Genetic diversity of porcine epidemic diarrhea virus spike gene in Thailand

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Introduction
Porcine epidemic diarrhea (PED) is a devastating enteric disease in pigs characterized by vomit and acute watery diarrhea leading to death due to severe dehydration (8). In Thailand, PED has been endemic causing sporadic outbreaks since 2007 (9). At present, PED virus (PEDV) was classified into 2 genogroups, the classical variant (G1) and the pandemic variant (G2). An early report investigating the genetic diversity of PEDV in Thailand suggested that the G2 was responsible for the outbreak and was the only variant in Thailand (12). However, several herds had experienced severe and recurrent PED outbreaks which the reason still unknown during 2014-2015, and, the G1 was found in modified live vaccine (MLV) vaccinated herd in 2014 (2). These findings suggest that, both genogroups are existing in the swine population. This study was aimed to characterize PEDV spike gene diversities in Thailand.

Materials and Methods
99 PEDV clinical samples from 24 pig farms located in Ratchaburi, Nakorn Pathom, Saraburi, Lopburi, Nakorn Ratchasima, Buriram, Chonburi, Chanthaburi and ChaCherngsao during 2008-2015 that experienced PED outbreaks (55 outbreaks) were sequenced for complete spike gene and combined with 18 sequences previously report in GenBank (3) to create the dataset for phylogenetic analysis. The sequences were clustered into genogroups using 94 reference sequences described previously (6) to conduct the phylogenetic tree. The dataset was also analyzed for their evolutionary pattern and geographic distribution using BEAST (5,4). Best-fitted model, TN93+G+I, and base frequency was set as empirical, all other settings were left as default. The analysis was performed until ESS>200. For evolutionary analysis, the dataset will be analyzed, separately, between the clusters, and, the strict molecular clock model (STR), the uncorrelated/lognormal relaxed-clock model (LOG), and the uncorrelated exponential relaxed-clock model (EXP) were applied for all datasets. The geographic distributions among the clusters were also analyzed as described previously providing times of outbreaks. The information will be displayed and interpreted using Tracer (10), FigTree (11), SPREAD (1), and Google Earth (7).

Results and Discussion
Thai PEDV are classified into 6 subgroups including TH1-TH6. Subgroups TH1 (n=79) and TH2 (n=30) were the two dominant groups responsible for the outbreaks in Thailand. TH1 included the first Thai PEDV strain that was published in 2008 along with the latest samples we found in 2015. In TH1 and TH2, there were the samples with the 9 nucleotides insertion when aligned with the outbreak samples in Thailand and reference sequence. TH3 might be a new introduction of the virus or the recombinant. TH1-3 were included into G2a, whereas, subgroups TH4 and TH6 were in genogroup G2b. TH4 was applied for CBR1 and CBR2 (KR610993 and KR610994) sequences in July 2014, these sequences belong to G2b and very close to Vietnam sequences in 2013. TH6 is the latest subgroup which contained the new introduction of the exotic strain in 2015 that belong to G2b or US-like subgroup. Subgroup TH5 was in genogroup G1a. TH5 belongs to the latter group of samples in October 2014, EAS1 and EAS2 which the first strain that belong to G1 that we found in Thailand.

Evolutionary analysis show that subgroup TH1 is higher in evolutionary rate and become predominant subgroup of the strains in Thailand since 2014 when compare with TH2. Based on the phylogeographical analysis, there was geographic separation between PEDV stains in Thailand. TH2 subgroup was confined mainly in the western regions. In contrast, TH1 subgroup was found across Thailand, especially the northern east regions of Thailand.
Thai PEDV was genetically diverse and the genetic development has been influenced by the multiple introduction of exotic strains. Such introductions were not developed into epidemic stage. In contrast, Thai PEDV strains developed their own subgroups separated from other countries and underwent relatively high in evolutionary rate. There was an evidence of intra-recombination between sub-groups that can be one factor to accelerate the evolutionary rate of Thai PEDV. Samples in the sub-group with high evolutionary rate become dominant causing not only geographic separation, but also influence the development of other subgroup as the unique insertion could be found in both TH1 and TH2 whereas the identity between subgroup is greater than 97%.

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