotide sequence of 294 bp, we used the maximum-likelihood method with the Kimura 2-parameter model in MEGA version 5.1 (<http://www.megasoftware.net>) (bootstrap, 1,000 replicates). The nucleotide distance was calculated by using PAUP version 4.0 software (<http://paup.csit.fsu.edu/>). The sequences obtained in this study have been assigned GenBank accession nos. KJ469442–KJ469466.

Of the 300 serum samples, 25 (8.3%) were positive for NPHV by nested reverse transcription PCR; these results were confirmed by nucleotide sequencing. This prevalence of NPHV infection among horses in Brazil is higher than that reported for other countries (6–8).

The prevalence among competition horses (19.1%) was remarkably higher than that among farm (6.3%)

and cart horses (6.2%). Many possibilities for the higher prevalence in the first group, such as exposure to non-sterile needles shared by a large number of animals, must be considered; however, further studies are needed to investigate this hypothesis.

No mules or donkeys tested in this study were infected by NPHV. A similar finding for another study has been reported (8), which might suggest that these species are not natural hosts for NPHV.

Until now, it has been impossible to associate *Hepacivirus* infection in horses with clinical hepatic disease (7,8). All infected horses for which information was available were healthy at the time of sampling. Our finding of high NPHV prevalence among horses is consistent with findings of previous studies in which NPHV-mediated disease was not found, suggesting that the course of infection is chronic and largely asymptomatic, similar to that of HCV infection in humans.

The sequences reported in this study shared close genetic relationships with previously reported NPHV sequences from dogs and horses (Figure) (2,6–8). The nucleotide distances of the sequences ranged from 0 to 19.9%.

Many of these new isolates were grouped according to geographic origin, except for 2 sequences that grouped distantly from other isolates from Neotropical regions. One isolate (KJ469449), from a Quarter horse, was closely related to NPHV detected in horses in the United States (7); similarity was  $\approx 94\%$ . The other sequence from a Neotropical region (KJ469466), from a Marajoara horse, grouped closer to the NPHV from horses in Germany (6); similarity was as high as 95%.

NPHV, one of the viruses most closely genetically related to HCV, is present in the Neotropics and was identified in the eastern Brazilian Amazon. The infection seems to be enzootic in the studied horse population and to be geographically dispersed in northeastern Pará State.

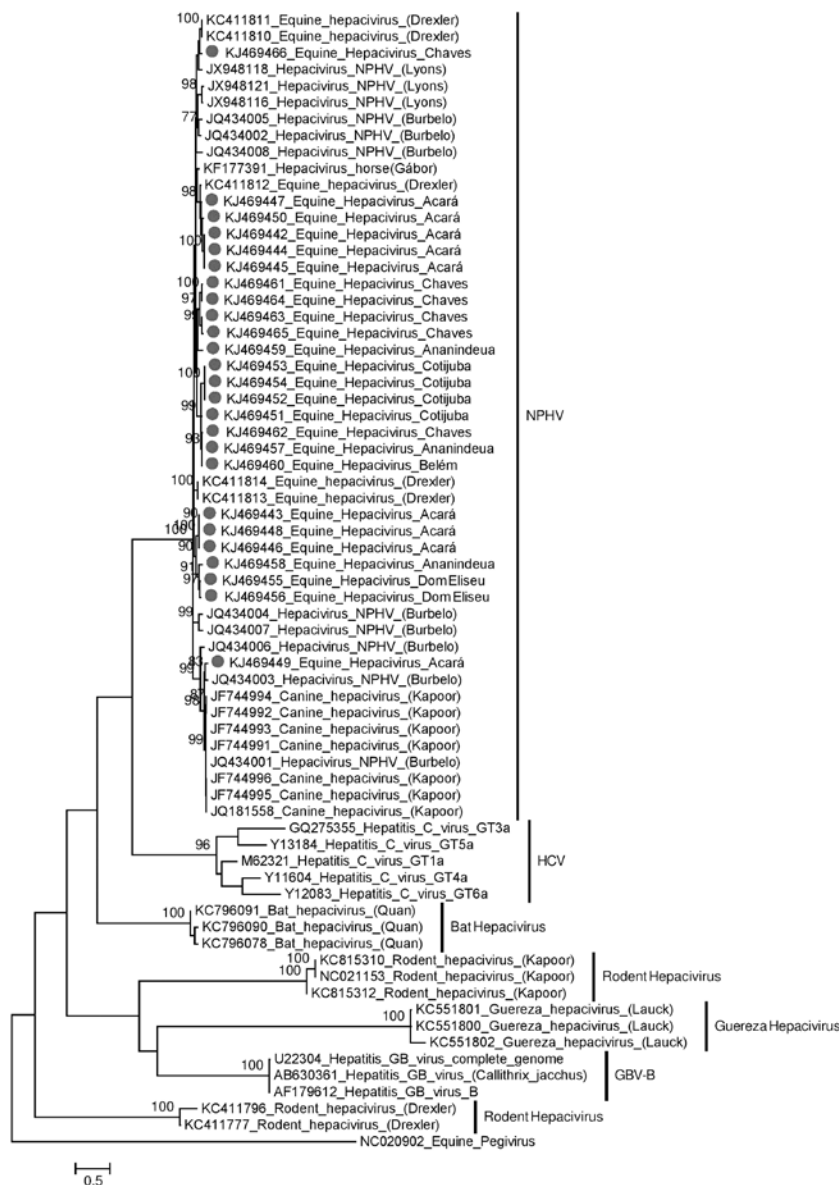


Figure. Maximum-likelihood phylogenetic tree of the partial nucleotide sequences of the nonstructural protein 3 region (294 bp) of *Hepacivirus*. The retrieved sequences from GenBank are indicated by the accession number followed by the species from which each was isolated. The 25 sequences obtained from 300 equids in 7 cities and 1 district of the State of Pará, Brazil, during January 2011–November 2013, are indicated with a dot and are identified by their GenBank accession numbers followed by their community of origin. Bootstrap values (1,000 replicates) >70% are listed at the nodes. One sequence of equine Pegivirus was used as an outgroup. GBV-B, GB virus B; HCV, hepatitis C virus; NPHV, nonprimate hepaciviruses. Scale bar indicates nucleotide substitutions per site.

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## Hepatitis E Virus Genotype 4 in Yak, Northwestern China

**To the Editor:** Hepatitis E virus (HEV; family *Hepeviridae*, genus *Hepevirus*) is a positive-stranded RNA virus with a genome of  $\approx 7.2$  kb that contains 3 open reading frames (1,2). On the basis of sequence analysis, mammalian HEVs are classified into 4 recognized genotypes (3,4). HEV genotypes 1 and 2 are restricted to humans and are often associated with large outbreaks and epidemics

in developing countries, especially in Africa and Asia. Genotypes 3 and 4 are zoonotic and have been detected in humans, pigs, and other animal species (1,3–6).

Yaks (*Bos grunniens*) live on the cold highland (altitude  $>3,000$  m, average annual temperature  $<0^{\circ}\text{C}$ ) surrounding the Qinghai-Tibet Plateau, which includes Qinghai and Gansu Provinces in northwest China. Domestic yaks are usually slaughtered for meat at 3 years of age. Infectious pathogens in yaks have been reported only recently (7,8). On the basis of the high prevalence of HEV in human and pigs in China and close human–yak contact in the Tibet region (4,5), we sought to determine if HEV infects yaks.

During March–September 2013, we collected 167 fecal samples from yaks  $<3$  years of age; 92 were from Qinghai Province (56  $<1$  year of age) and 75 from Gansu Province (48  $<1$  year of age). Soon after sampling, 10% (wt/vol) fecal suspensions were prepared by using sterile phosphate-buffered saline (0.01 mmol/L phosphate, pH 7.2–7.4; 0.15 mmol/L NaCl, 0.1% diethyl pyrocarbonate). After centrifugation, supernatants were separated, and total RNAs were extracted by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNAs were used as templates to amplify full-length cDNA by reverse transcription PCR (RT-PCR; SuperScript III Synthesis Kit, Invitrogen), according to the manufacturer's instructions. A positive control sample (GenBank accession no. JU119961) and negative control (water) were included.

Briefly, 10 pairs of primers were designed based on HEV genotype 4 (GenBank accession nos. JU119961, JQ740781, AB291965, and AB602440) (online Technical Appendix Table 1, <http://wwwnc.cdc.gov/EID/article/20/12/13-1599-Techapp1.pdf>) to obtain the HEV genome consisting of all the 3 open reading frames. RT-PCR was

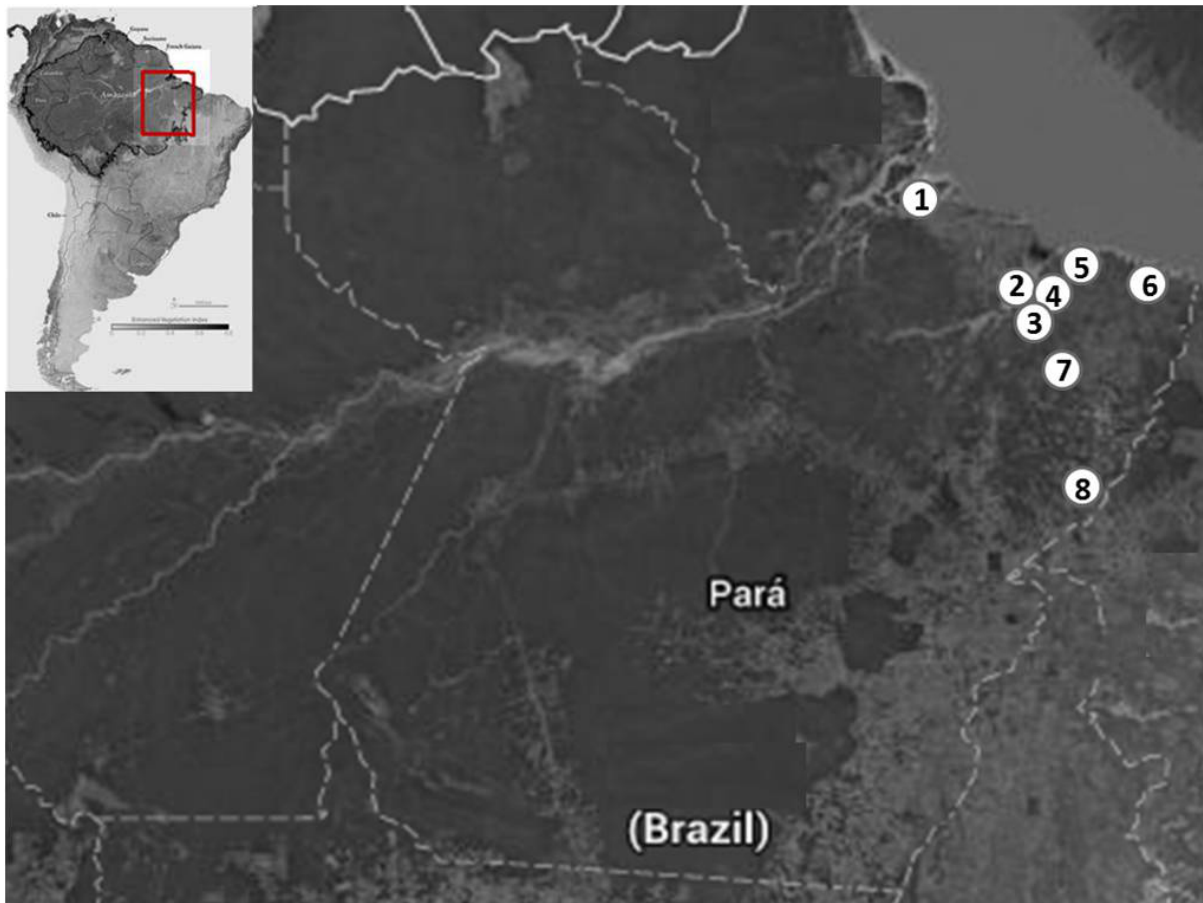
then performed in a 25- $\mu\text{L}$  volume containing 2- $\mu\text{L}$  templates and 0.1  $\mu\text{mol/L}$  of each primer; cycles were  $94^{\circ}\text{C}$  for 2 min, followed by 38 cycles of  $94^{\circ}\text{C}$  for 30 s,  $59^{\circ}\text{C}$  for 30 s, and  $72^{\circ}\text{C}$  for 1 min, with a final extension step of 10 min at  $72^{\circ}\text{C}$ . RT-PCR–amplified DNA fragments of the expected sizes were sequenced in a 310 Genetic Analyzer/Sanger Sequencer (Invitrogen). The complete genome sequences were assembled on the basis of 10 amplified sequences. Phylogenetic analysis was performed for the complete genome sequences of the detected sequences compared with all other mammalian HEV sequences available in GenBank.

We found that 3 (3.26%) of the 92 samples were positive for the HEV genotype 4 genome sequence; all samples were from yaks  $<1$  year of age from Qinghai Province (online Technical Appendix Table 2). Sequence analysis revealed 100% identity of the full-length genomes of the 3 HEV sequences (7,234 bp; sequence submitted to GenBank as CHN-QH-YAK, accession no. KF736234). Phylogenetic analysis demonstrated that all 19 mammalian HEVs grouped into 4 clades corresponding to the 4 HEV genotypes. The sequence we identified belonged to genotype 4 and was most closely related to China/Xinjiang/swine (GenBank accession no. JU119961; 99.14% identity) and to China/Nanjing/human (GenBank accession no. JQ740781; 93.84% identity) (Figure).

We found HEV genotype 4 infection in yaks, but prevalence was low (3.26%), and only young yaks in Qinghai Province were affected. In comparison, studies have shown that swine are an established reservoir of HEV worldwide (3,5,9) and that 20%–100% of pigs are infected with HEV (3,4,9,10). Our findings suggest that yak is an emerging but imperfect host for this virus. The HEV genome sequence derived from infected yak shared 99.14% identity with the

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## Technical Appendix



Technical Appendix Figure. Locations in the State of Pará (eastern Brazilian Amazon), Brazil, where blood samples were collected from 300 equids for nonprimate hepacivirus testing. The numbers correspond to municipalities, followed by latitude and longitude. 1, Chaves (00°09'36"S; 49°59'18"W); 2, Cotijuba (01°13'04"S; 48°32'44"W); 3, Belém (01°27'21"S; 48°30'16"W); 4, Ananindeua (01°21'56"S; 48°22'20"W); 5, Santo Antonio do Tauá (01°09'07"S; 48°07'46"W); 6, Peixe-boi (01°11'31"S; 47°18'44"W); 7, Acará (01°57'39"S; 48°11'48"W); 8, Dom Eliseu (04°17'06"S; 47°30'18"W).