J Vet Sci. 2026 Jan;27(1):e4
https://doi.org/10.4142/jvs.25092
pISSN 1229-845X·eISSN 1976-555X

Journal of Veterinary Science

Research Report
Virology

Check for updates

Molecular characterisation and histopathological analysis of canine distemper virus in a Malayan tiger (*Panthera tigris jacksoni*)

Muhammad Farris Mohd Sadali [1], Abdul Razak Mariatulqabtiah [2,3], Annas Salleh [4], Nurul Izzati Uda Zahli [1], Tengku Rinalfi Putra Tengku Azizan [5], Hafandi Ahmad [5], Mohd Arifin Kaderi [6], Khor Kuan Hua [7], Ridhwan Abdul Wahab [8], Ahmad Lutfi Abdullah [9], Millawati Gani [9], Farina Mustaffa-Kamal [1,3,*]

[1]Department of Veterinary Pathology & Microbiology, Faculty of Veterinary Medicine, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[2]Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[3]Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[4]Department of Veterinary Laboratory Diagnosis, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[5]Department of Veterinary Preclinical Science, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[6]Department of Biomedical Science, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia
[7]Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[8]Department of Microbiology, Faculty of Medicine, MAHSA University, Bandar Saujana Putra, 42610 Jenjarom, Selangor, Malaysia
[9]Ex-Situ Conservation Division, Department of Wildlife and National Parks Peninsular Malaysia, 56000 Kuala Lumpur, Malaysia

OPEN ACCESS

Received: Apr 6, 2025
Revised: Aug 18, 2025
Accepted: Aug 23, 2025
Published online: Dec 18, 2025

***Corresponding author:**
Farina Mustaffa-Kamal
Department of Veterinary Pathology & Microbiology, Faculty of Veterinary Medicine, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
Email: farina@upm.edu.my
https://orcid.org/0000-0002-5015-3836

ABSTRACT

Importance: Canine distemper virus (CDV) has a broad host range, spanning mammals of the order Carnivora and other orders, often proving fatal. The detection of CDV in a Malayan tiger (*Panthera tigris jacksoni*) in Malaysia in 2019 marks the first such case diagnosed in Malayan tigers, suggesting possible exposure from domestic animals or wildlife reservoirs.
Objective: This study aims to describe histological manifestations and characterise the CDV strain in the tiger to determine its molecular epidemiology and postulate viral pathogenicity.
Methods: Histopathology characterisation of the infected tissues were performed through haematoxylin and eosin and immunohistochemistry staining, respectively. We conducted virus isolation and titration in Chinese hamster ovarian expressing the dog signalling lymphocytic activation molecule (CHO-SLAM) cells. Reverse transcription polymerase chain reaction was performed to confirm the presence of CDV RNA, nucleotide and amino acid sequence analyses for comprehensive characterisation of the CDV strain.
Results: Notable histopathological changes were observed primarily in the brain, lung, liver, kidney, spleen, and stomach, with viral antigens localised in the lung, liver, kidney, and stomach tissues. CDV-induced cell cytopathic effects yielding 4.27×10^6 $TCID_{50}$/mL were observed at 48 h post-inoculation in CHO-SLAM cells. Phylogenetic analysis suggested that the virus originated from the Asia-1 clade. Notably, 549H and 519I mutations in the hemagglutinin protein were observed, indicating adaptation to a non-canid wildlife species.

ORCID iDs

Muhammad Farris Mohd Sadali
https://orcid.org/0009-0002-8972-1690
Abdul Razak Mariatulqabtiah
https://orcid.org/0000-0001-6999-0207
Annas Salleh
https://orcid.org/0000-0003-4142-0418
Nurul Izzati Uda Zahli
https://orcid.org/0000-0001-6635-7561
Tengku Rinalfi Putra Tengku Azizan
https://orcid.org/0000-0003-1161-9258
Hafandi Ahmad
https://orcid.org/0000-0001-8096-9863
Mohd Arifin Kaderi
https://orcid.org/0000-0002-6294-4002
Khor Kuan Hua
https://orcid.org/0000-0003-1500-9705
Ridhwan Abdul Wahab
https://orcid.org/0000-0003-1866-5062
Ahmad Lutfi Abdullah
https://orcid.org/0000-0002-3472-9858
Millawati Gani
https://orcid.org/0000-0002-9508-7481
Farina Mustaffa-Kamal
https://orcid.org/0000-0002-5015-3836

Author Contributions

Conceptualization: Mustaffa-Kamal F, Salleh A, Azizan TRPT, Ahmad H, Kaderi MA, Hua KK, Wahab RA, Abdullah AL, Gani M; Data curation: Sadali MFM, Mustaffa-Kamal F, Salleh A, Zahli NIU, Mariatulqabtiah AR, Abdullah AL, Gani M; Formal analysis: Sadali MFM, Mustaffa-Kamal F, Salleh A, Zahli NIU, Mariatulqabtiah AR; Funding acquisition: Mustaffa-Kamal F, Azizan TRPT, Kaderi MA, Hua KK, Wahab RA; Investigation: Sadali MFM, Mustaffa-Kamal F, Azizan TRP, Salleh A, Zahli NIU, Mariatulqabtiah AR, Abdullah AL, Gani M; Methodology: Sadali MFM, Mustaffa-Kamal F, Salleh A, Zahli NIU, Mariatulqabtiah AR; Project administration: Mustaffa-Kamal F, Azizan TRPT, Ahmad H, Kaderi MA; Resources: Mustaffa-Kamal F, Azizan TRPT, Salleh A, Zahli NIU, Mariatulqabtiah AR; Software: Sadali MFM; Supervision: Mustaffa-Kamal F, Salleh A, Zahli NIU, Mariatulqabtiah AR, Azizan TRPT; Validation: Mustaffa-Kamal F, Mariatulqabtiah AR, Salleh A, Zahli NIU, Azizan TRPT; Visualization: Sadali MFM, Salleh A, Zahli NIU, Mariatulqabtiah AR; Writing - original draft: Sadali MFM; Writing - review & editing: Sadali MFM, Maraitulqabtiah AR, Salleh A, Zahli NIU, Azizan TRPT, Ahmad H, Kaderi MA, Hua KK, Wahab RA, Abdullah AL, Gani M, Mustaffa-Kamal F.

Conclusions and Relevance: Overall, this study enhances our understanding of the molecular characterisation and evolutionary dynamics of the CDV strain present in the Malayan tiger and serves as a benchmark for developing effective preventative measures to protect Malayan tigers and mitigate their risk of extinction.

Keywords: Morbillivirus; felidae; histology; hemagglutinin; phylogenetic analysis

INTRODUCTION

Canine distemper virus (CDV) is a highly contagious pathogen affecting mammals in the order Carnivora, including domestic and wildlife populations, often leading to severe illness and, in some cases, fatal outcomes. Belonging to the *Morbillivirus* genus within the *Paramyxoviridae* family, CDV is characterised by its enveloped structure and negative-sense, single-stranded RNA genome [1]. To date, the virus has been implicated in mass deaths of various mammals of the order Carnivora and other orders. Being an RNA virus, CDV exhibits high mutation rates and broad cell tropism, which contribute significantly to its ability to infect diverse host species. Mutations in surface proteins, particularly the fusion (F) and hemagglutinin (H) proteins are crucial for determining host specificity and pathogenicity. These proteins demonstrate the highest nucleotide variability and are commonly utilised for molecular identification and lineage differentiation, emphasising the H protein [2,3].

While initially considered immune to CDV, the virus has increasingly affected felids, with mass mortality events observed in wild lion populations [4]. CDV outbreaks in felids have been linked to mutations in the virus surface proteins, particularly in the H protein binding site, which is crucial for interactions with host cell receptors, namely the signalling lymphocyte activation molecule (SLAM) and nectin-4 [5]. Despite the rarity of CDV infections in domestic cats [6,7], outbreaks in wild felids highlight the possibility of severe consequences in big cat species.

Panthera tigris, listed as endangered species under the International Union for Conservation of Nature (IUCN) Red List, has an estimated population of fewer than 200 individuals in Malaysia [8]. Factors such as anthropogenic disruption, environmental disturbances and infectious diseases could contribute to the potential extinction of the species. In mid-2019, the first confirmed case of CDV in a Malayan tiger (*P. tigris jacksoni*) occurred in Peninsular Malaysia. The affected tiger, a young adult male, was first sighted in July 2019 near Kampung Besul Lama, Bukit Besi, Terengganu, where it was observed roaming close to human settlements. The animal exhibited timid behaviour upon capture. During captivity, sudden seizures and decreased appetite were observed from the tiger, which led to investigation for disease. CDV infection was subsequently confirmed through molecular testing via reverse transcription polymerase chain reaction (RT-PCR) by the Department of Wildlife and National Parks (PERHILITAN) Peninsular Malaysia [9]. The case raised concerns about the potential threat of CDV to the endangered Malayan tiger populations and the surrounding wildlife, highlighting the need for further research into the characteristics and pathogenicity of the virus in this species.

The emergence of the CDV strain has raised significant concern regarding its capability to infect Malayan tigers in their natural habitat. While studies on CDV in other tiger subspecies have shown virus manifestation via histological analysis and highlighted the

Conflict of Interest
The authors declare no conflicts of interest.

Data Availability Statement
Data supporting the findings are available from the corresponding author upon reasonable request. Sequence data are available in GenBank under accession numbers PP894824.1 (F gene) and PP894823.1 (H gene).

Funding
This study was financially supported by the National Conservation Trust Fund (NCTF) under the Ministry of Natural Resources, Environment and Climate Change, Malaysia with reference number KeTSA (S) 600-2/1/48/4 (JLD2).

association between surface protein mutations and pathogenicity through comprehensive molecular characterisation [10,11], such comprehensive analysis is lacking for the Malayan tiger infected by the virus. As the affected tiger in our study is the first reported case of CDV infection among the Malayan tiger subspecies, our understanding of the CDV strain and its pathogenicity in this subspecies was limited, especially its origin and strain lineage.

This study aimed to address critical gaps in understanding the CDV isolate infecting the Malayan tiger, including its pathological features and *in vitro* infective capability. Subsequently, a comprehensive molecular characterisation of the *F* and *H* genes and amino acid sequence, was conducted to investigate the origin of the strain and the potential implications of its mutations on tiger infection.

METHODS

Study approval and sample background

This study was conducted under the approval of the PERHILITAN Peninsular Malaysia (Permit number: JPHL&TN (IP): 100-34/1.24 Jld 20(11)). Upon complaints by the public, a male Malayan tiger named Awang Besul seen roaming close to the human population, was captured by the PERHILITAN. Awang Besul was treated symptomatically at Sungkai Wildlife Conservation Centre; however, it died naturally in captivity one week post-capture, and a post-mortem examination was conducted at the Faculty of Veterinary Medicine, Universiti Putra Malaysia.

Sample processing for histopathological analysis

Tissues from brain, lung, kidney, spleen, liver and stomach of Awang Besul were collected during necropsy. The sections of each tissue were fixed with 10% formalin solution before being embedded into paraffin wax blocks for histopathological and immunohistochemical evaluation. The tiger's brain, lung, kidney, and spleen tissues were used for viral isolation and molecular characterisation and kept in a phosphate-buffered saline (PBS), pH 7.0 solution at −80°C. A total of 0.3 g of each tissue was pooled in 1 mL PBS solution and stored at −80°C until further analysis.

CDV histopathological analysis

Paraffin-fixed tissues were cut into 5 μm thick sections and stained with haematoxylin and eosin. The paraffin-embedded tissues were cut into 3 μm sections for immunohistochemistry (IHC) using an anti-CDV monoclonal primary antibody. The sections were deparaffinised in xylene solution, rehydrated in alcohol solution at gradual concentrations, and microwaved at 50 W in pH 6.0 citrate buffer for 15 min for antigen retrieval. After washing with PBS solution 3 times at 3 min each, endogenous peroxidase activity was blocked with 3% hydrogen peroxide solution in PBS for 30 min before undergoing another washing step. The sections were then incubated with 1% bovine serum albumin (BSA) in PBS solution for 30 min to block non-specific antibody binding.

The sections were incubated for 1 h at 37°C with mouse anti-CDV monoclonal antibody (clone DV2-12) (Custom Monoclonal International, USA) (1:1,500 dilution in PBS containing 0.1% BSA). After washing, the sections were incubated with a rabbit anti-mouse secondary antibody (Nichirei Biosciences, Japan) for 3 min at 37°C, washed, and incubated for 30–50 sec with Liquid 3,3′-diaminobenzidine (DAB; DAKO, USA) for chromogen staining. They were

then counter-stained with methylene blue (Bendosen, Malaysia), dried, and mounted with DPX (Sigma-Aldrich, USA) for qualitative immunolabelling. Brain tissues from a CDV-positive dog (courtesy of Dr. Apisit Pornthummawat, Mahidol University) and tissues of brain, lung, kidney from a CDV-negative Malayan tiger were subjected to the same protocol as positive and negative control, respectively.

Cell culture and virus isolation

Chinese hamster ovary (CHO) cells expressing the dog signalling lymphocytic activation molecule receptor (CHO-SLAM) were used for CDV identification and propagation [12]. Cells were maintained in culture medium, consisting of RPMI medium (Gibco, USA) supplemented with 10% foetal bovine serum (FBS; Gibco), 1% Penicillin-Streptomycin-Glutamine (PSG) (Gibco), and Geneticin^{TM} (G418 sulfate; Gibco) in a T25 cell culture flask (Jet BioFil, China) at 37°C in 5% CO_2. Cells at 70%–80% confluency were used for virus isolation. CDV was collected from the tiger pooled tissues. The tissues were ground with sterile sand and homogenised in 5 mL PBS solution. The homogenate was centrifuged at 1,500 rpm for 5 min and filtered through a 0.22 μm filter to collect the virus. Flask with confluent cells were rinsed twice with PBS solution, and 1 mL of infection medium, consisting of RPMI medium supplemented with 5% FBS and 1% PSG was added. A total of 100 μL of virus was then inoculated into the cell culture and incubated at 37°C with 5% CO_2 for 45 min, after which an additional 4 mL of infection medium was added. Cells were monitored daily for qualitative cytopathic effect (CPE). Upon confirming CPE, the culture underwent a freeze-thaw cycle three times to release the virus. The media supernatant containing the virus was collected.

The CHO-SLAM cells were sub-cultured in 24-well plates (Jet BioFil) for viral titration using a 50% tissue culture infectious dose ($TCID_{50}$) assay with virus dilutions from 10^{-1} to 10^{-10} [13]. The virus-inoculated cells were maintained at 37°C, 5% CO_2 and monitored daily for CPE. Once CPE was confirmed, the average $TCID_{50}$ evaluation (done in triplicates) was calculated using the Spearman-Kärber formula [14,15].

Primer design, RT-PCR and genomic sequencing

CDV reference sequences from the Asian region were retrieved from GenBank and aligned using Molecular Evolutionary Genetics Analysis Version 10 (MEGA-X) software to design primers specific to the CDV *F* and *H* genes coding regions (**Supplementary Table 1**). The primers were designed using the Primer-Basic Local Alignment Search Tool (Primer-BLAST) tool. Their specificity to CDV was validated via the Basic Local Alignment Search Tool for nucleotides (BLASTn).

CDV viral RNA was extracted from the cell culture using the Nucleospin RNA Virus Kit (Macherey-Nagel, Germany), followed by cDNA synthesis using the SensiFAST cDNA Synthesis Kit (Bioline, UK) according to the manufacturer's protocol. PCR amplification of the cDNA was performed using the MyTaq Red Mix kit (Bioline) according to the manufacturer's protocol, utilising the primers designed. The PCR cycle included initial denaturation at 95°C for 1 min; 30 cycles of denaturation at 94°C for 1 min, annealing following the primers' T_m for 1 min, and extension at 72°C for 50 sec; and a final extension at 72°C for 3 min. Recombitek C3 vaccine (Boehringer Ingelheim, Germany) and nuclease-free water were used as positive and negative controls, respectively. Gel electrophoresis were done on the amplified PCR products and visualised using the GeneGenius Bio Imagining System (Syngene, India). The target bands were excised, purified with GeneJET Gel Extraction kit (Thermo

Fisher Scientific, USA) and sequenced on an ABI PRISM 3730xl Genetic Analyser (Applied Biosystems, USA).

Full-length *F* and *H* genes sequences

The sequences of the amplified amplicons were aligned using the MEGA-X software for each gene segment to get the full *F* and *H* genes sequence and deposited in the GenBank. Afterwards, each sequence was converted into the amino acid sequence using the Expasy translating tool. The sequences for each protein region were compared to the sequences of their closest CDV strain relatives and the chemical composition was analysed. The cysteine residue and the N-glycosylation sites were determined using the Expasy ProtParam tool and the NetNGlyc software. Each amino acid sequence was evaluated for the mentioned characteristics above by comparing them to reference genes from the National Centre for Biotechnology Information (NCBI) via the BioEdit sequence alignment tool [16]. The complete sequence of both genes were deposited in GenBank as PP894824.1 and PP894823.1.

Phylogenetic analyses

Phylogenetic analysis was done on *F* and *H* genes sequence against a list of CDV sequences from various lineages of different geographical locations retrieved from the NCBI GenBank. Phocine and dolphin morbillivirus from the genus of morbillivirus were used as outgroup in the phylogenetic analysis. The phylogenetic analysis was done using the MEGA-X software, and the maximum likelihood tree was constructed using a substitution model with a bootstrap value of 1,000 replicates calculating the topology, and branch lengths likelihood in addition to, applying the Tamura 3-parameter substitution model parameter on the CDV sequences for the highest overall likelihood score [17].

RESULTS

Histopathological findings

Histopathological manifestations of CDV in the brain, lung, kidney, spleen, liver, and stomach of Awang Besul were observed under a light microscope (**Fig. 1**) for any histological abnormalities pertaining to CDV infection against the positive and negative controls (**Supplementary Fig. 1**). In the brain, diffuse oedema was observed in the leptomeninges along with mild mononuclear cell infiltration with a gemistocytic astrocyte, which may indicate reactive gliosis associated with demyelination. In the lungs, bronchointerstitial pneumonia, characterised by alveolar spaces filled with leukocytes and fibrin, along with vascular congestion, thickened alveolar septa, and diffuse pulmonary oedema, was evident. In addition, histological examination of the kidney revealed cytoplasmic lipid vacuolation and mild tubular epithelial degeneration. No significant glomerular or peritubular congestion, lymphocytic infiltration, or inclusion bodies were observed. Hepatic cord dissociation and individualisation with degeneration with mononuclear cells infiltration at the area of liver necrosis were present. The stomach showed cytoplasmic viral inclusion bodies.

Through IHC, positive immunostaining indicated by dark brownish deposits was observed in various tissues of Awang Besul, including the lung, liver, kidney, and stomach (**Fig. 2**). The localisation of CDV antigen in the lung tissue was noted in the alveolar epithelium besides the presence of CDV antigen in the liver parenchyma where the degeneration of hepatocytes occurred. Presence of high immunolabelling was noted on the tubular

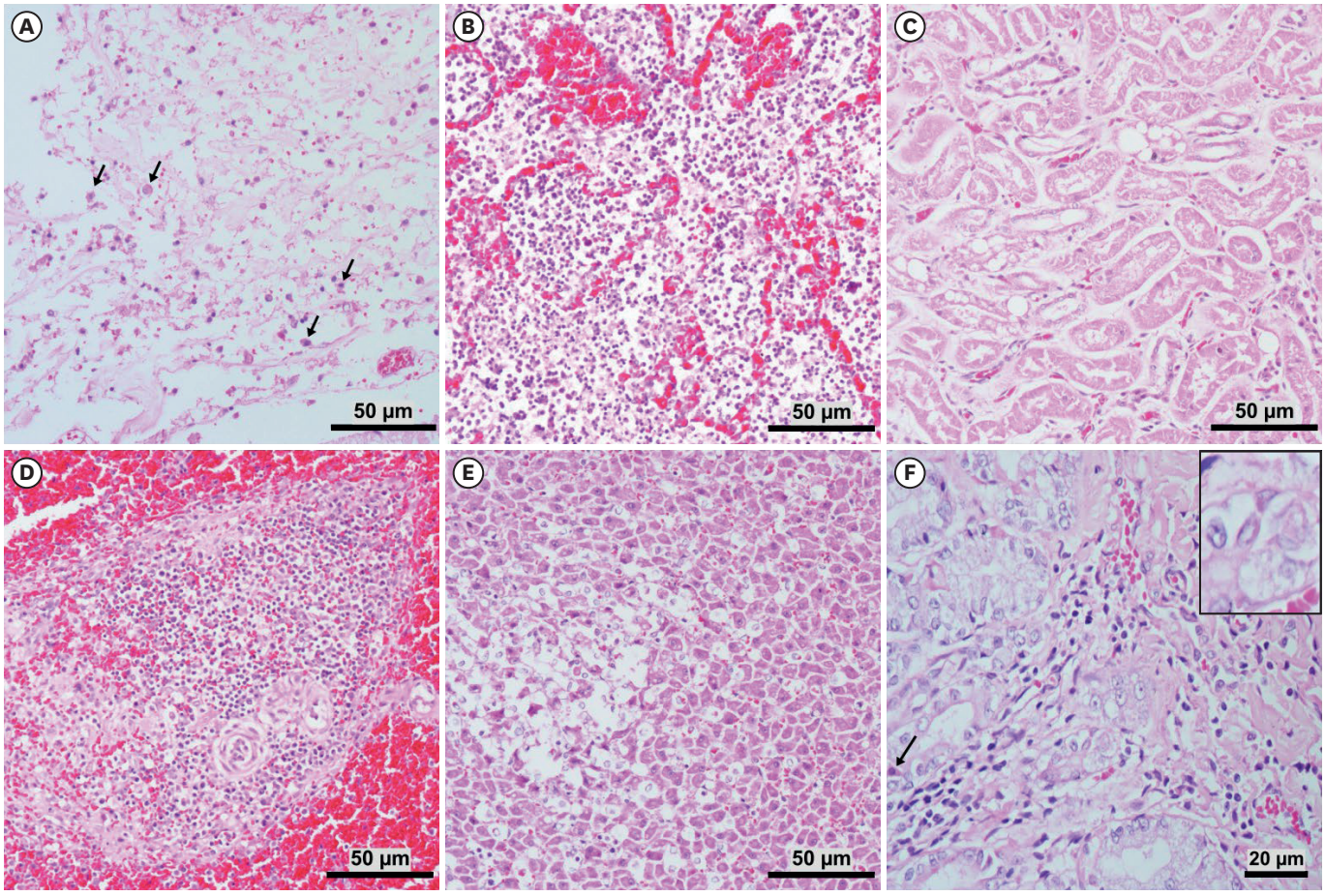

Fig. 1. Histological observation on canine distemper virus lesions in the tiger's tissues: (A) Brain showing diffused oedema in the leptomeninges and mild mononuclear cells infiltration with the presence of gemistocytic astrocyte (arrows), H&E, scale bar = 50 μm. (B) Lung showing bronchointerstitial pneumonia with thickened septa and alveoli filled with leukocytes and fibrins, H&E, scale bar = 50 μm. (C) Kidney showing cytoplasmic lipid vacuolation of renal tubule epithelium with mild tubular degeneration, H&E, scale bar = 50 μm. (D) Spleen showing severe lymphoid necrosis with lymphocytolysis, H&E, scale bar = 50 μm. (E) Liver showing hepatic cord dissociation and individualisation and degeneration with mononuclear cells infiltration at the area of necrosis, H&E, scale bar = 50 μm. (F) Stomach showing cytoplasmic viral inclusion bodies (inset) and macrophage (arrow), H&E, scale bar = 20 μm. H&E, haematoxylin and eosin.

epithelium of the kidney, indicating high CDV antigen. Similarly, positive immunolabelling of cytoplasmic antigens was detected in the gastric glands of the stomach lining.

Virus isolation

The CDV from the infected tiger pooled tissues was isolated by inoculating the virus into the CHO-SLAM cells and monitoring for CPE daily. The manifestation of CPE in the CHO-SLAM cells was observed 48 h post-infection (**Supplementary Fig. 2**). The CPE in the CHO-SLAM was represented by cell fusion and clumping, minor syncytium formation, and cell detachment aligning with the CPE characteristics of most morbillivirus [12]. At 72 h post-infection, no difference in CPE manifestation was detected, despite more cell being detached due to cell death. $TCID_{50}$ assay was done on the CDV-infected CHO-SLAM to determine the viral load in the infected tiger tissues. The tiger's pooled tissues showed a high CDV $TCID_{50}$ value of 4.27×10^6 $TCID_{50}$/mL at 48 h post-CDV infection.

RT-PCR analysis

The optimised RT-PCR for verification of the CDV isolate in the infected tiger was done using the H_CDV1 primer targeting a region in the *H* gene of the virus, which yielded the

https://doi.org/10.4142/jvs.25092

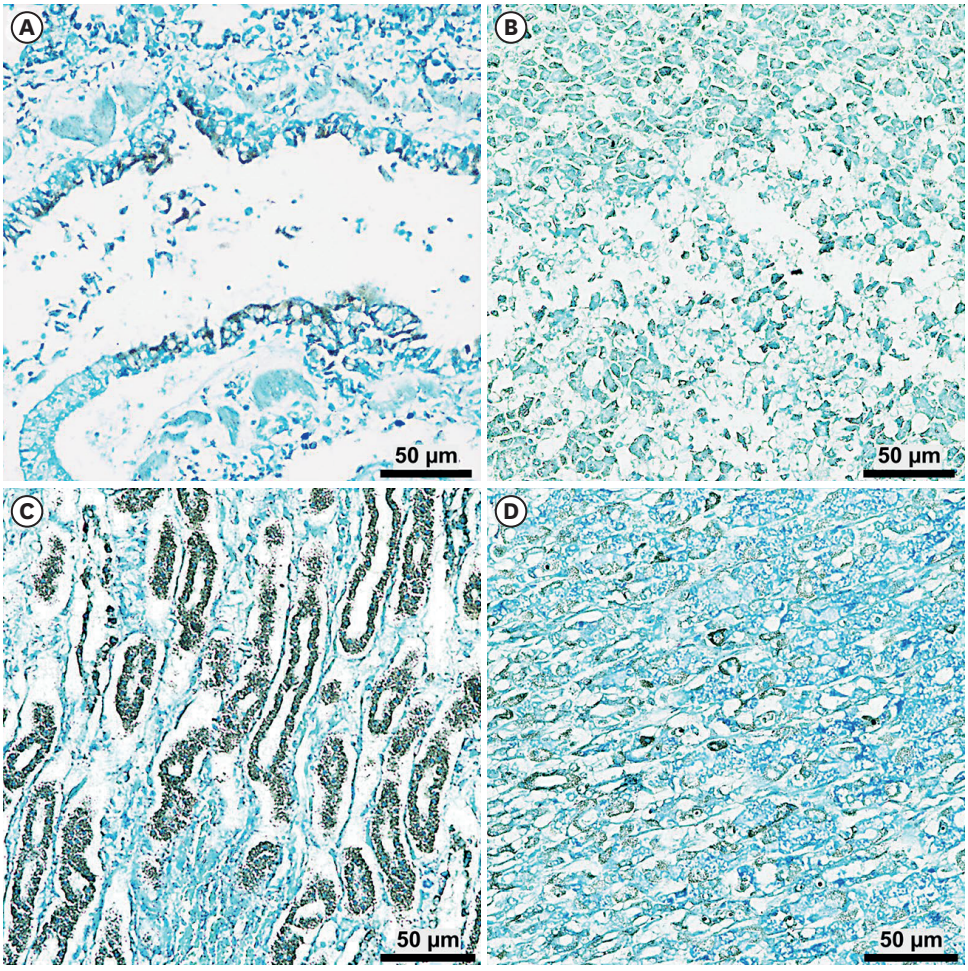

Fig. 2. Immunohistochemical detection of CDV antigen in the tiger's tissues. (A) Lung showing positive CDV antigen immunolabelling in the bronchiolar epithelium. (B) Liver showing strong immunolabelling of cytoplasmic CDV antigen thoroughly in degenerate hepatocytes. (C) Kidney section with dense accumulation of intracellular viral antigens in the tubular walls. (D) Positive immunolabelling was observed in the gastric mucosa of the stomach lining. Scale bars = 50 μm.
CDV, canine distemper virus.

desired amplicon of 326 bp, followed by a qualitative observation via gel electrophoresis (**Supplementary Fig. 3**). The RT-PCR result done after viral isolation via cell culture confirmed the presence of the CDV antigen that had been successfully propagated in the CHO-SLAM cells.

CDV sequencing and characterisation

The amplicons from the PCR gel electrophoresis for each gene were amplified using the optimised PCR method and designed primers and were retrieved and sequenced. The sequences from BLAST analysis that showed the highest similarity with BesulMY sequence were assembled and aligned using MEGA-X software to obtain the full *F* and *H* genes. The completed sequences were translated to their amino acid sequences via the Expasy translating tool. The pairwise comparison revealed that the *F* gene exhibited 99.20% nucleotide similarity to the HeB(07)/EU327874.1 CDV isolate from China and 98.79% amino acid similarity to the CDV_TH/2014/AZO92836 strain from Thailand. In contrast, the BesulMY *H* gene demonstrated 99.18% nucleotide similarity to the SD(09)3/

https://doi.org/10.4142/jvs.25092

HM448834 isolate and 99.01% amino acid similarity to the SD(09)1/ADM26778.1 strain, both originating from China.

To deduce the phylogenetic evolution of the BesulMY CDV strain, a phylogenetic analysis was performed on the full-length nucleotide coding sequences of the *F* (**Fig. 3**) and *H* genes (**Fig. 4**). Overall, the BesulMY CDV isolate identified was classified within the Asia-1 strain, demonstrating a high genetic evolutionary relationship with CDV isolates in China. Notably, the BesulMY *H* gene formed a distinctive cluster within a novel divergent clade, suggesting a distinct clade from other Asia-1 isolates.

The BesulMY CDV F amino acid sequence was subjected to comparative evaluation with Asia-1 reference isolates of close similarity from the NCBI GenBank (**Fig. 5**). We observed a high variability of the amino acid sequence in the Fsp of the isolates, while the F1 and F2 regions were considered more conserved. Furthermore, the BesulMY CDV strain lacked the potential N-glycosylation site (N-X-S/T) at amino acid residue 62–64 compared to the reference amino acids. Conversely, all 17 cysteine residues were conserved in all compared

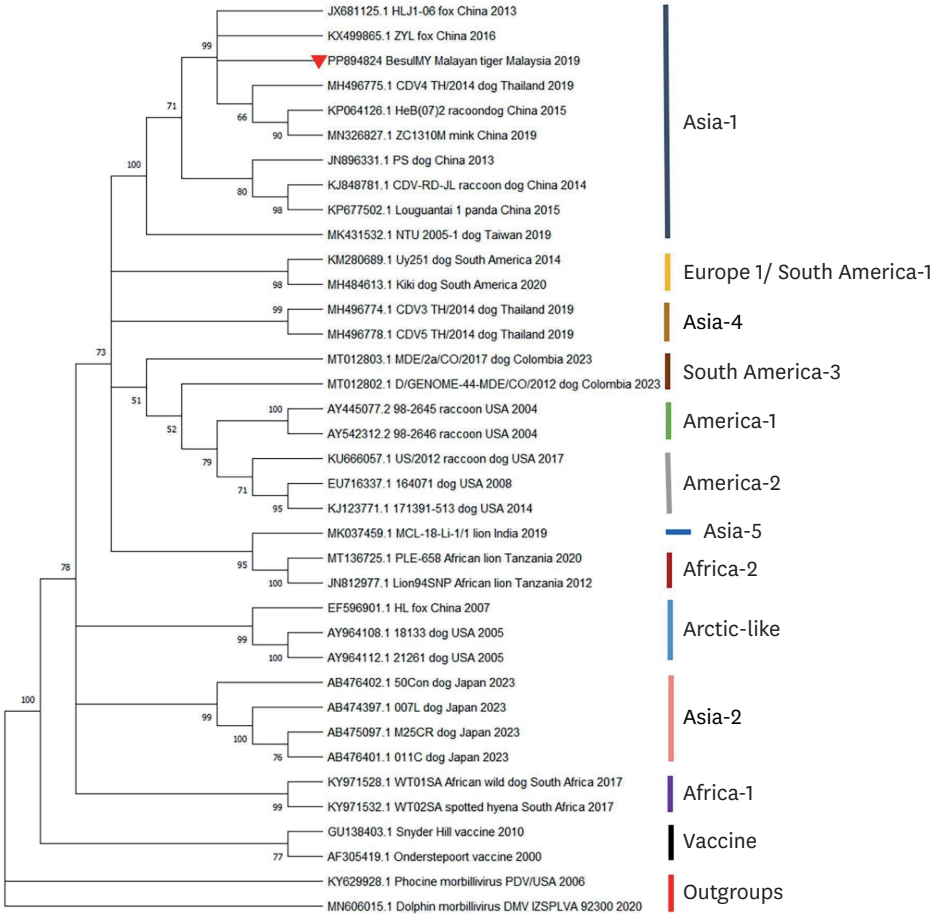

Fig. 3. Phylogenetic tree of the full-length *F* gene of CDV isolates based on nucleotide sequence alignment. The phylogenetic tree was constructed using the MEGA-X software. The evolutionary history was inferred using the Maximum Likelihood method with 1000 bootstrap. Bootstrap values of > 50% where the associated taxa are clustered together, are shown on the branch points. The complete *F* gene coding sequence of BesulMY CDV strain (PP894824) is indicated by the symbol (▼).
CDV, canine distemper virus.

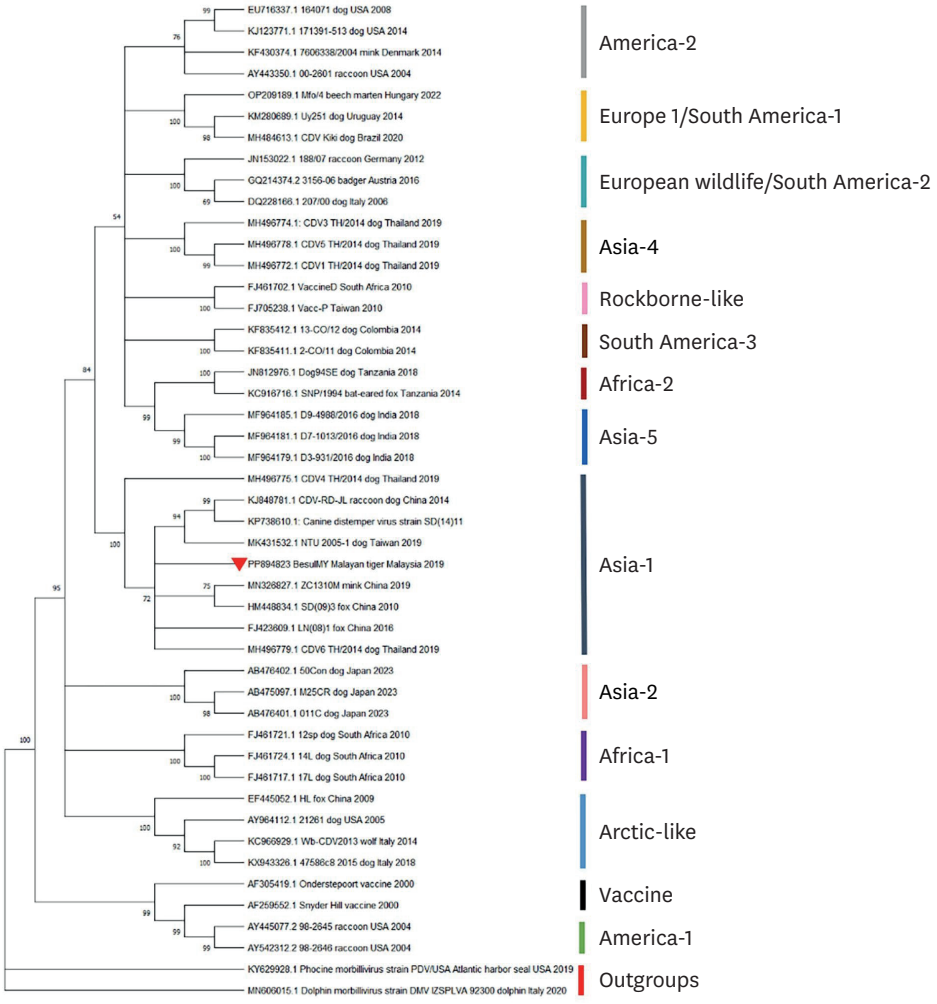

Fig. 4. Phylogenetic tree of the full-length *H* gene of CDV isolates based on nucleotide sequence alignment. The phylogenetic tree was constructed using the MEGA-X software. The evolutionary history was inferred using the maximum likelihood method with 1000 bootstraps. Bootstrap values of > 50% where the associated taxa clustered together, are shown on the branch points. The complete *H* gene coding sequence of the BesulMY CDV strain (PP894823) is indicated by the symbol (▼).
CDV, canine distemper virus.

strains. **Fig. 6** illustrates the deduced amino acid sequence of the *H* gene against the closest CDV isolates, as well as the isolate from an African lion from the Serengeti epidemic (AOV62807.1). Despite conserved cysteine residues, the BesulMY CDV strain exhibited a mutation at the N456 glycosylation site (N456H), while other sites, including N149, N422, and N587, were preserved.

We also observed mutations in the key residues of the H amino acid that determine host specificity in the SLAM-binding region (**Supplementary Table 2**). Amino acid substitution combinations of 519I and 549H were observed in the H amino acid of the CDV isolate when compared to other canid and non-canid species, similar to previous observations on the CDV isolate in the Serengeti African lion (AOV62807.1).

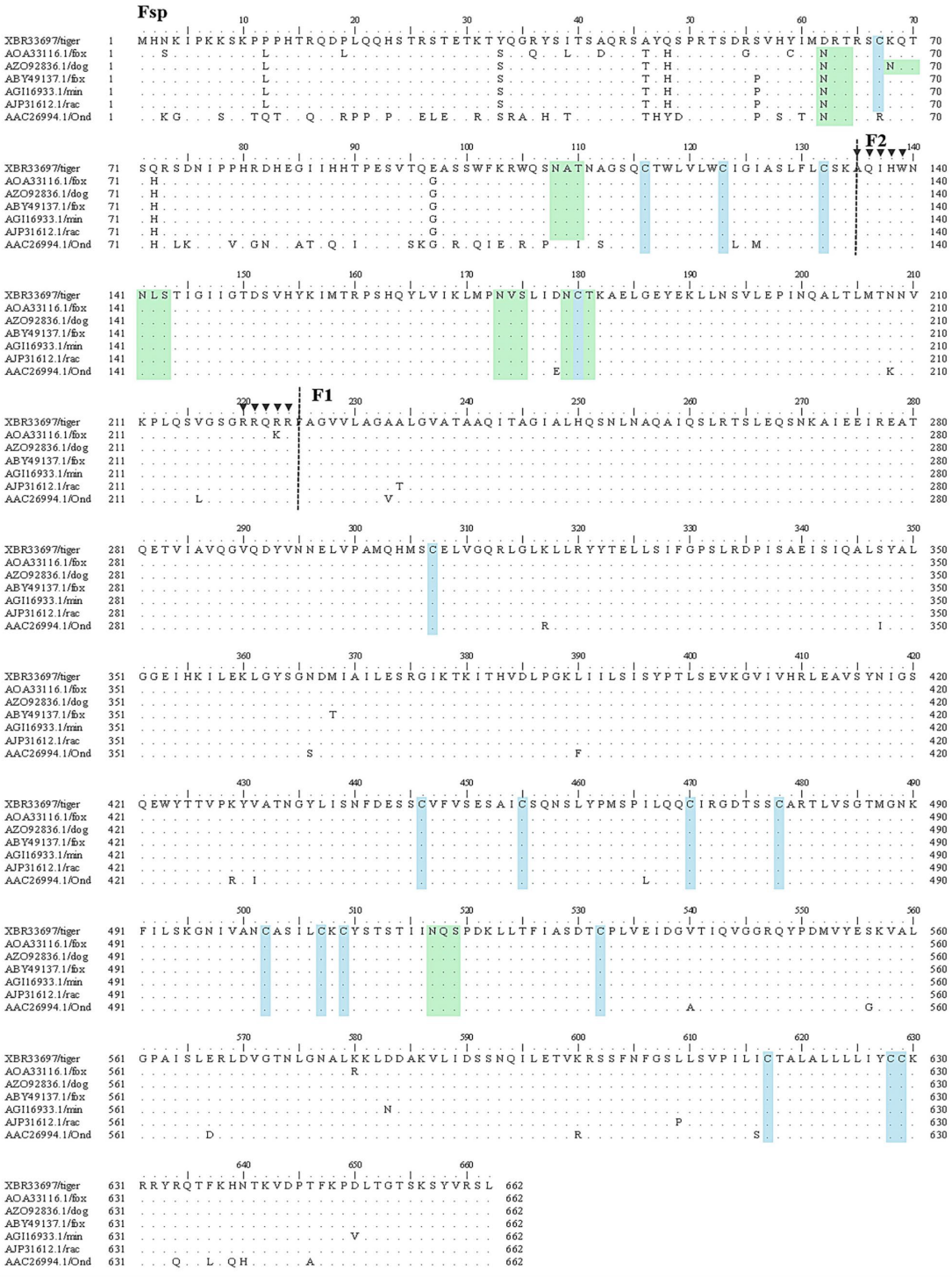

Fig. 5. F amino acid sequence alignment divided by their respective regions (Fsp, F1, and F2). The green region represents potential N-glycosylation sites, and the blue region represents cysteine residues, while ▼ signifies cleavage sites. The BesulMY CDV strain (XBR33697) has the highest F protein amino acid similarity to AZO92836.1/dog/CDV_TH/2014/Thai/2014 strain at 98.79%. The alignment showed the highest amino acid variability in the Fsp region among all isolates. The CDV isolate studied showed a lack of potential N-glycosylation site in this region.
CDV, canine distemper virus.

https://doi.org/10.4142/jvs.25092

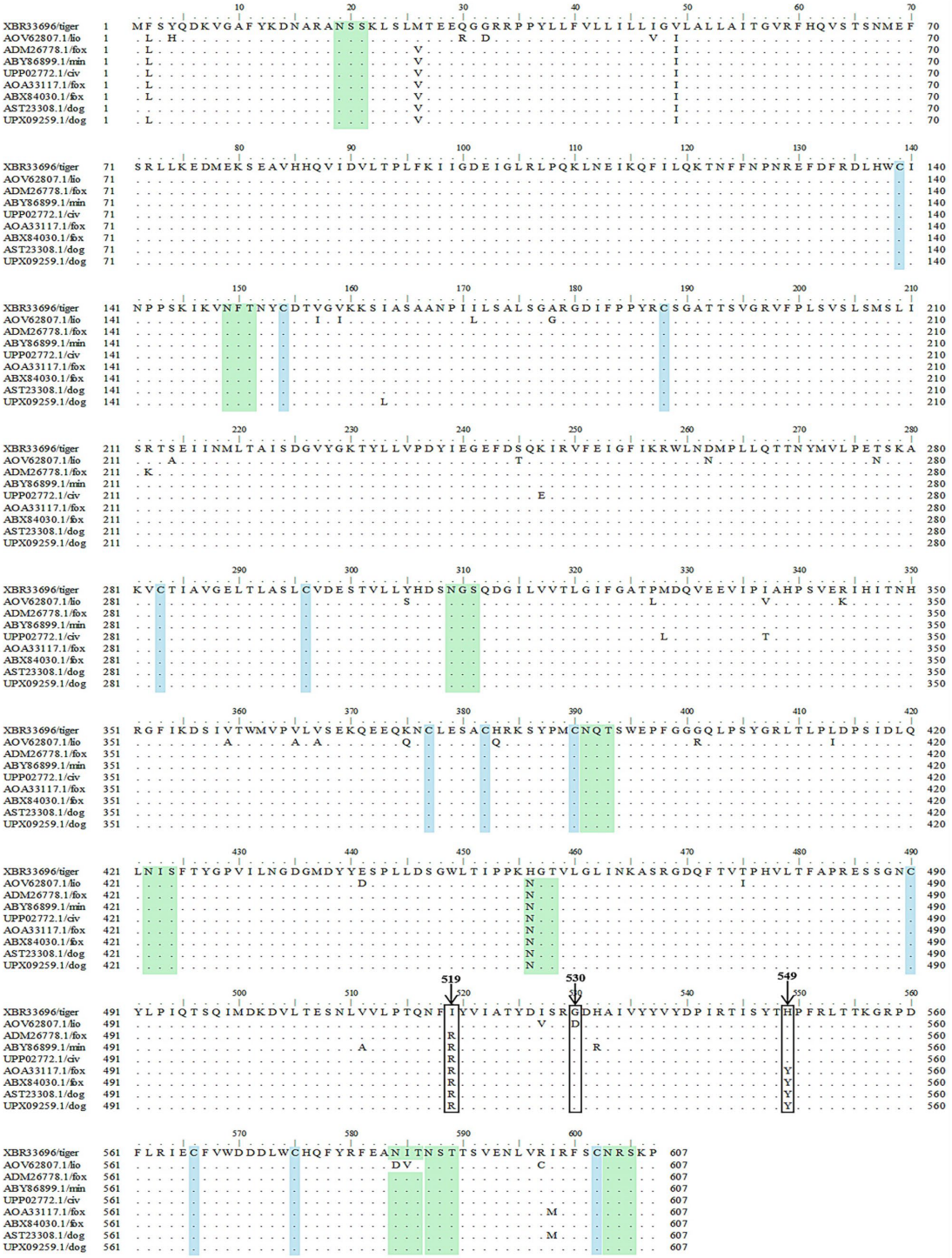

Fig. 6. H amino acid sequence alignment highlights residues of interest for viral mutation regarding host specification. The green region represents potential N-glycosylation sites, and the blue represents cysteine residues. The BesulMY CDV strain (XBR33696) has the highest H protein amino acid similarity to ADM26778.1/fox/SD(09)1/Chi/2010 strain at 99.01%. The CDV isolate from the Malayan tiger exhibits the 549H mutation, while the 530G are conserved in all isolates. Mutation at the 519 amino acid position is also observed, where the CDV isolate harbours a 519I mutation.
CDV, canine distemper virus.

https://doi.org/10.4142/jvs.25092

DISCUSSION

In this study, tissue samples from the first reported CDV-positive Malayan tiger were analysed and the presence of CDV was confirmed by RT-PCR. The tiger exhibited clinical signs of CDV infection, including respiratory issues, seizures, and neurological symptoms [9]. Histopathological findings in the tiger's tissues were consistent with those observed in other CDV-infected hosts, including lymphocytolysis, pneumonia, oedema, tissue haemorrhage and degeneration from viral disruption, and the presence of cytoplasmic inclusion bodies [18]. These lesions resembled those documented in other felids infected with CDV, including lymphoid tissue depletion and hepatic necrosis [10,19]. Previous studies also reported lymphoid tissue depletion in the lymph nodes as well as spleen and hepatocytes necrosis in other CDV-infected felids, namely lions and leopards. Pathological changes noted in commonly affected organs, such as the lungs, kidneys, and brain, have been documented, although their severity varies among different species [20-22]. The variation in pathological changes among different CDV-infected animals is due to its multi-systemic nature and complex pathogenicity as a multi-host virus [10].

Although histopathological analysis offers valuable insights into the pathological manifestations of pathogens in host tissues, general histological changes proved unreliable for detecting CDV antigens [23,24]. Inclusion bodies typically emerge in the later stages of the disease, and there is a potential for inaccurately perceiving other structures as virus-induced inclusions [25]. Therefore, IHC provides significant value to pathological assays following the observed pathological lesions by confirming the presence of CDV antigens in the infected tissues through specific interactions with anti-CDV antibodies. This study confirmed CDV tissue lesions through histopathological and immunostaining methods using CDV-specific antibodies, followed by the isolation of the wild-type CDV from the infected tiger. The observed high CPE at 48 h post-infection aligns with a previous study indicating distinct CPE development and high viral titres between 24 and 48 h post-infection of wild-type CDV [26].

Despite previous challenges in infecting cell cultures with wild-type CDV strains as opposed to attenuated vaccine and recombinant strains [27], this study successfully induced CPE in CHO-SLAM cells following infection with CDV obtained from the tiger's pooled tissues. The severe CPE observed at 48 h post-infection suggests robust viral replication, possibly attributed to stable SLAM receptor expression by CHO cells, facilitating virus-cell interaction and fusion. Furthermore, the high viral titre, as evidenced by the robust CPE observed, indicates successful viral replication, contributing to infection severity and eventual host demise. The correlation between the elevated viral load and the observed CPE manifestation directly translates to viral replication efficiency and the resultant pathological consequences in the CDV-infected tiger.

Complementary molecular tests are crucial for precise strain identification and characterisation, providing insight into genetic composition and pathogenic mechanisms. In this study, CDV antigen verification was successfully performed on tiger tissue samples propagated in CHO-SLAM cell culture. Virus propagation was limited to a single passage to prevent nucleotide mutations introduced through cell culture adaptation [28]. Successful in vitro infection facilitated the identification and analysis of the isolated CDV isolate from the infected tiger, focusing on the characteristics of the CDV *F* and *H* full genes governing its pathogenicity.

Phylogenetic analysis revealed that our CDV isolate clustered within the Asia-1 lineage and shared a closer relationship with isolate from China than those from Thailand. This pattern may reflect the extensive CDV strain heterogeneity in Thailand [29], resulting in selective genetic spillover between countries. Selective spillover from China to Malaysia through Thailand borders could be driven by transmission dynamics of stable strains influenced by ecological factors. Notably, the CDV *H* gene formed a divergent clade from other Asia-1 isolates, suggesting a distinct Asia-1 clade in Malaysia. The observed divergence in this clade is hypothesised to result from the genetic drift of the virus and its adaptive capacity to thrive in the Malaysian environment and ecology. The study highlights the distinctive nature of the H gene, which displays the highest divergence among CDV genomes due to the protein's crucial role in the initial virus-host interaction [29].

Furthermore, the study identified specific point mutations in CDV F and H amino acids to assess CDV virulence in tigers. Generally, the F protein is a crucial component of the viral envelope, which is responsible for mediating the fusion of the viral membrane with the host cell membrane, allowing the virus to enter the cell [30]. In this study, the signalling peptide (Fsp) region of the CDV F amino acid sequence exhibited the highest variability. The Fsp region is known to have the highest variations in all CDV isolates and is commonly used for strain identification when targeting the *F* gene region [31]. In addition, changes in the N-glycosylation site and the cysteine residues may alter protein structure and affect disease induction. These alterations may affect the activation of the Fsp protein precursor through cleavage or the protein transfer to the proteolytic cleavage site [26].

The N-glycosylation site, commonly found in most enveloped viruses, is crucial in enhancing virus infectivity and facilitating immune evasion within the host cells. Overall, the CDV isolate in this study lacks the potential N-glycosylation sites in both the F and H amino acid sequences compared to other CDV isolates. Reduced potential N-glycosylation sites in the F and H amino acids may diminish glycosylation activity, affecting viral biogenesis, antigenicity, and disease attenuation [32]. However, a previous study challenged the notion by demonstrating the effect of non-glycosylated CDV H protein on the pathogenesis of CDV [33]. Despite its reduced expression and virulence, they suggested glycosylation is not essential for maintaining virus immunosuppressive characteristics. The report conclusively demonstrated that the absence of N-glycosylation on the CDV H protein did not compromise its functional integrity. Therefore, the absence of potential N-glycosylation sites in the CDV strain in this study did not impact the virus infectivity as observed *in vitro*. However, future laboratory evaluations on other possible effects of reduced glycosylated sites in the CDV isolate are warranted to observe its pathogenicity in nature. In recent years, most molecular studies have focused on the H protein analysis due to its association with host recognition, resulting in pronounced genetic variability, specifically in residues 530 and 549 related to the SLAM-binding receptor region. While residue 530 was often found conserved in most species, residue 549 was often observed for its mutative capability that may be responsible for host range specificity and increased virulence. In this study, the CDV strain showed 549H residue in the H amino acid sequence, suggesting that the strain may originate and circulate among the wildlife population in Malaysia. A previous study proposed that dog species tend to exhibit a 549Y mutation, while 549H was observed in most wildlife species (canid and non-canid), which showed the mutation observed in this study was associated with host species specificity [34]. Additionally, they suggested that the Y549H mutation can occur due to selection pressure for viral adaptation from domestic species to the wildlife population. This study observed different mutations in the CDV isolate

from the infected tiger but did not investigate how and when these mutations occurred. Therefore, a larger pool of CDV isolates among domestic and wildlife species in the vicinity needs to be obtained to determine the origin of the isolate and the factors causing these mutations, specifically in the Malayan tiger species.

Additional mutations in other residues of the H amino acid generally do not correlate towards positive selection. However, the R519I substitution found in CDV isolates suggests potential host adaptability to non-canid species, particularly big cats in the felid family [2,35]. This specific mutation, observed in other feline species like the Serengeti African lion, has been associated with fatal outcomes in non-canid species. The rare combination of mutations 519I and 549H substitution has been exclusively linked to 100% mortality in non-canids [4,35]. A similar finding in this study postulated that the observed mutations influence viral entry in host cells and may contribute to the fatal outcome of the Malayan tiger. High viral replication, often correlated with increased disease severity, indicates heightened viral burden and tissue damage [36]. The mutations observed in the viral protein may enhance viral replication efficiency, which exacerbated the severity of CDV-induced pathology and led to the tiger's mortality. Considering the present findings, the extent of CDV spread in the wild Malayan tiger population needs to be addressed further and monitoring this disease should be incorporated into the current conservation strategy.

ACKNOWLEDGMENTS

We would like to express our thanks to Professor Hironobu Tatsuo, Kyushu University for graciously providing the CHO-SLAM cell line. Thank you to all individuals from the Virology and Histopathology laboratories, Faculty of Veterinary Medicine, Universiti Putra Malaysia for their technical assistance.

SUPPLEMENTARY MATERIALS

Supplementary Table 1

Eight CDV primer sets of *F* and *H* full-length genome sequencing and their melting temperatures

Supplementary Table 2

H amino acid sequence comparison between BesulMY CDV strain (XBR33696) and reference isolates from GenBank

Supplementary Fig. 1

DAB immunohistochemical staining on (A) brain of a canine distemper virus-positive dog showing positive immunolabelling (brown colouring) in the cytoplasm of the neurons of the cerebral cortex: positive control, scale bar = 50 μm. (B) Kidney of a Malayan tiger: negative control, scale bar = 100 μm, (C) lung of a Malayan tiger: negative control, scale bar = 50 μm.

Supplementary Fig. 2

CDV BesulMY infection in the CHO-SLAM cell culture observed for cytopathic effect 48 h post-infection: (A) Normal CHO-SLAM cell culture, 100X and (B) CHO-SLAM cell culture infected with the CDV isolated from the infected tiger showing cell clumping and detachment. Scale bars = 100 μm.

Supplementary Fig. 3

Gel electrophoresis showing result for CDV antigen reverse transcription polymerase chain reaction verification in the infected tiger (BesulMY) shows the desired amplicon (326 bp) related to CDV based on a 100-bp DNA ladder (Vivantis Technologies, Malaysia) and compared with against a positive control (+ve; Recombitek C3 vaccine; Boehringer Ingelheim; Germany) and negative control (-ve; nuclease-free water).

REFERENCES

1. Deem SL, Spelman LH, Yates RA, Montali RJ. Canine distemper in terrestrial carnivores: a review. *J Zoo Wildl Med*. 2000;31(4):441-451. PUBMED | CROSSREF
2. Trogu T, Castelli A, Canziani S, Tolini C, Carrera M, Sozzi E, et al. Detection and molecular characterization of canine distemper virus in wildlife from Northern Italy. *Pathogens*. 2022;11(12):1557. PUBMED | CROSSREF
3. Romanutti C, Gallo Calderón M, Keller L, Mattion N, La Torre J. RT-PCR and sequence analysis of the full-length fusion protein of canine distemper virus from domestic dogs. *J Virol Methods*. 2016;228:79-83. PUBMED | CROSSREF
4. Duque-Valencia J, Sarute N, Olarte-Castillo XA, Ruíz-Sáenz J. Evolution and interspecies transmission of canine distemper virus-an outlook of the diverse evolutionary landscapes of a multi-host virus. *Viruses*. 2019;11(7):582. PUBMED | CROSSREF
5. Beineke A, Baumgärtner W, Wohlsein P. Cross-species transmission of canine distemper virus-an update. *One Health*. 2015;1:49-59. PUBMED | CROSSREF
6. Appel MJG, Summers BA. Pathogenicity of morbilliviruses for terrestrial carnivores. *Vet Microbiol*. 1995;44(2-4):187-191. PUBMED | CROSSREF
7. Harder TC, Kenter M, Vos H, Siebelink K, Huisman W, van Amerongen G, et al. Canine distemper virus from diseased large felids: biological properties and phylogenetic relationships. *J Gen Virol*. 1996;77(Pt 3):397-405. PUBMED | CROSSREF
8. Ten DCY, Jani R, Hashim NH, Saaban S, Abu Hashim AK, Abdullah MT. *Panthera tigris* jacksoni population crash and impending extinction due to environmental perturbation and human-wildlife conflict. *Animals (Basel)*. 2021;11(4):1032. PUBMED | CROSSREF
9. Department of Wildlife and National Parks. 'Awang Besul' The Tiger Dies [Internet]. New Straits Times. Published [2019]. Updated [2019]. Accessed [Feb 10, 2025]. https://www.nst.com.my/news/nation/2019/07/506553/awang-besul-tiger-dies
10. Kadam RG, Karikalan M, Siddappa CM, Mahendran K, Srivastava G, Rajak KK, et al. Molecular and pathological screening of canine distemper virus in Asiatic lions, tigers, leopards, snow leopards, clouded leopards, leopard cats, jungle cats, civet cats, fishing cat, and jaguar of different states, India. *Infect Genet Evol*. 2022;98:105211. PUBMED | CROSSREF
11. Roelke-Parker ME, Munson L, Packer C, Kock R, Cleaveland S, Carpenter M, et al. A canine distemper virus epidemic in Serengeti lions (*Panthera leo*). *Nature*. 1996;379(6564):441-445. PUBMED | CROSSREF
12. Tatsuo H, Ono N, Yanagi Y. Morbilliviruses use signaling lymphocyte activation molecules (CD150) as cellular receptors. *J Virol*. 2001;75(13):5842-5850. PUBMED | CROSSREF
13. Muench HR. A simple method of estimating 50 per cent end points. *Am J Hyg*. 1938;27:493-497.
14. Spearman CI. The method of 'right and wrong cases' ('constant stimuli') without Gauss's formulae. *Br J Psychol*. 1908;2(3):227-247. CROSSREF
15. Kärber G. Beitrag zur kollektiven behandlung pharmakologischer reihenversuche. *Naunyn Schmiedebergs Arch Exp Pathol Pharmakol*. 1931;162:480-484.
16. Alzohairy A. BioEdit: an important software for molecular biology. *GERF Bull Biosci*. 2011;2:60-61.
17. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol*. 2013;30(12):2725-2729. PUBMED | CROSSREF
18. Creevy KE, Evans JB. Canine distemper [Internet]. Merck & Co., Inc. Published [2022]. Updated [2025]. Accessed [Feb 10, 2025]. https://www.msdvetmanual.com/infectious-diseases/canine-distemper/canine-distemper
19. Appel MJG, Yates RA, Foley GL, Bernstein JJ, Santinelli S, Spelman LH, et al. Canine distemper epizootic in lions, tigers, and leopards in North America. *J Vet Diagn Invest*. 1994;6(3):277-288. PUBMED | CROSSREF

20. Beineke A, Puff C, Seehusen F, Baumgärtner W. Pathogenesis and immunopathology of systemic and nervous canine distemper. *Vet Immunol Immunopathol*. 2009;127(1-2):1-18. PUBMED | CROSSREF
21. Lempp C, Spitzbarth I, Puff C, Cana A, Kegler K, Techangamsuwan S, et al. New aspects of the pathogenesis of canine distemper leukoencephalitis. *Viruses*. 2014;6(7):2571-2601. PUBMED | CROSSREF
22. Summers BA, Greisen HA, Appel MJG. Canine distemper encephalomyelitis: variation with virus strain. *J Comp Pathol*. 1984;94(1):65-75. PUBMED | CROSSREF
23. Ducatelle R, Coussement W, Hoorens J. Demonstration of canine distemper viral antigen in paraffin sections, using an unlabeled antibody-enzyme method. *Am J Vet Res*. 1980;41(11):1860-1862. PUBMED | CROSSREF
24. Miry C, Ducatelle R, Thoonen H, Hoorens J. Immunoperoxidase study of canine distemper virus pneumonia. *Res Vet Sci*. 1983;34(2):145-148. PUBMED | CROSSREF
25. Haines DM, Martin KM, Chelack BJ, Sargent RA, Outerbridge CA, Clark EG. Immunohistochemical detection of canine distemper virus in haired skin, nasal mucosa, and footpad epithelium: a method for antemortem diagnosis of infection. *J Vet Diagn Invest*. 1999;11(5):396-399. PUBMED | CROSSREF
26. Romanutti C, Keller L, La Torre J, Panzera Y, Fuques E, Pérez R, et al. Virus isolation and full-length genome sequencing of a representative canine distemper virus wild type strain of the South America 2 clade. *J Virol Methods*. 2020;279:113857. PUBMED | CROSSREF
27. Tan B, Wen YJ, Wang FX, Zhang SQ, Wang XD, Hu JX, et al. Pathogenesis and phylogenetic analyses of canine distemper virus strain ZJ7 isolate from domestic dogs in China. *Virol J*. 2011;8(1):520. PUBMED | CROSSREF
28. Cherpillod P, Beck K, Zurbriggen A, Wittek R. Sequence analysis and expression of the attachment and fusion proteins of canine distemper virus wild-type strain A75/17. *J Virol*. 1999;73(3):2263-2269. PUBMED | CROSSREF
29. Piewbang C, Chansaenroj J, Kongmakee P, Banlunara W, Poovorawan Y, Techangamsuwan S. Genetic adaptations, biases, and evolutionary analysis of canine distemper virus Asia-4 lineage in a fatal outbreak of wild-caught civets in Thailand. *Viruses*. 2020;12(4):361. PUBMED | CROSSREF
30. Villanueva RA, Rouillé Y, Dubuisson J. Interactions between virus proteins and host cell membranes during the viral life cycle. *Int Rev Cytol*. 2005;245:171-244. PUBMED | CROSSREF
31. Sarute N, Calderón MG, Pérez R, La Torre J, Hernández M, Francia L, et al. The fusion protein signal-peptide-coding region of canine distemper virus: a useful tool for phylogenetic reconstruction and lineage identification. *PLoS One*. 2013;8(5):e63595. PUBMED | CROSSREF
32. Vigerust DJ, Shepherd VL. Virus glycosylation: role in virulence and immune interactions. *Trends Microbiol*. 2007;15(5):211-218. PUBMED | CROSSREF
33. Sawatsky B, von Messling V. Canine distemper viruses expressing a hemagglutinin without N-glycans lose virulence but retain immunosuppression. *J Virol*. 2010;84(6):2753-2761. PUBMED | CROSSREF
34. Karki M, Rajak KK, Singh RP. Canine morbillivirus (CDV): a review on current status, emergence and the diagnostics. *Virusdisease*. 2022;33(3):309-321. PUBMED | CROSSREF
35. Nikolin VM, Olarte-Castillo XA, Osterrieder N, Hofer H, Dubovi E, Mazzoni CJ, et al. Canine distemper virus in the Serengeti ecosystem: molecular adaptation to different carnivore species. *Mol Ecol*. 2017;26(7):2111-2130. PUBMED | CROSSREF
36. Murugesan A, Manoharan M. Dengue virus. In: Ennaji MM, ed. *Emerging and Reemerging Viral Pathogens*. Academic Press; 2020: p281-359.