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Hepatitis E virus genotype 3f sequences from pigs in Thailand, 2011–2012

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Abstract Phylogenetic analysis of partial ORF1 and ORF2 genes of Hepatitis E virus (HEV) strains from pigs in Thailand during 2011–2012 was performed. The result indicated that the current Thai strains belonged to the genotype 3 subgroup 3f, which were similar to the previous HEVs circulating in humans in Thailand.

Keywords Hepatitis E virus · Genotype 3 · Pigs · Thailand

Hepatitis E virus (HEV) belongs to the *Hepevirus* genus of the *Hepeviridae* family. It comprises spherical, non-enveloped, positive-polarity, single-stranded RNA of approximately 7.2 kb in length. HEV has been divided into at least 4 genotypes based on geographical distribution. Genotypes 1 (1a–e) and 2 (2a–b) are found exclusively in humans and have mainly been identified in Asia, Africa, and Mexico. However, genotypes 3 (3a–j) circulating worldwide except in Africa and genotype 4 (4a–g) mainly found in Asia are known as causative agents of zoonotic disease, with domestic and wild pigs representing potential reservoirs [1–3, 5, 7]. In Thailand, evidence of anti-HEV antibodies and detection of HEV genotype 3 in sera had been reported in 5 commercial pig farms from September 2006 to January 2007 [8]. In 2010, HEV genotype 3 recovered from Thai patients was found closely related to previous swine hepatitis E virus [9]. Although virus outbreak has not been reported, ongoing surveillance programs constitute the only effective means for preventing an HEV epidemic. The purpose of the present study was to characterize hepatitis E virus detected from pigs in central Thailand over a 1-year period from 2011 to 2012.

Fecal samples were collected from 237 one to 22-week old pigs from commercial pig farms. The sampling sites covered 12 provinces in the central ($n = 69$), western ($n = 121$), eastern ($n = 37$), northern ($n = 1$), and north-eastern ($n = 9$) regions of Thailand. Viral nucleic acid extraction and cDNA synthesis were performed. Viral cDNA were amplified using previously described HEV primers specific for the ORF1 and ORF2 genes encoding a non-structural and capsid protein, respectively [4, 6]. The three PCR-positive samples (1.27 %) detected from Prachuap khiri khan ($n = 1$) and Phitsanulok ($n = 2$) provinces were subjected to nucleotide sequencing. A maximum likelihood tree with 1,000 bootstraps was constructed to show the relationship between recently detected Thai HEVs and other previously published HEVs using Tamura-Nei with gamma distributed model for ORF1 and Tamura-Nei with gamma distributed and invariant site model for the ORF2 region. Phylogenetic analysis of the 318 bp ORF1 and 314 bp ORF2 sequences showed that the Thai strains clustered within genotype 3f of HEV, which are closely related to reported

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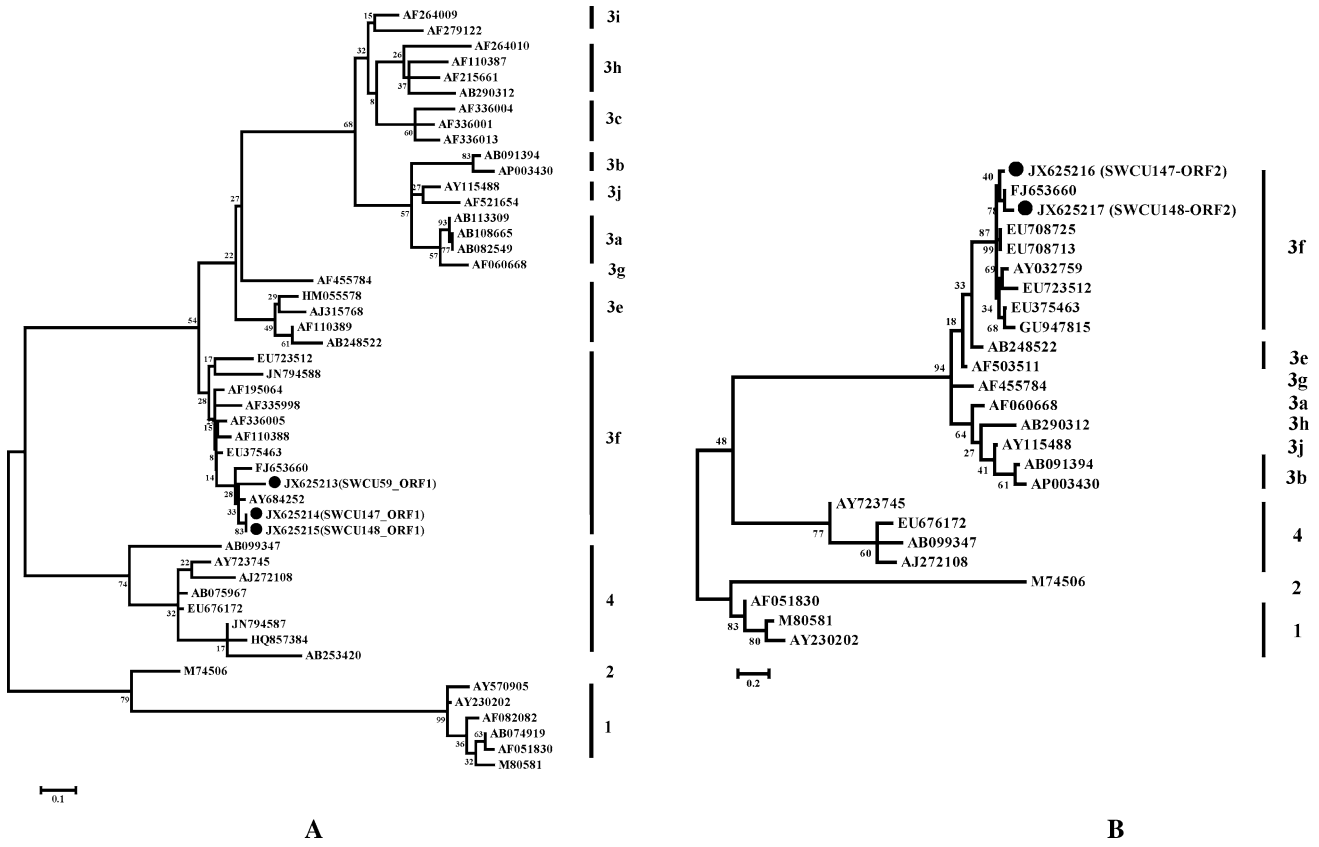

Fig. 1 Phylogenetic tree based on a 318 nt sequence of ORF1 (**a**) and a 314 nt sequence of ORF2 of hepatitis E virus (HEV). The current Thai ORF1 and ORF2 sequences have been submitted to the GenBank database under accession numbers JX625213–JX625217

swine and human HEV strains in Thailand (Fig. 1). The genetic distance of the ORF1 regions between Thai HEV strains ranged from 93.75 to 100 %. By comparison, the distance values between three Thai HEV strains and any published HEVs exhibited high percentage of nucleotide identity with genotype 3 (71.86–97.47 %), especially with subgroup 3f (84.01–97.47 %). Upon comparison with HEV genotype 1, genotype 2, and genotype 4 reference strains, the nucleotide identity amounted to 69.54–75.36 %, 73.93–79.38 %, and 69.51–76.52 %, respectively. The homology of ORF2 between two recent Thai strains indicated 93.45 % nucleotide identity. The highest percent identity was observed with HEV genotype 3 (78.01–93.44 %), particularly with subgroup 3f (84.61–93.44 %). The genetic distance showed low identity compared with HEV genotype 1 (66.78–71.38 %), genotype 2 (65.68–63.90 %), and genotype 4 (65.34–71.40 %).

As described in the present study, all current HEV strains detected in pigs in Thailand from 2011 to 2012 predominantly clustered together within genotype 3 subgroup 3f, similar to previous findings in pigs and humans [8, 9]. The risk of HEV transmission between pigs and humans has been a continuous concern. The comprehensive surveillance system and the public health education of HEV should be performed to prevent and control the disease outbreak both in pigs and humans in Thailand.

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References

1. S.D. Goens, M.L. Perdue, Anim. Health Res Rev. **5**, 145–156 (2004)
2. L. Lu, C. Li, C.H. Hagedorn, Rev. Med. Virol. **16**, 5–36 (2006)
3. X.J. Meng, J. Viral. Hepat. **17**, 153–161 (2010)
4. I. Nakai, K. Kato, A. Miyazaki, M. Yoshii, T.-C. Li, N. Takeda, H. Tsunemitsu, Am. J. Trop. Med. Hyg. **75**, 1171–1177 (2006)
5. H. Okamoto, Virus Res. **127**, 216–228 (2007)
6. H. Okamoto, M. Takahashi, T. Nishizawa, K. Fukai, U. Muramatsu, A. Yoshikawa, Biochem. Biophys. Res. Commun. **289**, 929–936 (2001)
7. E. Pelosi, I. Clarke, Emerg. Health Threats J. **1**, e8 (2008)
8. U. Siripanyaphinyo, D. Laohasinnarong, J. Siripanee, K. Kaeoket, M. Kameoka, K. Ikuta, J. Med. Virol. **81**, 657–664 (2009)
9. K. Suwannakarn, C. Tongmee, A. Theamboonlers, P. Komolmit, Y. Poovorawan, Arch. Virol. **155**, 1697–1699 (2010)