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Hepatitis E Virus in Yellow Cattle, Shandong, Eastern China

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To the Editor: Hepatitis E, caused by hepatitis E virus (HEV), is recognized as a zoonosis (*1*). HEV has been identified in a wide range of animals, and swine is the primary reservoir (*2*). In cattle, HEV strains have been recently described in yak (*3*), Holstein cows and their milk (*4*), and dairy cows in Xinjiang Province, China (*5*), but not in other cattle. Yellow cattle (*Bos taurus*), the predominant breed (≈80%) in China (*6*), widely distributed over the country, and commonly used for meat and milk production or as a draft animal, could act as a potential HEV reservoir. The objective of this study was to determine whether HEV strains are circulating among yellow cattle in Shandong Province of eastern China.

During April–November 2011, a total of 842 blood samples from yellow cattle of local breeds were collected monthly as part of a severe fever with thrombocytopenia syndrome virus study. These samples were obtained from Laizhou and Penglai Counties (≈100 km apart) of Yantai Prefecture in Shandong Province.

Because the prevalent seasons for human HEV in this region were winter and spring, 254 samples (Laizhou = 131; Penglai = 123) collected only in April and November were selected for detection of HEV. All 254 cattle appeared to be healthy. Sixteen were <1 year of age, 108 were 1–3 years of age, and 130 were >3 years of age. The cattle came from 20 villages (10 villages per county) and were raised by the local peasants, who owned an average of 2 cattle (range 1–8). The animals were bred mainly to produce meat and seldom to produce milk.

Additional serum samples from domestic sheep, dogs, and chickens were also collected in this region simultaneously (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/22/12/16-0641-Techapp1.pdf). All blood samples were centrifuged, and the separated serum was stored at -70°C until use. The protocol for animal sampling was approved by the Animal Care Committee of the Chinese Center for Disease Control and Prevention.

We tested serum samples for total antibodies against HEV by using a double-antigen sandwich ELISA kit (Wantai Biological, Beijing, China) that uses a recombinant peptide of HEV open reading frame 2 (aa 394–606) from the virus as the antigen (*7*). Overall, the proportion seropositive for antibodies against HEV in yellow cattle was 47% (120/254; 95% CI 41%–54%), in line with the 28.2% positivity ratio previously reported in cattle from 26 provinces of China (*8*), suggesting that a high proportion of yellow cattle were exposed to HEV in this region. The proportions seropositive among sheep, dogs, and chickens were 32% (70/222), 41% (80/194), and 8% (41/484), respectively (online Technical Appendix Table 1).

We used nested reverse transcription PCR to amplify 644 nt within HEV open reading frame 2 region, as described previously (*9*). We detected HEV RNA in 8 of 254 cattle samples; the overall proportion seropositive was 3%. Positive yellow cattle included one <1 year of age, three 1–3 years of age, and four >3 years of age. The 8 sequences obtained in this study (GenBank accession nos. KU904271, KU904273, KU904274, KU904278–KU904282) were subjected to phylogenetic analysis along with reference sequences for subtyping (*10*).

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Using MEGA 7.0 software (http://www.megasoftware.net) with the maximum-likelihood algorithm and a bootstrap of 1,000 replicates, we constructed a phylogenetic tree (online Technical Appendix Figure). All 8 sequences clustered within subtype 4d of HEV. The sequences were similar to each other (95.5%–99.8% similarity in nucleotide sequence) and similar to sequences reported for other cattle (83.3%–85.3%; online Technical Appendix Figure). Moreover, these sequences shared 96.1%–96.6% similarity with a human HEV strain (GenBank accession no. KC163335) from the Yantai Prefecture in 2012 and 95.7%–97.9% similarity with a swine strain (GenBank accession no. KF176351) isolated in Shandong Province the same year.

Our data strongly indicate that HEV infection occurs in yellow cattle and that they could also play a role as a reservoir of HEV. Because these animals serve mainly as a source of food, consumption of undercooked meat from yellow cattle, similar to pork, might also contribute to the transmission of HEV to humans. Additionally, we also detected HEV RNA in 8 of 70 sheep (online Technical Appendix Table 2). Eight sequences from yellow cattle had 95.1%–99.8% nt homology with 8 sheep-derived HEV strains, possibly because mixed raising of domestic livestock is popular in this region. Our finding of high sequence similarity between yellow cattle, sheep, swine, and human populations suggests a complicated interspecies transmission of HEV occurred in this province. Further studies are required to evaluate the contribution of the yellow cattle reservoir to human HEV infection.

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Introgressed Animal Schistosomes *Schistosoma curassoni* and *S. bovis* Naturally Infecting Humans

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To the Editor: Schistosomiasis, a disease caused by infection with parasitic worms (schistosomes), is a neglected tropical disease across many parts of the world. Numbers of infected livestock are unknown, but >250 million persons are infected; the greatest number of cases are in sub-Saharan Africa (*1*). Schistosome eggs are excreted through urine or feces, depending on the species, and hatch into miracidia upon contact with freshwater. Larvae are transmitted to the mammalian host indirectly through a molluscan intermediate host. Goals to eliminate schistosomiasis by 2020 in select countries in Africa have

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Technical Appendix

Technical Appendix Table 1. Proportion of domestic yellow cattle, sheep, dogs, and chickens seropositive for anti-hepatitis E virus, Shandong Province, China, 2011*

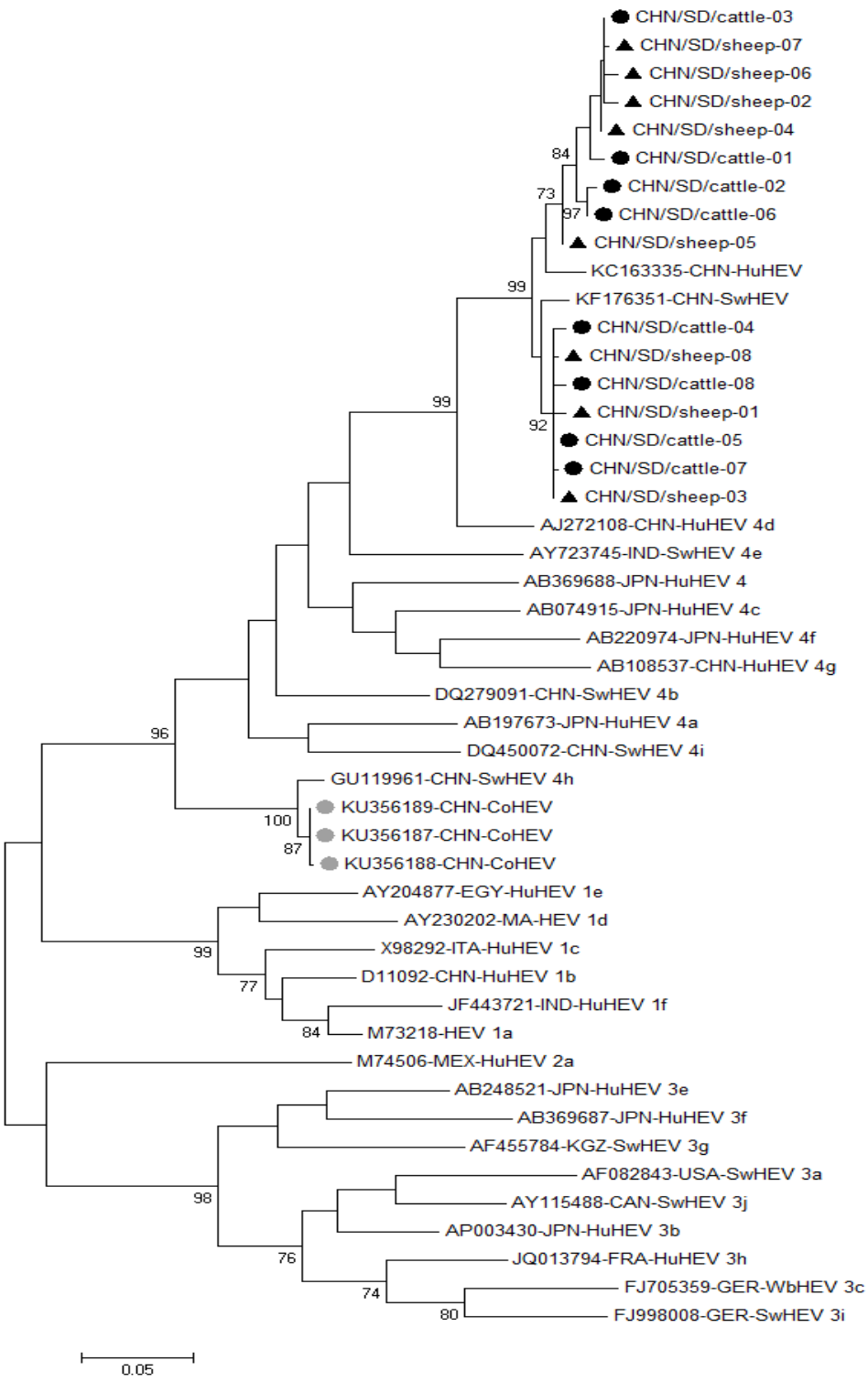
Sample category	Age, y	No. samples	No. positive	Proportion seropositive for anti-HEV %, (95% CI)
Yellow cattle	<1	16	5	31 (11–59)
	1–3	108	43	40 (31–50)
	>3	130	72	55 (46–64)
	Total	254	120	47 (41–54)
Sheep	<1	83	25	30 (21–41)
	1–3	103	32	31 (22–41)
	>3	36	13	36 (21–54)
	Total	222	70	32 (25–38)
Dogs	<1	32	15	47 (29–65)
	1–3	113	49	43 (34–53)
	>3	49	16	33 (20–48)
	Total	194	80	41 (34–49)
Chickens	–	484	41	8 (6–11)

* Blood samples of each species were obtained monthly during April–November 2011, as part of a severe fever with thrombocytopenia syndrome virus study. The total number of samples collected in April and November from domestic yellow cattle, sheep, dogs, and chickens were 254, 222, 194, and 484, respectively. Anti-HEV total antibodies were detected by a double-antigen sandwich ELISA kit (Wantai Biological, Beijing, China). Signal-to-cutoff values ≥1 were considered positive. HEV, hepatitis E virus.

Technical Appendix Table 2. Hepatitis E virus–positive strains isolated from domestic yellow cattle, sheep, dogs, and chickens, Shandong Province, China, 2011*

Source	Location	Age, y	GenBank accession no.
Yellow cattle	Penglai	>3	KU904273
	Penglai	1–3	KU904274
	Penglai	1–3	KU904282
	Laizhou	>3	KU904271
	Laizhou	<1	KU904278
	Laizhou	1–3	KU904279
	Laizhou	>3	KU904280
	Laizhou	>3	KU904281
Sheep	Penglai	>3	KU904272
	Laizhou	1–3	KU904267
	Laizhou	1–3	KU904268
	Laizhou	1–3	KU904269
	Laizhou	1–3	KU904270
	Laizhou	<1	KU904275
	Laizhou	<1	KU904276
	Laizhou	1–3	KU904277
Dogs†	–	–	–
Chickens†	–	–	–

* All 254 cattle and HEV total antibody–positive serum samples from sheep (n = 70), dogs (n = 80), and chickens (n = 41) were analyzed for HEV RNA. Positive results were obtained by reverse transcription PCR. HEV, hepatitis E virus.
† No samples from dogs or chicken were positive for HEV RNA.



Technical Appendix Figure. Phylogenetic analysis of hepatitis E virus strains based on the 540-nt open reading frame 2 fragment (positions 5765–6304 of reference sequence M73218). The phylogenetic tree was constructed with MEGA 7.0 software (http://www.megasoftware.net) by using the maximum-likelihood algorithm and Jukes-Cantor model with 1,000 bootstrap replicates. Bootstrap values (%) >70

are indicated at branch nodes. HEV sequences derived from cattle and sheep in this work are indicated with black circles and triangles, respectively. The reference sequences included in the figure were the ones suggested by Smith et al. 2016 for genotype subtyping. We also included for comparison HEV sequences isolated from cattle in other studies (gray circles). CAN, Canada; CHN, China; Co, cow; EGY, Egypt; FRA, France; GER, Germany; Hu, human; IND, India; ITA, Italy; JPN, Japan; KGZ, Kyrgyzstan; MA, Morocco; MEX, Mexico; Sw, swine; USA, United States of America; Wb, wild boar. All sequences obtained in this study are deposited in GenBank (accession nos. KU904267–KU904282). Scale bar represents nucleotide substitutions per site.