Genetic characterization of duck Tembusu virus isolated from domestic ducks in Thailand, 2016

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Introduction
A new contagious disease characterized by severe neurological problems and severe drop in egg production has emerged in broiler and layer duck farms in China, Malaysia and Thailand (1,3,6, 9). The causative agent of the outbreaks was identified as a duck Tembusu virus (DTMUV), which is a single-stranded RNA virus, belonging to the Nyata group, a member of mosquito-borne Flavivirus. Previous studies showed that Thai DTMUVs were closely related to Chinese DTMUVs (1, 9). After the initial outbreak, the DTUMV cases have been continuously detected in several duck producing areas in Thailand. Therefore, the systemic surveillance and the genetic characterization of currently circulating DTMUVs in ducks in Thailand are necessary for understanding the divergence of circulating DTMUV strains. This study aims to investigate the genetic characteristics and the geographic distribution of DTMUVs in ducks in Thailand during July-October 2016.

Materials and Methods
A total of 27 pooled organ samples from DTMUVs suspected cases were obtained from duck farms during July-October 2016. Then, organ samples were processed to 10% tissue suspension for inoculation into the allantoic cavities of 9-day-old embryonated duck eggs. Viral RNAs were extracted from tissue suspensions and allantoic fluid by using NucleoSpin Extract Viral RNA Kit (Macherey-Nagel, Düren, Germany). The samples were then examined for the presence of DTMUV by RT-PCR using specific primers for E gene (6). All samples tested negative for Avian Influenza virus (AIV), Newcastle Disease virus (NDV) and Duck Enteritis virus (DEV) by RT-PCR (4,5,7). DTMUV positive samples were subjected to partial E gene sequencing. Then, the nucleotide sequences were assembled using SeqMan software v.5.03 (DNASTAR Inc., Wisconsin, USA). The nucleotide sequences were aligned using Clustal W v.2.0 (3). Phylogenetic analysis was analysed by comparing the partial E gene sequences from new Thai DTMUVs identified in our study with previously reported Thai, Chinese, Malaysian DTMUVs and selected reference avian-origin TMUVs from GenBank database. Phylogenetic analysis was constructed in MEGA6 v.6.0 using neighbor-joining algorithm with the Kimura-2 parameter model applied to 1000 replications of bootstrap (8). Nucleotide identities among Thai, Malaysian, Chinese DTMUVs and avian-origin TMUVs were determined by MegAlign software v.5.03 (DNASTAR Inc., Wisconsin, USA).

Results and Discussion
A total of 6 (22.22%) out of 27 samples tested positive for DTMUV as determined by E-specific RT-PCR. DTMUV-positive samples were collected from duck farms located in 5 provinces in the eastern (Chonburi and Prachinburi), the central (Lopburi and Singburi) and the northeastern (Nakhon Ratchasima) regions of Thailand during July-October 2016 (Table 1). Interestingly, DTMUV-positive cases were first identified in Lopburi and Singburi. This finding demonstrated that DTMUV has continuously caused outbreaks and has currently spread to new areas of Thailand, indicating the extensive distribution of this virus.

Table 1 Details of DTMUVs characterized in this study

<table>
<thead>
<tr>
<th>Virus name</th>
<th>Province</th>
<th>Duck type</th>
<th>Time of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>DK/TH/CU85</td>
<td>Lopburi</td>
<td>Layer</td>
<td>July 2016</td>
</tr>
<tr>
<td>DK/TH/CU89</td>
<td>Singburi</td>
<td>Layer</td>
<td>July 2016</td>
</tr>
<tr>
<td>DK/TH/CU100</td>
<td>Nakhon</td>
<td>Layer</td>
<td>August 2016</td>
</tr>
<tr>
<td>DK/TH/CU126</td>
<td>Chonburi</td>
<td>Layer</td>
<td>October 2016</td>
</tr>
<tr>
<td>DK/TH/CU128</td>
<td>Chonburi</td>
<td>Layer</td>
<td>October 2016</td>
</tr>
<tr>
<td>DK/TH/CU132</td>
<td>Prachinburi</td>
<td>Broiler</td>
<td>October 2016</td>
</tr>
</tbody>
</table>

Phylogenetic analysis revealed that 6 DTMUVs identified from this study were closely related and grouped into the same cluster with the previously reported Thai DTMUVs and Chinese DTMUVs, while grouped into different cluster with Malaysian
DTMUVs. It is noted that Thai DTMUVs were grouped into subcluster II-a, while Chinese DTMUVs were located in subcluster II-b (Figure 1). The partial E gene sequences of these new Thai DTMUVs shared 97-99.5% nucleotide (nt) identity with previously reported Thai DTMUVs, and 96-98.3% nt identity Chinese DTMUVs, while shared only 88.5-90.3% nucleotide identity with Malaysian DTMUVs. This indicated that these 2016 Thai DTMUVs were most closely related to previously reported Thai DTMUVs when compared with Chinese and Malaysian DTMUVs. Moreover, our results indicated that subcluster II-a of DTMUV was associated with the current DTMUV outbreaks in Thailand (Figure 1).

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References

Figure 1 Phylogenetic analysis of DTMUVs generated from partial E genes of Thai DTMUVs identified in this study, the previously reported Thai, Chinese, Malaysian DTMUVs and the selected avian-origin TMUVs. Time of sample collection was shown after the name of each virus. Blue circle indicates Thai DTMUVs identified in this study.

In summary, our study demonstrated the expanded geographic distribution and the genetic divergence of DTMUVs circulating in ducks in Thailand. Further DTMUV surveillance should be routinely conducted to provide the information on the evolution of DTMUVs for the effective disease control and prevention.