

## Comparative genomic analysis of duck plague virus reveals evolutionary divergence between vaccine and contemporary field isolates in Thailand

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### ABSTRACT

Duck Plague Virus (DPV), an alphaherpesvirus, causes significant morbidity and mortality in waterfowl and remains a persistent threat to duck farming across Asia. This study reports the first complete genomic characterization of DPV isolates from Thailand including a commercial vaccine strain (DPVac) and two contemporary field isolates (DPV7 and DPV8). Using Oxford Nanopore Technologies (ONT) and hybrid Illumina-ONT sequencing, we successfully assembled complete genomes ranging from 160,511 to 163,789 bp. The assembled genomes showed high sequence identity to the European reference strain DEV 2085. Comparative genomic analysis revealed structural differences in the UL and US regions. Phylogenetic reconstruction based on core genome single-nucleotide polymorphisms (SNPs) placed DPV7 within a clade of recent Chinese field strains, while DPV8 clustered closely with vaccine-associated lineages from Germany, India, and Bangladesh. Notably, SNP analysis identified multiple virulence-associated mutations uniquely present in DPV7. These mutations, absent in DPAvac and DPV8, were located within or near genes involved in viral replication (UL54), host immune evasion (UL41, UL14), viral entry (UL44, UL8), intracellular trafficking (US3, US8), and virulence modulation (LORF3). These findings suggest that DPV7 may be undergoing adaptive evolution under immune pressure, potentially compromising vaccine effectiveness. Our results underscore the critical need for continuous molecular surveillance and functional studies to evaluate the impact of emerging DPV variants. The complete genome sequences reported herein provide a valuable resource for future research on DPV evolution, diagnostics, and vaccine development in endemic regions.

### Introduction

Duck plague (DP), also known as duck virus enteritis (DVE), is a highly contagious and often fatal disease that affects domestic and wild waterfowl, particularly ducks, geese, and swans (Dhama et al., 2017). The causative agent, duck plague virus (DPV), belongs to the family *Herpesviridae* and poses a significant threat to the duck farming industry,

leading to high mortality rates, production losses, and severe economic impacts (Metwally and Cheng, 2020).

Despite the long-standing presence of DPV in Thailand, where the duck industry plays a critical role in food supply and rural livelihoods, comprehensive research on the genetic characteristics of circulating field strains remains limited. The primary method of duck plague control in Thailand relies on vaccination using the Jansen vaccine strain, which

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has been used for decades. However, the genetic composition of this vaccine strain is poorly understood, and its relationship to contemporary field isolates remains unclear. Concerns persist regarding residual virulence, reversion to virulence, and potential recombination events between the vaccine strain and field isolates, as observed in other herpesvirus-based vaccines (Elshafiee et al., 2022). These factors may compromise vaccine efficacy and contribute to continued outbreaks of duck plague (Jia et al., 2025).

Due to the limited genomic data on DPV in Thailand, understanding the genetic diversity and evolutionary dynamics of local field strains is essential for effective disease control. Whole-genome sequencing (WGS) provides a powerful tool to characterize DPV isolates and identify genetic differences between vaccine strains and field isolates (Gilchrist et al., 2015). Such insights can inform vaccine improvements, enhance diagnostic capabilities, and contribute to the development of targeted control measures (Apinda et al., 2022; Bisht et al., 2024).

This study aimed to address these knowledge gaps by performing WGS of DPV isolates in Thailand. It represents the first successful isolation and WGS of DPV field strains in Thailand. By comparing the genetic differences between the Jansen vaccine strain and the first field isolates sequenced in the country, this research provides valuable insights into DPV genetic diversity, evolution, and potential adaptation in comparison with WGS data from other countries for epidemiological studies. The findings have significant implications for improving vaccine design, monitoring vaccine efficacy, and developing more effective disease control strategies, ultimately helping to mitigate the impact of duck plague on Thailand's duck farming industry and contributing to global DPV genomic databases.

## Material and methods

### *Clinical sample isolation and propagation condition*

The DPV vaccine was manufactured by the Bureau of Veterinary Biologics, Department of Livestock Development, Ministry of Agriculture and Cooperative, Thailand. One dose of the vaccine (0.5 mL) contained  $10^3$  TCID<sub>50</sub> (median Tissue Culture Infectious Dose) of DEV Jansen strain.

Two native DPV isolates (DPV7 and DPV8) used in this study were obtained from different duck farms located in the western provinces of Thailand. The carcasses were submitted to the Center of Duck Health Science, Kasetsart University, Kamphaeng Saen Campus, for confirmation of duck plague virus (DPV) infection. DPV-positive cases were confirmed by gross pathology and histopathological examination. Tissue samples (0.5 g each) from the heart, liver, spleen, kidney, and pancreas were collected and suspended in 1 mL of phosphate-buffered saline (PBS; pH 7.4). The tissues were homogenized in Eagle's Minimal Essential Medium (EMEM) supplemented with 200 IU/mL penicillin and 200 µg/mL streptomycin to form a 20% (w/v) suspension. The homogenates were centrifuged at 12,000 rpm for 5 minutes, and the resulting supernatants were filtered through a 0.2 µm syringe-driven filter (Corning®, Corning Incorporated, Germany) for subsequent viral isolation, following the method described previously (Wolf et al., 1974). Briefly, duck embryo fibroblasts (DEF) (CCL-141™, ATCC) were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37°C with 5% CO<sub>2</sub>. DPV isolates were further propagated in DEF cultures for five passages, after which the supernatants were collected, aliquoted, and stored at -80°C for further analysis.

### *DEV-strain PCR verification*

DEF cells were infected at a multiplicity of infection (MOI) of 1 plaque-forming unit (PFU) cell<sup>-1</sup> for 48 h at 37°C. When 90 to 100% cytopathic effect was observed, the supernatant and cells were collected and centrifuged at 300 × g for 10 min; the cell pellet was extracted using the viral genomic DNA extraction kit following the instructions (DNeasy

Blood & Tissue Kit, QIAGEN, Germany). The purified DNA samples were identified through polymerase chain reaction (PCR) using DEV-specific primer pairs for the UL2 gene (UL2-F: TGACCAACCAACGTCTACATGC and UL2-R: CAGAAAGCCTTAAATTCAGCGTG) following previous study (Huo et al., 2024).

### *DPV genome isolation and sequencing*

Total genomic DNA of DPV samples were extracted using DNeasy Blood & Tissue Kit (QIAGEN, Germany). The samples were qualified by TapeStation 4150 (Agilent Technologies, USA) and was quantified with the 1 × dsDNA High Sensitivity assay kit on a Qubit 4.0 Fluorometer (Invitrogen, USA) according to the manufacturer protocols. Subsequently, the DNA library was prepared using the Native Barcoding Sequencing Kit 24 V14 (SQK-NBD114.24, ONT, UK). The library was loaded into an R10.4.1 nanopore flow cell (FLO-PRO114M) on a PromethION 2 Integrated (P2i) (ONT, UK) with the super accuracy mode (SUP) for base calling. For short-read sequencing, only the DPV sample was used to prepare paired-end libraries with an insert size of 150-bp using the TruSeq Nano DNA kit (Illumina, USA). Sequencing was performed on the Illumina HiSeq 2500 system, utilizing the HiSeq Rapid SBS kit v2 (Illumina, USA).

### *Data pre-processing and DPV genome construction*

The adapters in the ONT raw reads were trimmed using Porechop v0.2.4 (<https://github.com/rrwick/Porechop>) and quality-filtered with NanoFilt v2.8.0 (De Coster et al., 2018), applying a minimum read length of 200 bp and a quality score (Q) threshold of ≥10. Short-read data for the DPV samples were trimmed and quality-controlled using Trimmomatic v0.39 with the following parameters; LEADING:3, TRAILING:3, SLIDINGWINDOW:4:15, and MINLEN:36 (Bolger et al., 2014). *De novo* whole-genome assembly of long-read data was performed using Flye v2.9.5-b1801 (Lin et al., 2016), followed by polishing with corresponding short-read data using Pilon v1.24 (Walker et al., 2014) to improve assembly accuracy for the DPV assemblies. For the field isolates (DP7 and DP8), the genome was constructed using reference-based method using Minimap2 v2.24-r1122 (Li, 2018) by aligning to the anatid herpesvirus 1 strain 2085 reference genome (GenBank ID: JF999965.1) (Wang et al., 2011). Then, variant calling and consensus sequence generation were carried out from the aligned BAM files using BCFtools v1.17 (Lefouili and Nam, 2022). Average nucleotide identity (ANI) was calculated between our three DPV assembled genomes and DPV strain 2085 reference genomes using FastANI v1.31 (Jain et al., 2018). Sequencing depth of each contig in the genome was analysed by Samtools v1.21 (Danecek et al., 2021). Genome quality assessment was conducted using QUAST v5.3.0 (Gurevich et al., 2013).

### *DPV genome sequences analysis*

Core single nucleotide polymorphism (SNP) analysis was conducted using Snippy v4.6.0 (<https://github.com/tseemann/snippy>), and recombining regions were identified and removed using Gubbins v3.1.6 (Croucher et al., 2015). The SNPs were called using Snippy with herpesvirus 1 strain 2085 as the reference, and annotated with BCFtools to determine a unique long (UL), inverted repeat sequence (IRS), unique short (US), and terminal repeat sequence (TRS) in the assembled DPV genomes. To compare the assembled genomes with available DPV genomes in the NCBI database, eighteen DPV genomes were retrieved from the NCBI GenBank database (Table S1). Phylogenetic reconstruction was performed with RaxML-NG v1.0.1 (Kozlov et al., 2019) using the GTR+G model and 1,000 bootstrap replicates, based on 147 core SNP sites. The resulting maximum likelihood phylogenetic tree was visualized with FigTree v1.4.4 (<https://github.com/ambaut/figtree>).

### Data available

The genome sequences of DPVac, DPV7, and DPV8 have been deposited in GenBank under accession numbers PQ679937, PQ679938, and PQ679939, respectively.

## Results

### DPV isolation and detection

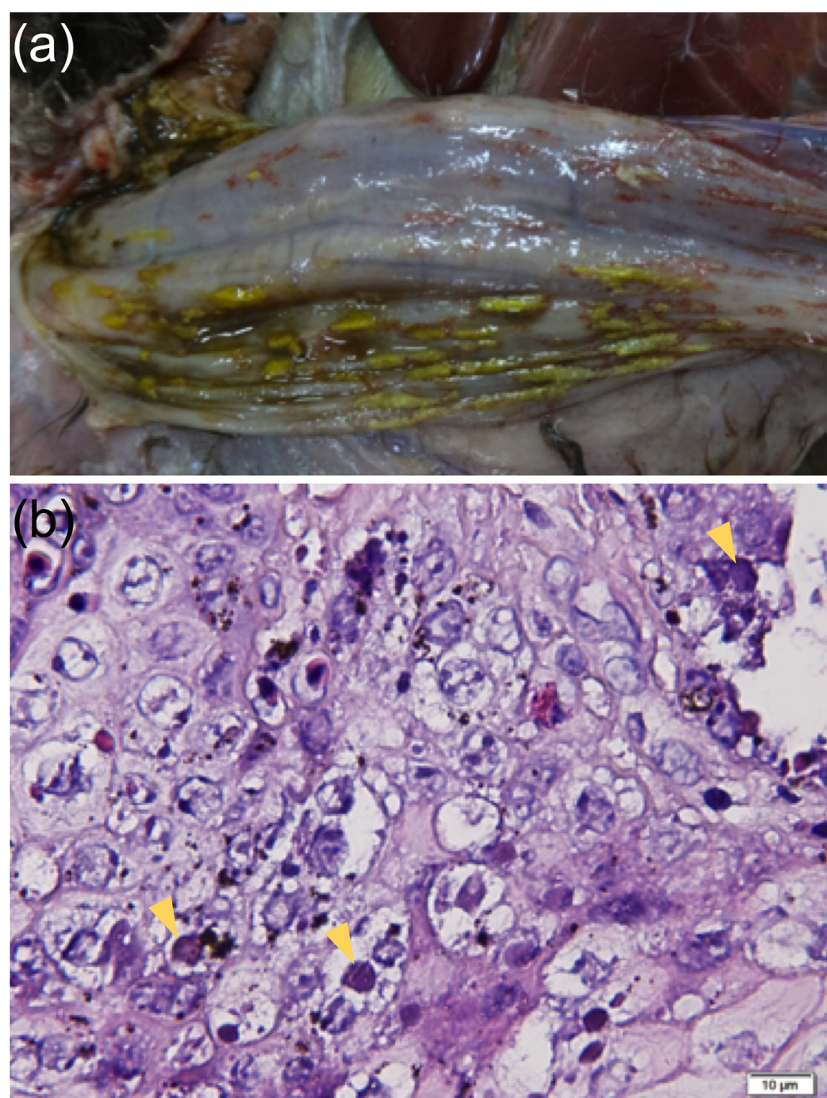
Duck Plague Virus (DPV) infection in this study was confirmed by the Center of Duck Health Science, Kasetsart University Kamphaeng Saen Campus, Thailand. Post-mortem examination of affected carcasses revealed hallmark gross lesions, including a diphtheritic membrane in the esophagus and multifocal yellowish necrotic plaques along the intestinal mucosa (Fig. 1a). Histopathological analysis showed hepatocellular degeneration with prominent eosinophilic intranuclear inclusion bodies, classic microscopic features of herpesvirus replication (Fig. 1b). These pathological findings confirmed the diagnosis of DPV and validated the samples for further virological analysis.

Following isolation, 200  $\mu$ L of viral suspension was used to infect

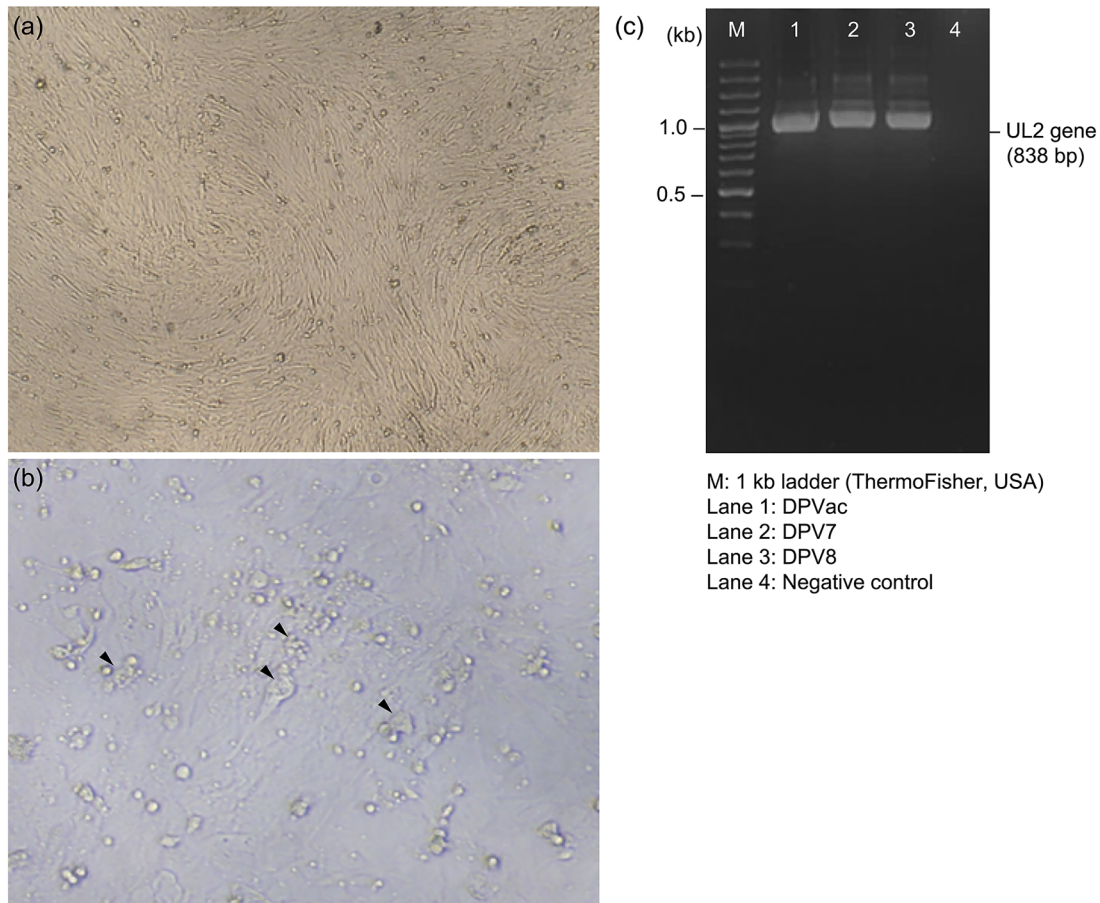
duck embryo fibroblast (DEF) cell cultures (CCL-141™, ATCC). Cytopathic effects (CPE), including cell rounding and detachment, were observed within 24 hours (Fig. 2b), in contrast to the uninfected DEF cells (Fig. 2a). By 48 hours post-infection, the infected cells exhibited increased cytoplasmic granulation and progressive degeneration. Viral DNA extracted from infected cultures was subjected to PCR amplification targeting the UL2 gene, yielding an 838-bp product specific for DPV (Fig. 2c). The isolates were subsequently purified through five rounds of plaque purification and designated as the first Thai field isolates: DPV7 and DPV8.

### Characteristics of DPV genomes

Whole-genome sequencing of three DPVs, DPVac (the Jansen vaccine strain predominantly used in Thailand), DPV7, and DPV8 (field isolates), was successfully performed using either solely ONT or hybrid assembly with Illumina short-reads. Raw read sequencing analysis was shown in Table S2. In total, ONT sequencing of the DPVac strain produced 18,850 reads, yielding approximately 66.17 Mb of data with a raw read *N50* of 4,937 bp. In parallel, Illumina short-read sequencing generated approximately 17,708,800 reads, corresponding to ~2,610



**Fig. 1.** Clinical symptoms, gross lesions, and histopathological findings in DPV-infected ducks. (a) Diphtheritic membrane formation in the esophagus and multifocal yellowish necrotic plaques along the intestinal mucosa of infected ducks. (b) Histopathological section of liver tissue showing hepatocellular degeneration with prominent eosinophilic intranuclear inclusion bodies (yellow arrowheads).



**Fig. 2.** Propagation of clinical DPV isolate in duck embryo fibroblasts. (a) Control: growth pattern of uninfected duck embryo fibroblasts at 400 × magnification, showing confluent monolayers of stellate-shaped fibroblast cells. (b) DPV-infected cells: duck embryo fibroblasts infected with DPV exhibit cytopathic effects (indicated by black arrows) at 400 × magnification. (c) PCR amplification of the UL2 gene showing an 838-bp product, confirming the presence of DPV.

Mb of data with a read length of 150 bp. Hybrid genome assembly of the DPVAc strain successfully yielded a complete genome size of 163,789 bp (Figure S1), with an average sequencing depth of  $371.58 \times$ . In contrast, the two field strains, DPV7 and DPV8, were exclusively sequenced using ONT, resulting in lower read counts and sequencing yields, 10,085 reads (8.51 Mb) for DPV7 and 11,714 reads (8.64 Mb) for DPV8, respectively (Table S2). Therefore, reference-based genome assembly was applied to construct in both field isolated in this work. The DPV7 isolate resulted in an assembled genome size of 160,511 bp with an average sequencing depth of  $41.86 \times$ . Likewise, the DPV8 isolate yielded a genome size of 160,591 bp and an average depth of  $42.07 \times$ . All genomes were assembled into a single contig, confirming high-quality and complete genome. Comparative analysis with the complete genome of DPV strain 2085 demonstrated sequence identities ranging from 99.91% to 99.96%, further supporting the accuracy and completeness of the assemblies (Table 1).

**Table 1**  
Genomic characteristics of three assembled DPV isolates.

| Isolate | Genome size (bp) | G+C (%) | Depth ( $\times$ ) | ANIb (%) <sup>a</sup> |
|---------|------------------|---------|--------------------|-----------------------|
| DPVAc   | 163,789          | 44.96   | 371.58             | 99.91                 |
| DPV7    | 160,511          | 44.90   | 41.86              | 99.76                 |
| DPV8    | 160,591          | 44.94   | 42.07              | 99.96                 |

<sup>a</sup> Percentage of average nucleotide identity (ANI) when compared to DPV genome strain 2085.

#### DPV genomic organization

Genomic segmentation analysis revealed structural differences among the DPV strains, particularly in the UL (unique long), US (unique short), IRS (inverted repeat), and TRS (terminal repeat) regions. The vaccine strain DPVAc exhibited an extended UL region (125,227 bp) and reduced US region (11,764 bp) compared to the reference virulent strain 2085 and the two Thai field isolates. Notably, the US region in DPVAc was approximately 960 base pairs shorter than those of DPV7 and DPV8, which both retained the full length of  $\sim 12,724$  bp. On the other hand, the IRS of the DEVac contains an additional  $\sim 514$  bp when compared with strain 2085. These structural variations contribute to the increased total genome size of DPVAc (163,789 bp), compared to the field strains DPV7 (160,511 bp) and DPV8 (160,591 bp) (Table 2).

**Table 2**  
The number of unique long (UL) region, internal repeat sequence (IRS), unique short (US) region, and terminal repeat sequence (TRS) in the different duck plague isolates.

| Strain | UL (bp) | IRS (bp) | US (bp) | TRS (bp) | Source             |
|--------|---------|----------|---------|----------|--------------------|
| 2085   | 122,141 | 12,892   | 12,724  | 12,892   | Wang et al. (2011) |
| DPVAc  | 125,227 | 13,400   | 11,764  | 13,398   | This study         |
| DPV7   | 122,161 | 12,814   | 12,722  | 12,814   | This study         |
| DPV8   | 122,099 | 12,884   | 12,724  | 12,884   | This study         |

Wang J, Höper D, Beer M, Osterrieder N. Complete genome sequence of virulent duck enteritis virus (DEV) strain 2085 and comparison with genome sequences of virulent and attenuated DEV strains. *Virus Res.* 2011. 160(1-2):316-25. doi: 10.1016/j.virusres.2011.07.004.

Phylogenetic relationship of Thai DPV strains with global isolates

To elucidate the evolutionary relationships among Thai DPV strains, a phylogenetic tree was constructed based on core genome single nucleotide polymorphisms (cgSNPs), incorporating reference strains from China, India, Bangladesh, and Germany. The cgSNP-based analysis delineated four distinct phylogenetic clades, corresponding broadly to continental-scale geographic distributions (Fig. 3). Thai field strain DPV7 clustered with recent Chinese isolates (e.g., PQ736106 and OR757570), supported by high bootstrap values (100), indicative of a close evolutionary relationship and a likely shared ancestry. Conversely, the DPVac Jansen strain and DPV8 were positioned within a separate clade that encompassed the vaccine reference strain DEV 2085 (JF999965.1, Germany), alongside Indian and Bangladeshi isolates. The close phylogenetic proximity between DPVac and DPV8 suggests that DPV8 may have undergone evolutionary adaptation under vaccine-induced selective pressure or may derive from a vaccine-associated lineage (Fig. 3).

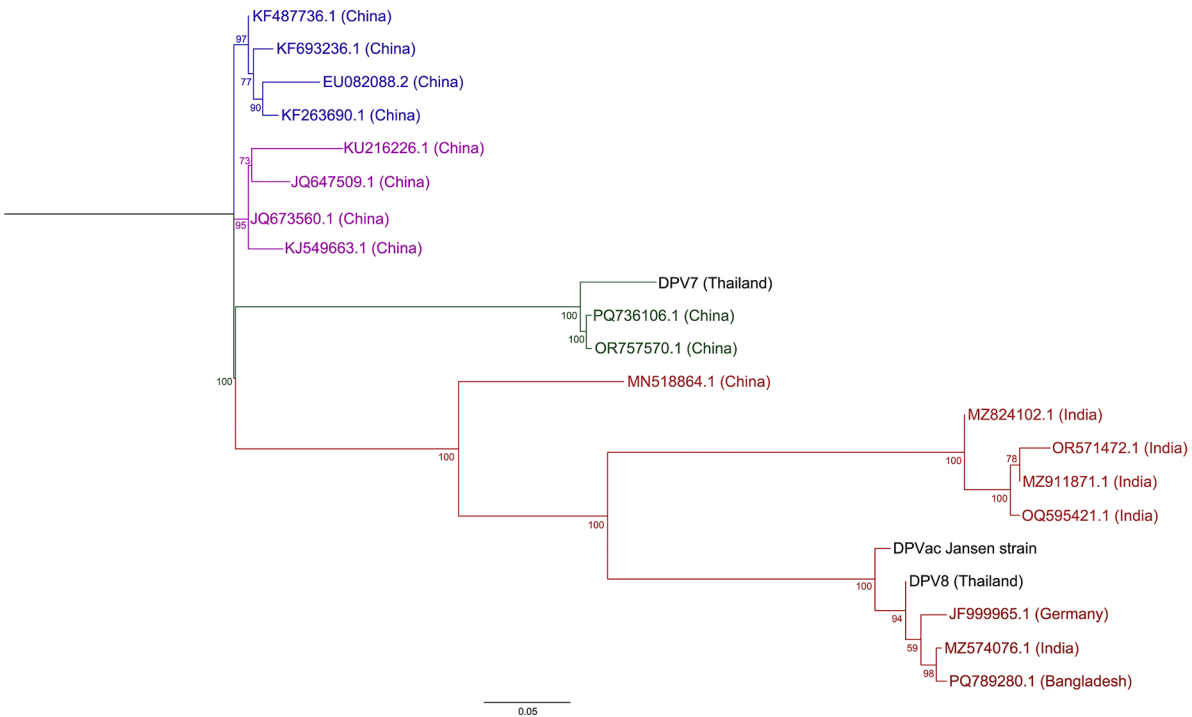
Virulence-linked SNP signatures in Thai DPV strains

Expanded SNP analysis of genes associated with virulence revealed numerous unique polymorphisms in the DP7 strain, whereas DPVac and DP8 remained relatively conserved at these loci. In the UL54 gene, DP7 exhibited two distinct SNPs (positions 7,871 and 7,883), both absent in DPVac and DP8. The UL44 gene, important for viral attachment, also carried two DP7-specific substitutions (positions 26,221 and 27,320). Similarly, the UL41 gene, linked to host shutoff and immune suppression, contained four mutations exclusive to DP7 (positions 31,987, 32,065, 32,218, and 32,520). Furthermore, the UL14 gene, involved in the modulation of host immune responses, harbored two distinct mutations (positions 95,613 and 95,879) unique to DP7 (Table 3). In addition, SNPs in the immune-modulatory gene US3 (positions 142,973 and 143,984) were also unique to DP7. The glycoprotein gene US8 and the less-characterized virulence gene LORF3 (position 115,902) likewise showed divergence in DP7, with US8 exhibiting three SNPs and

**Table 3**  
Comparison of SNP positions among three assembled DPV genomes and strain 2085. Predicted function of targeted genes were obtained from previously reports, –, deletion SNP position.

| Gene  | SNP position | Strain |       |      |      | Predicted function                                                                                             |
|-------|--------------|--------|-------|------|------|----------------------------------------------------------------------------------------------------------------|
|       |              | 2085   | DPVac | DPV7 | DPV8 |                                                                                                                |
| UL54  | 7,871        | T      | –     | C    | –    | Gene regulation; immediate-early protein (Li et al., 2009)                                                     |
|       | 7,883        | C      | –     | T    | –    |                                                                                                                |
| UL44  | 26,221       | G      | G     | A    | G    | Viral entry (virion attachment); type I membrane protein; binds to heparan sulfate (Li et al., 2009)           |
|       | 27,320       | C      | C     | T    | C    |                                                                                                                |
| UL41  | 31,987       | A      | A     | AGAC | A    | Gene regulation (inhibitor of gene expression); virion host cell shutoff factor (Li et al., 2009)              |
|       | 32,065       | T      | T     | C    | T    |                                                                                                                |
|       | 32,218       | C      | C     | T    | C    |                                                                                                                |
|       | 32,520       | G      | G     | A    | G    |                                                                                                                |
| UL14  | 95,613       | G      | G     | A    | G    | Minor tegument protein/modulation of host immune responses (Wan et al., 2022)                                  |
|       | 95,879       | T      | T     | G    | T    |                                                                                                                |
|       | 95,613       | G      | G     | A    | G    |                                                                                                                |
|       | 95,879       | T      | T     | G    | T    |                                                                                                                |
| LORF3 | 115,902      | T      | T     | G    | T    | Viral replication (Zhao et al., 2009)                                                                          |
|       | 135,679      | A      | A     | G    | A    |                                                                                                                |
|       | 136,618      | T      | T     | C    | T    |                                                                                                                |
| US8   | 136,887      | G      | G     | A    | G    | Cell-cell spread; type I membrane protein; complexed with gI; C terminus interacts with UL49 (Li et al., 2009) |
|       |              |        |       |      |      |                                                                                                                |
| US3   | 142,973      | G      | G     | A    | G    | Protein kinase (Deng et al., 2022)                                                                             |
|       | 143,984      | C      | C     | T    | C    |                                                                                                                |

LORF3 one. These findings indicate that DP7 has accumulated a distinct set of virulence-linked SNPs not observed in either DPVac or DP8, supporting the hypothesis of adaptive divergence under field conditions (Table 3).



**Fig. 3.** Core genome SNP (cgSNP) phylogenetic tree and geographic distribution of DPV genomes. A phylogenetic tree of eighteen DPV reference genomes, including three newly obtained DPV genomes (DPVac, DPV7, and DPV8), was inferred from cgSNP analysis based on 147 core SNP sites and visualized using FigTree.

## Discussion

This study represents the first comprehensive genomic characterization of Duck Plague Virus (DPV) isolates from Thailand, including both vaccine-derived and field strains. Using either Oxford Nanopore Technology (ONT) or hybrid with Illumina sequencing for the vaccine strain, we successfully obtained complete genome sequences for three DPV strains: DPV<sub>vac</sub> (Jansen vaccine), DPV7, and DPV8. All genomes were assembled into single contigs, confirming high completeness, with genome sizes and GC content consistent with that of the Anatid herpesvirus 1 European reference strain 2085 (Wang et al., 2011).

Notably, the DPV<sub>vac</sub> genome (163,789 bp) was larger than the field strains, due to structural expansions primarily in the UL and inverted repeat regions, while the US region was comparatively shortened. These findings align with earlier reports suggesting that genomic variation in terminal and unique regions of herpesviruses may influence virulence and replication efficiency (Dhama et al., 2017; Shawky and Schat, 2002). Minor structural rearrangements and an extended UL region in the DPV<sub>vac</sub> strain may reflect its attenuation and cell-adapted history (Bindu et al., 2024).

Phylogenetic analysis grouped DPV7 with recent Chinese field isolates, such as PQ736106 and OR757570, suggesting regional evolution and potential cross-border transmission (Aasdev et al., 2021). In contrast, DPV8 grouped with the vaccine strain DPV<sub>vac</sub> and the German reference strain DEV 2085, forming a distinct clade that also included isolates from India and Bangladesh (Dandapat et al., 2024; Khan et al., 2021; Priyadharsini et al., 2015). This clustering pattern may indicate a shared vaccine-derived lineage or reflect evolutionary adaptation under vaccine-induced selective pressure. Such findings align with prior studies reporting circulation of genetically similar vaccine-associated strains and the potential for back-mutation and field adaptation following prolonged use of live-attenuated herpesvirus vaccines.

Importantly, SNP analysis identified multiple virulence-associated mutations uniquely present in DPV7 but absent in both DPV<sub>vac</sub> and DPV8. These mutations were located in or near genes critical for DPV pathogenesis, including UL54 (viral replication) (Cao et al., 2012), UL41 and UL14 (host shutoff and immune evasion) (He et al., 2021; Wan et al., 2022), UL44 and UL8 (viral attachment and entry) (Sun et al., 2014), US3 and US8 (intracellular trafficking) (Deng et al., 2022), and LORF3 (virulence modulation) (Huo et al., 2024; Shen et al., 2021). The presence of multiple non-synonymous and frame-altering mutations, especially in DPV7, suggests functional divergence that could impact viral fitness, transmissibility, or immune escape.

The absence of these mutations in DPV<sub>vac</sub> and DPV8 implies that DPV7 may be undergoing independent field evolution, possibly driven by immune pressure or incomplete vaccine coverage. This evolutionary trend mirrors findings in other alphaherpesviruses, where immune-evasive mutations accumulate under prolonged or suboptimal vaccination campaigns (Guo et al., 2023; Loncoman et al., 2018). Similar evolutionary patterns have been reported in India and China, where long-term use of live-attenuated vaccines has contributed to viral adaptation (Aasdev et al., 2021; Dandapat et al., 2024). In addition, circulation of diverse field strains has been documented in Bangladesh, highlighting the regional risk of cross-border spread (Khan et al., 2021). Together, these findings support the observed phylogenetic and SNP divergence in DPV7 and underscore the importance of continuous genomic surveillance and functional validation of emerging mutations. Monitoring virulence-associated SNPs remains essential to assess their impact on vaccine efficacy and to guide updates to disease control strategies.

Duck plague continues to cause major economic losses worldwide through high mortality and disruptions in duck production (Hanson and Willis, 1976; Islam et al., 2024). Our finding of genomic divergence between vaccine and field strains in Thailand aligns with reports from other endemic regions, including China and India, where vaccine-derived viruses have shown evidence of adaptation and immune

escape (Dandapat et al., 2024; Liang et al., 2022). Effective prevention and control measures are therefore critical for sustaining and advancing the waterfowl industry, as recurrent outbreaks not only lead to direct mortality and reduced productivity but also hinder long-term genetic improvement programs and restrict international trade. These results highlight the need to account for viral evolution when developing global vaccine strategies and biosecurity frameworks, particularly in countries that continue to rely on live-attenuated vaccines such as Thailand. Although the complete Thai DPV genomes generated here contribute valuable data to the international genomic surveillance network, essential for tracking viral evolution across borders and detecting emerging strains of concern (Ning et al., 2022; Shen et al., 2023), expanded whole-genome sequencing across wider geographic and temporal scales will be necessary to fully characterize DPV evolutionary dynamics and link genetic variation with pathogenic potential.

In summary, our genomic, phylogenetic, and SNP analyses collectively demonstrate that Thai DPV field strains are diverging structurally and functionally from the vaccine lineage. This highlights the dynamic evolution of DPV in Thailand and underscores the urgent need for integrated genomic surveillance systems to detect emerging escape variants and ensure continued vaccine effectiveness. Importantly, the complete genome sequences generated in this study serve as a valuable resource for future research into DPV evolution, diagnostics, and vaccine development.

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## Institutional review board statement

The study was conducted in accordance with relevant guidelines and was approved by the Chiang Mai University Institutional Biosafety Committee (CMU-IBC), approval number CMUIBC A-0763002. The use of laboratory animals was overseen by the Animal Care And Use Committee of the Faculty of Veterinary Medicine Chiang Mai University (FVM – ACUC), approval number R13/2568.

## Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## CRediT authorship contribution statement

**Worarat Kruasuwan:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Tantip Arigul:** Visualization, Validation, Software, Methodology. **Piroon Jenjaroenpun:** Validation, Software. **Thidathip Wongsurawat:** Validation, Software, Conceptualization. **Kanokwan Sangkarakam:** Methodology. **Anucha Muenthaisong:** Methodology. **Nattawooti Sthitmatee:** Supervision, Resources. **Korakot Nganvongpanit:** Supervision. **Venugopal Nair:** Supervision. **Sittinee Kulprasertsri:** Methodology. **Thaweesak Songserm:** Resources. **Nisachon Apinda:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

## Disclosures

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.psj.2025.105892](https://doi.org/10.1016/j.psj.2025.105892).

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