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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 ≈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°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-μL volume containing 2-μL templates and 0.1 μmol/L of each primer; cycles were 94°C for 2 min, followed by 38 cycles of 94°C for 30 s, 59°C for 30 s, and 72°C for 1 min, with a final extension step of 10 min at 72°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

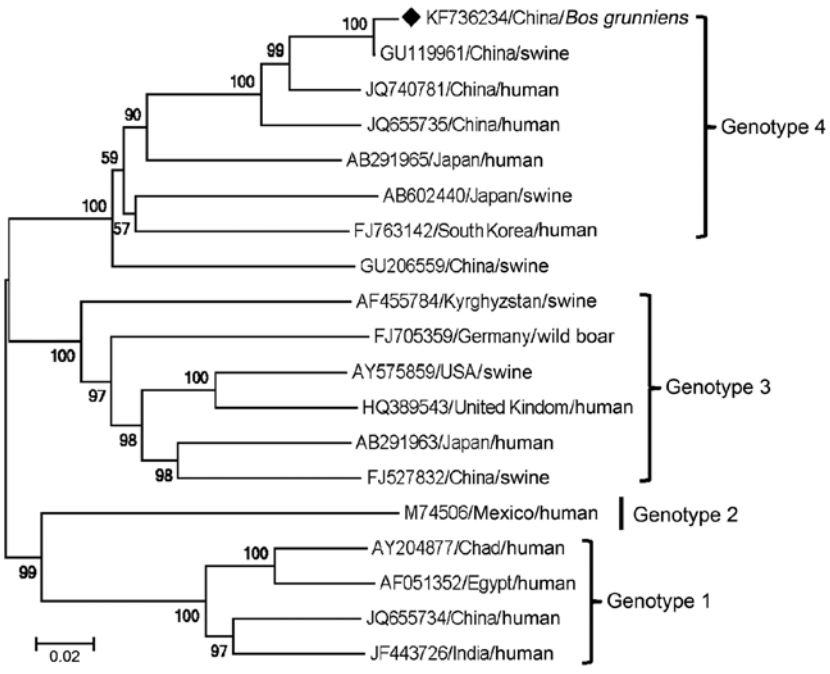

Figure. Phylogenetic analysis of hepatitis E virus (HEV) based on the complete genome sequences of HEVs using the neighbor-joining method with MEGA 4.0 software (http://www.megasoftware.net). Black diamond indicates the newly identified yak HEV sequence from Qinghai, China (GenBank accession no. KF736234). Another 18 sequences were collected from GenBank, including 7 sequences of genotype 4 (GU119961, JQ740781, JQ655735, AB291965, AB602440, FJ763141, GU206559), 6 of genotype 3 (AF455784, FJ705359, AY575859, HQ389543, AB291963, FJ527832), 1 of genotype 2 (M74596), and 4 of genotype 1 (AY204877, AF051352, JQ655734, JF443726). Bootstrap values of >50% are indicated for the corresponding nodes based on a bootstrapping with 1,000 replicates. GenBank accession numbers and geographic and animal species origin are shown. Scale bar indicates nucleotide substitutions per site.

China/Xinjiang/swine isolate, suggesting the Qinghai isolate evolved and was transmitted from the Xinjiang swine isolate. Like the swine isolate, this yak isolate probably possesses the potential to infect humans. Because persons in the Tibet region eat undercooked yak milk and meat, yaks may become an emerging reservoir of HEV genotype 4.

In addition, the high sequence identity (93.84%–99.14%) among isolates from China, including China/Qinghai/yak, China/Xinjiang/swine, China/Nanjing/human, and China/Beijing/human, demonstrates a complicated, transregional and cross-species transmission cycle for HEV genotype 4 in China. We cannot determine why yaks in Qinghai Province were affected and those in Gansu Province were not, but a neighboring pig farm may have served as a source for HEV transmission. More research is needed to determine the prevalence of HEV genotype 4 in various human and animal populations with concomitant virus isolation and phylogenetic analysis.

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Peste des Petits Ruminants Virus, Tunisia, 2012–2013

To the Editor: Peste des petits ruminants (PPR) is a viral disease of sheep and goats caused by peste des petits ruminants virus (PPRV), a negative-sense, single-stranded RNA virus of the genus *Morbillivirus*. Illness and death can be high (>90%) when PPR occurs in populations of immunologically naive sheep and goats (*1*). Mortality rates are ≈10%–40% in disease-endemic areas (*2*). Because of its economic effects and ability to spread rapidly, PPR has been included among reportable diseases by the World Organization for Animal Health.

In the past 20 years, PPR has shown rapid spread throughout large areas of Africa and Asia (*2*). A unique serotype of PPRV circulates and is classified into 4 genetically distinct lineages (*3*). The geographic distribution of lineages I and II is restricted mainly to western and central Africa and that of lineage III to eastern Africa. Lineage IV is more widely distributed throughout eastern Africa (*4,5*), the Near and Middle East, and large areas of Asia (*3*).

In 2008, PPR occurred in Morocco, and 257 cases were reported in goats and sheep over a 6-month period (*3*). In 2011, PPR was officially reported in Algeria (*3*). Genetic analysis showed that PPRV strains isolated in Morocco and Algeria belonged to lineage IV (*4–6*). Although PPR has been reported in Tunisia since 2011 (*7*), no data are available on the molecular characterization of PPRV circulating in this country.

During September 2012–January 2013, clinical signs compatible with PPR in ovine and caprine flocks were reported to the Tunisian veterinary service. Ocular, nasal, oral, and rectal swab specimens were obtained from animals showing clinical signs of this disease. Swab specimens were sent to the Institute de la Recherche Vétérinaire de Tunisie in Tunis for laboratory confirmation. Total RNA from swab samples was extracted by using the NucleoSpin RNA Virus Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. The presence of the PPR viral RNA was determined in samples by using a specific reverse transcription PCR reported by Polci et al. (*8*). Laboratory tests confirmed circulation of PPRV in farms near Kairouan and Sidi Bouzid.

Aliquots of RNA samples were shipped to the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise in Teramo, Italy, for genetic characterization. RNA was amplified by using the reverse transcription PCR reported by Couacy-Hymann et al. (*9*). Amplicons from virus- positive samples were purified by using the QIAquick PCR Purification Kit (QIAGEN, Valencia, CA, USA) and used for direct sequencing. Sequencing reactions were performed by using the Big Dye Terminator Kit (Applied Biosystems, Foster City, CA, USA), and nucleotide sequences were determined by using the ABI PRISM 3100 DNA sequencer (Applied Biosystems). Amplification and sequencing were repeated twice to avoid introduction of artificial substitutions. Raw sequence data were assembled by using Contig Express (Vector NTI suite 9.1; Invitrogen, Carlsbad, CA, USA), and a 351-nt fragment of the nucleoprotein coding sequence was obtained after deletion of primer sequences.

Two sequences were obtained, 1 from the Kairouan outbreak and 1 from the Sidi Bouzid outbreak. The 2 sequences generated in this study were submitted to GenBank (accession nos. KM068121 and KM068122). Sequences showed nearly complete identity at nucleotide and amino acid levels; there was 1 nucleotide substitution. The BLAST (http://www.ncbi.nlm.nih.gov) was used to detect homologous regions in sequence databases. Sequences were aligned by using ClustalW (http://www.genome.jp/tools/clustalw/) and MEGA version 6 (*10*). Phylogenetic analysis was performed with a 255-nt sequence of the PPRV nucleoprotein gene by using the neighbor-joining method with bootstrap support (1,000 replicates) in MEGA version 6 (*10*) and reference strains representing the 4 lineages of PPRV that have been isolated in different years or countries (Figure).

Our results indicate that lineage IV of PPRV is present in Tunisia. PPRV isolates from the outbreaks in Sidi Bouzid in 2012 and Kairouan in 2013 are closely related to viruses responsible for PPR outbreaks in Morocco in 2008 (*4*) and Algeria in 2010 (*6*). These isolates are closely related to strains from Saudi Arabia, which were detected in Eritrea in 2005 (*5*) and in Sudan in 2008 (*4*). These data suggest that a unique PPRV strain is circulating across this area of the Maghreb. PPRV circulation is maintained probably by the abundant trade in ruminants between Tunisia and neighboring countries. This information highlights the need for a regional approach to control PPR in northern Africa.