

# ความชุกและความแปรผันทางพันธุกรรมของเชื้อไวรัสพาร์โวในแมวที่พบในภาคกลางและภาคตะวันออกของประเทศไทย

## Prevalence and genetic variation of Feline Parvovirus (FPV) infection in the central and eastern regions of Thailand

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Received: 21 April 2025 ; Revised: 19 June 2025 ; Accepted: 14 July 2025

### บทคัดย่อ

เชื้อไวรัสพาร์โวในแมว หรือ Feline Parvovirus (FPV) เป็นเชื้อไวรัสที่ทำให้เกิดอาการท้องเสียรุนแรงและทำให้เกิดโรคลำไส้อักเสบในแมว ซึ่งมีอัตราการเสียชีวิตค่อนข้างสูง โดยเฉพาะในแมวเด็กที่ไม่ได้ทำวัคซีน อีกทั้งยังมีรายงานพบว่าแมวมีความไวต่อการติดเชื้อได้ทั้ง FPV และ Canine Parvovirus type2 (CPV-2) การศึกษานี้มีวัตถุประสงค์ในการหาความชุกของโรคลำไส้อักเสบในแมวจากเชื้อไวรัสพาร์โวหรือโรคลำไส้อักเสบที่พบในภาคกลางและภาคตะวันออกของประเทศไทย โดยทำการเก็บตัวอย่างตั้งแต่เดือน พฤศจิกายน 2565 ไปจนถึง เดือนเมษายน 2567 รวมระยะเวลาทั้งสิ้น 1 ปี 6 เดือน และวิเคราะห์ความแปรผันทางพันธุกรรมของเชื้อไวรัสพาร์โวในแมวร่วมกับ ผลที่ได้พบความชุกของโรคลำไส้อักเสบในแมวจากเชื้อไวรัสพาร์โวหรือโรคลำไส้อักเสบเท่ากับร้อยละ 14.29 และมีอัตราความชุกของโรคสูงในเดือน พฤศจิกายน ธันวาคม มกราคม และ กุมภาพันธ์ โดยอยู่ในช่วงของฤดูหนาวของทุกปี ส่วนฤดูฝนพบการเกิดโรคได้บ้าง และฤดูร้อนพบอัตราการเกิดโรคต่ำที่สุด ซึ่งยังคงสอดคล้องกับการติดเชื้อตามธรรมชาติของไวรัสและการแพร่กระจายของโรคในอดีตที่ผ่านมา การหาความสัมพันธ์ความชุกของการเกิดโรคลำไส้อักเสบในแมวจากเชื้อไวรัสพาร์โวในพื้นที่ภาคกลางและภาคตะวันออกของประเทศไทย ด้วยวิธี Spearman rank correlation บ่งชี้ว่า อาการป่วยในระบบทางเดินอาหารของแมวและการติดเชื้อลำไส้อักเสบในแมวจากไวรัสพาร์โว หรือ โรคลำไส้อักเสบ มีความสัมพันธ์กันอย่างมาก โดยค่าสัมประสิทธิ์สหสัมพันธ์ = 0.84 ที่ระดับนัยสำคัญ 0.05 นอกจากนี้ผลการเปรียบเทียบวิธีการตรวจวินิจฉัยโรคลำไส้อักเสบในแมวจากเชื้อไวรัสพาร์โวหรือโรคลำไส้อักเสบระหว่างชุดทดสอบไวรัสพาร์โวแบบรวดเร็วทางการค้า (Immunochromatography; IC Test) และการตรวจสอบยีน VP2-FPV โดยปฏิกิริยาลูกโซ่พอลิเมอร์ (PCR) พบค่าความไวของการตรวจคัดกรอง (Sensitivity) เท่ากับ ร้อยละ 62.96 (17/27) และ ค่าพยากรณ์ความถูกต้องของผลบวก (Positive predictive value; PPV) คือ สัดส่วนผลการตรวจที่ได้ผลเป็นโรคจริงจากจำนวนทั้งหมดที่ผลตรวจบ่งชี้ว่าเป็นโรค) เท่ากับ ร้อยละ 65.38 (17/26) ดังนั้นการใช้ชุดทดสอบแบบรวดเร็วทางการค้าจะให้ผลบวกอยู่ที่ร้อยละ 62.96% และความถูกต้องของผลบวกของโรคนั้นอยู่ร้อยละ 65.38% เมื่อเทียบกับวิธีการตรวจแบบมาตรฐาน (Gold Standard) หรือ PCR ผลการวิเคราะห์ลำดับนิวคลีโอไทด์บางส่วนของยีน VP2 ทั้ง FPV (236 bp) และ CPV-2 (529 bp) พบว่าแมวที่ติดเชื้อไวรัสพาร์โวหรือโรคลำไส้อักเสบทั้งหมดมีสารพันธุกรรมเฉพาะ FPV เท่านั้น แสดงให้เห็นว่ายังไม่มีความผันแปรของพันธุกรรมของไวรัสในการติดเชื้อข้ามสายพันธุ์ในประชากรแมวที่อาศัยในพื้นที่ภาคกลางและภาคตะวันออกของประเทศไทย ในขณะนี้

**คำสำคัญ:** เชื้อพาร์โวไวรัสในแมว, โรคลำไส้อักเสบ, เชื้อพาร์โวไวรัสในสุนัข, ยีน VP2, ความแปรผันทางพันธุกรรม

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## Abstract

Feline Parvovirus (FPV) causes severe bloody diarrhea and feline enteritis, with a relatively high mortality rate, especially in unvaccinated kittens. Additionally, previous studies have indicated that cats are susceptible to infection by both FPV and Canine Parvovirus Type 2 (CPV-2). This study aimed to determine the prevalence of FPV, also known as feline distemper or feline panleukopenia, in the central and eastern regions of Thailand. Cat sample collection was conducted from November 2022 to April 2024, spanning a total period of 1.5 years, to investigate the prevalence of the disease and analyze the genetic variation of FPV. The findings revealed that the prevalence of FPV infection was 14.29%. The highest prevalence was observed in November, December, January, and February, which correspond to the winter season. In contrast, some cases were detected during the rainy season, while the lowest prevalence was recorded during the summer. These findings align with the natural infection patterns and past transmission trends of the virus. The correlation analysis using Spearman's rank correlation method indicated a strong relationship between gastrointestinal illness in cats and feline enteritis caused by FPV. The correlation coefficient was 0.84 at a significance level of  $p \leq 0.05$ . In addition, a comparative evaluation of diagnostic methods for FPV was conducted between a commercial rapid immunochromatographic test (IC Test) and polymerase chain reaction (PCR) detection of the VP2 gene of FPV/CPV-2. The sensitivity of the rapid IC test was 62.96% (17/27), while the positive predictive value (PPV), which is the proportion of true positive cases among all positive test results, was 65.38% (17/26). This indicated that the commercial rapid IC test detected 62.96% of positive cases, with a 65.38% accuracy rate compared to the gold standard PCR method. Moreover, the nucleotide sequence analysis of the VP2 gene in both FPV (236 bp) and CPV-2 (529 bp) revealed that all cases contained only FPV-specific genetic material. This suggested that at present, no genetic variation leading to cross-species transmission has occurred among the feline population in the central and eastern regions of Thailand.

**Keyword:** Feline Parvovirus, Feline distemper, Canine Parvovirus type 2, VP2 gene, genetic variation

## Introduction

The Feline Parvovirus (FPV) can cause disease in felids, including both domestic and wild animals, within the Suborder *Feliformia* and Suborder *Caniformia*, such as raccoons and foxes. However, it does not cause disease in domestic dogs (Yang *et al.*, 2021). FPV belongs to the *Parvoviridae* family, *Parvovirinae* subfamily, and *Protoparvovirus* genus, classified under the *Carnivore protoparvovirus-1* species along with *Canine parvovirus-2* (CPV-2), *Mink enteritis virus* (MEV), and *Raccoon parvovirus* (RPV) (Cotmore *et al.*, 2019). Feline Parvovirus (FPV) is a small, non-enveloped virus with a spherical capsid. Its genetic material consists of linear, single-stranded DNA approximately 5.1 kb in length. The genome contains two open reading frames (ORFs): *ORF-1* encodes two non-structural proteins, NS1 and NS2, while *ORF-2* encodes two structural proteins, VP1 and VP2 (Christensen & Tattersall, 2002). VP2 is the major component of the viral capsid, accounting for 90% of the

viral particle. It plays a crucial role in determining the virus's antigenic properties and inducing the production of neutralizing antibodies. Additionally, VP2 is associated with the genetic diversity of the virus and is responsible for defining the host range and receptor binding properties of FPV (Steinel *et al.*, 2000). Feline Parvovirus (FPV), also known as Feline distemper or Feline Panleukopenia, is a viral disease that induced severe enteritis. It is commonly found in young cats and primarily affects the digestive system. This disease can occur in all breeds of domestic cats and other feline species (Horiuchi *et al.*, 1998). Additionally, it has been found in other animal families, such as skunks, ferrets, minks, and raccoons. In adult cats, the symptoms of Feline distemper or FPV are often mild or unnoticeable. However, in unvaccinated kittens, the symptoms are more pronounced. The most susceptible age range for infection is between 3 to 5 months. In some cases, sudden death may occur in kittens between 4 weeks and 1 year old. Common

symptoms of Feline parvovirus infection include high fever, lethargy, loss of appetite for 3–4 days, vomiting of yellowish fluid, severe dehydration, diarrhea (sometimes with fresh blood), and foul-smelling feces. Cats may develop oral ulcers, tremors, or seizures in severe cases. Furthermore, affected cats may have a lower-than-normal body temperature. The pregnant cats infected with the virus may experience miscarriage. Without timely treatment, the disease can be fatal (Battilani *et al.*, 2011; Barrs, 2019). Then, Feline Parvovirus is one of a member in the *Carnivore protoparvovirus 1* group. Studies indicate that approximately 95% of infected cats contract Feline parvovirus (FPV), while the remaining 5% may be infected with Canine Parvovirus (CPV), particularly the CPV-2a, CPV-2b, and CPV-2c variants (Ikeda *et al.*, 2000 and Inthong *et al.*, 2019). Interestingly, cases of co-infection with both FPV and CPV have been reported in the same cat. A notable case was documented by Battilani (2011) and colleagues, who reported a 3-month-old mixed-breed kitten in Italy infected with both FPV and CPV-2a. The kitten ultimately succumbed to severe clinical symptoms associated with Feline distemper. At present, FPV outbreaks can occur throughout the year, unlike in the past when the disease was predominantly prevalent only in winter (Kolappan, 2023). The aforementioned data indicate that information regarding FPV in Thailand remains quite limited, particularly in terms of molecular genetics, which provides insight into the genetic diversity of FPV.

## Objectives

This study aims to determine the prevalence of feline viral enteritis caused by Feline Parvovirus (FPV) or Feline distemper in cats found in the central and eastern regions of Thailand. The cat samples were collected from November 2022 to April 2024, covering a total period of 1 year and 6 months and determined FPV-infection with the different diagnostic method compared with a commercial rapid test kit for disease screening and detecting the VP2 gene of both FPV and CPV-2 by PCR. Finally, the genetic variation of Feline Parvovirus was performed by nucleotide sequencing to assess genetic diversity currently.

Genetic variations of FPV has influence to severity, cross-species transmission, and host specificity. Regular genetic surveillance of the virus will help tracking the evolution of FPV and CPV-2, providing valuable insights for improving prevention against FPV infection in cats. Moreover, this findings serve as a foundation for future research in related fields.

## Materials and Methods

### 1. Ethical statement of the study

This research has obtained approval in accordance with the ethical guidelines and regulations for the use of experimental animals in research at Rajamangala University of Technology Tawan-ok (IRD RMUTTO) and has been granted an animal research permit with the license number U1-00943-2558. The study strictly adheres to the ethical regulations experimental animals in research at Rajamangala University of Technology Tawan-ok, ensuring compliance with animal welfare principles. Sample collection was conducted with care and in a manner that ensured both the physical and psychological well-being of the animals. Additionally, cat owners were informed about the objective of the study, and their consent was voluntarily obtained before any sample collection was carried out.

### 2. Randomized cat samples

The research team employed purposive sampling, selecting samples based on the discretion and judgment of veterinarians to determine whether the subjects were suitable and representative for the study. Fecal samples were collected exclusively from cats exhibiting clinical symptoms of Feline parvoviral enteritis or FPV. The clinical symptoms considered included diarrhea, lethargy, and fever, with diarrhea being the predominant and essential symptom in all selected cats.

### 3. Collecting fecal swab samples from domestic/ stray cats

Collecting samples of cats showing gastrointestinal symptoms, particularly with prominent diarrhea, resembling FPV infections, in the cat population found in the central

and eastern regions of Thailand. This included the districts of Sriracha, Chonburi, Ayutthaya, Ang Thong, and Bangkok respectively. The cats have been treated at private pet clinics or animal hospitals. Fecal samples were collected from both pet and stray cats and tested for feline parvovirus or feline distemper infection to determine the prevalence of the disease. This study took place over a period of 1 year and a half, from November 2022 to April 2024. The 40 fecal swab samples of 280 cats suspected of FPV were chosen for analysis. The fecal swabs were stored at 4°C no more than 48 hours or at -20°C if storage exceeded 48 hours. Each fecal sample was homogenized in 1 mL of phosphate-buffered saline (PBS) at pH 7.4. They were then centrifuged at 8,000 g for 10 minutes at 4°C using a refrigerated centrifuge. The supernatant was collected and stored at -80°C for further analysis.

#### 4. Screening of Feline Parvovirus infection (FPV/CPV-2) with the commercial rapid test Kit

Collected fecal swab samples from each cat exhibiting symptoms of diarrhea suspected to be caused by FPV were screened. The commercial rapid antigen test kit for FPV, the Bionote® (CCV/CPV) test kit was used to differentiate FPV infection from other digestive diseases, such as Feline Coronavirus infection (CCV), severe bacterial infections of the gastrointestinal tract, intestinal parasitic infestations, or protozoa infection, which may present with similar symptoms as gastrointestinal disorders. However, some cats were not screened due to certain owner-related factors.

#### 5. Primer design

The primers used in this study consisted of two

pairs: FPV-F/R and CPV-2F/R. These primers were specifically designed for detecting FPV and CPV-2 using the conventional PCR method. They are highly specific to the VP2 gene of each viral strain. The details of the primers were shown in Table 1.

#### 6. DNA extraction

DNA extraction was performed on all collected test samples using the commercial GF-1 Viral Nucleic Acid Extraction Kit (Vivantis, Malaysia) following the manufacturer's instructions. The process began by adding 50 µL of Proteinase K to 200 µL of the sample, mixing thoroughly, and then adding 200 µL of Buffer VL along with 15 µL of DNA, making a total volume of 215 µL. The mixture was homogenized and incubated at 65°C for 10 minutes to facilitate sample lysis. Next, 280 µL of absolute ethanol was added, and the mixture was immediately shaken to prevent nucleic acid precipitation by ethanol. The sample was then transferred to a GF-1 column and centrifuged at 5,000 g for 1 minute. The column was subsequently washed with 500 µL of Wash Buffer 1 and centrifuged at 5,000 g for 1 minute. This was followed by two washes with 500 µL of Wash Buffer 2, with the first wash being centrifuged at 5,000 g for 1 minute and the second at 14,000 g for 3 minutes. After washing, the filter was removed from the column and transferred to a new 1.5 mL microcentrifuge tube. DNA elution was performed by adding 25 µL of Elution Buffer, incubating at room temperature for 2 minutes, and centrifuging at 5,000 g for 1 minute. This elution step was repeated with another 25 µL of Elution Buffer, followed by 2 minutes incubation at room temperature and then centrifugation at 5,000 g for 1 minute. The extracted DNA samples were stored at -20°C until further analysis.

**Table 1** The primers were used in this experiment

| Specific gene  | Primers   | Sequences (5' 3')        | Gene | PCR product (bp) | References           |
|----------------|-----------|--------------------------|------|------------------|----------------------|
| Specific FPV   | FPV-F     | CATACATGGCAAACAAATAGAGCA | VP2  | 236              | (Zhang et al., 2019) |
|                | FPV-R     | TGTTTTAAATGGCCCTTGTGTAGA |      |                  |                      |
| Specific CPV-2 | CPV-2 (F) | AGCAGATGGTGATCCAAGAT     | VP2  | 529              | (Torre et al., 2018) |
|                | CPV-2 (R) | TGGATTCCAAGTATGAGAGG     |      |                  |                      |

## 7. Detection of Feline Parvovirus (FPV) / Canine Parvovirus-2 (CPV-2) Infection by Polymerase Chain Reaction (PCR)

The detection of FPV from fecal swab samples was performed by PCR with specific primers of the VP2 gene (Table 1). The PCR product was 236 bp in size of FPV and which in 529 bp of CPV-2 respectively. The thermal cycling conditions for DNA amplification were as following: initial denaturation at 94°C for 2 minutes, followed by 34 PCR cycles consisting of denaturation at 94°C for 30 seconds, annealing at 57.7°C for 40 seconds, and extension at 72°C for 40 seconds. A final extension was performed at 72°C for 7 minutes. The PCR products were analyzed using gel electrophoresis on a 1.2% (w/v) agarose gel in Tris-acetate EDTA (1x TAE) buffer at a constant voltage of 50 V for 50 minutes. The gel was then stained with SYBR® Green DNA dye and visualized using a Dark Reader blue light transilluminator (Major Science, Taiwan).

## 8. DNA purification

The amplified FPV- VP2 gene obtained from each sample was subjected to DNA purification using the GenepHlow™ Gel/PCR Kit (Geneaid, Taiwan), following the manufacturer's instructions. The DNA purification process consists of four mainly steps as following: the first step was sample preparation, the sample (PCR product) was placed into a 1.5 mL microcentrifuge tube and then added 5 volumes of PCR buffer to 1 volume of the sample (if the sample volume is X, add 5X PCR buffer) with mixed thoroughly. If the mixture turned in purple color, 10 µL of 3M Sodium Acetate (pH 5.0) was added and mixed well. The second was DNA Binding step: to place the DFH column into a 2 mL collection tube and added the preparing DNA sample to the DFH column, then centrifuged at 14,000 g for 45 seconds. After that, the flow-through was discarded and reassembled the DFH column into the collection tube. The third was washing step by adding 600 µL of washed buffer to the center of the DFH Column and leave at room temperature for 1

minute. The DFH Column was centrifuged at 14,000 g for 45 seconds and discarded the flow-through and reassembled the DFH Column into the collection tube, then the DFH Column was centrifuged repeat at 14,000 g for 3 seconds to dry the column. Finally, DNA Elution: the DFH Column was transferred into a 1.5 mL microcentrifuge tube. Then, 50 µL of elution buffer was added at 65°C into the center of the column (separate to each round of 25 µL) and left for 2 minutes until the elution buffer is fully absorbed by the column. Then, the column was centrifuged at 14,000 g for 2 minutes to elute the purified DNA. Afterwards, the DNA was sent to MacroGen Company in South Korea for DNA sequence analysis using Capillary Electrophoresis Sequencing (Sanger Sequencing) on an automated genetic analyzer.

## 9. DNA Sequencing

The DNA fragments of the FPV strain (VP2-FPV gene) were sent for sequencing of both the Forward and Reverse strands, as well as for analysis of the DNA sequencing. The nucleotide sequence similarity (% Nucleotide similarity) was analyzed by performing a Multiple Alignment comparison of the DNA samples with reference sequences of FPV and CPV-2 from the GenBank database, using the ClustalW method within the MEGALIGN program (DNASTAR).

## 10. Statistical analysis

All the data obtained from the study were subjected to statistical analysis. The Shapiro-Wilk method was used to test the distribution of numerical data, and the relationship between the prevalence of Feline parvovirus infection in the central and eastern regions of Thailand was analyzed using the Spearman's rank correlation. This analysis aimed to identify factors associated with or influencing the outbreak of the disease. Additionally, the study described the patterns of the disease spread in cats across different seasons within the cat populations of the central and eastern regions of Thailand. The statistical significance was set at  $p \leq 0.05$ .

## Results

### 1. The prevalence of Feline Parvovirus infection (FPV) or feline distemper, in cats during November 2022 to April 2024 (a total time of 1 year and a half).

Fecal sample of 280 cats exhibiting clinical signs of gastrointestinal disorders including diarrhea, lethargy, fever, and vomiting, with all cats showing prominent diarrhea; were collected from animal hospitals and private pet clinics in the central and eastern regions of Thailand over a period of 1 year and a half (from November 2022 to April 2024). Upon 40 cats were determined positive for FPV infection (Figure 1). This corresponds to a disease prevalence of 14.29% (prevalence = number of infected individuals in a population divided by the total population). The highest prevalence was observed during the winter months in November, December, January, and February. Some cases were detected during the rainy season, while the lowest incidence occurred during the summer. These findings are consistent with the natural infection patterns and historical transmission trends of the virus. The Spearman rank correlation method was used to assess the correlation between the prevalence of FPV and gastrointestinal diseases in cats in the central and eastern regions of Thailand. The results revealed a strong correlation between gastrointestinal diseases and FPV infection, with a correlation coefficient ( $r$ ) of 0.84 at a significance level of 0.05 (Data not shown).

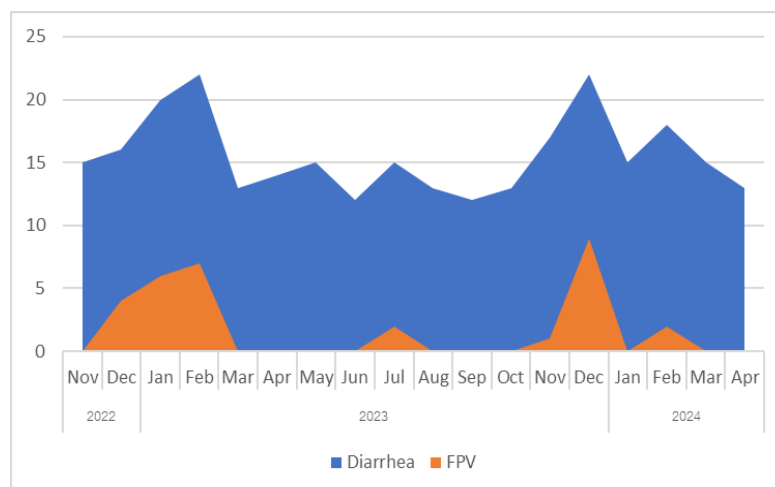
### 2. Confirmation of Feline Viral Enteritis caused by FPV/CPV-2 by Conventional PCR

A total of 40 fecal swab samples were determined the VP2 gene of FPV and CPV-2 using the conventional PCR. Specific primers targeting the VP2 gene of each virus strain were employed. The VP2 gene of FPV was a DNA fragment of 236 bp in size, while the VP2 gene of CPV-2 produces a 529 bp of PCR product size. The results showed that only VP2-FPV gene was detected.

However, VP2-CPV-2 gene was negative in all samples. Of the 40 total samples, 30 were positive for the VP2 gene of FPV, representing 75% (30/40). The distribution of positive samples by province is as follows: Chonburi: 100% positive (17/17), Ayutthaya: 50% positive (1/2), Bangkok: 100% positive (1/1) and Ang Thong: 55% positive (11/20) respectively. The VP2-FPV gene was performed on gel electrophoresis and PCR products of 236 bp was shown in Figure 2.

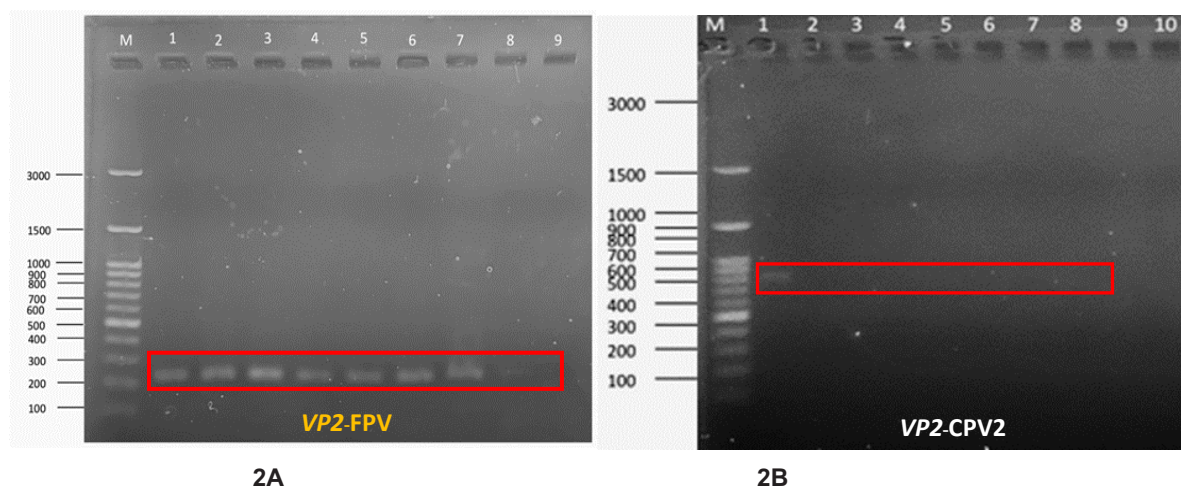
### 3. Comparative of diagnostic methods for Feline Parvovirus (FPV) infection between rapid commercial Immunochromatographic Test (IC Test) and VP2 gene detection by Polymerase Chain Reaction (PCR)

Total of 40 feline fecal swab samples were collected from animal hospitals and private veterinary clinics in the central and eastern regions of Thailand: thirty-six samples were identified for FPV infection using a commercial rapid immunochromatographic test kit, excluding 4 samples were not tested by IC. Of these, 26 samples were tested positive for FPV while 10 samples were tested negative. Then, all of 40 feline fecal swab samples were also examined for FPV and CPV2 infection using polymerase chain reaction (PCR) targeting each strain of VP2 gene. PCR results showed 30 positive and 10 negative samples of FVP infection (Table 2). However, all of them were negative of CPV-2 infection. Based on these findings, the sensitivity of the rapid screening test or IC test (defined as the proportion of true positives detected among all actual cases) was 62.96% (17/27). The positive predictive value (PPV), which is the proportion of true positive results among all positive test results, was 65.38% (17/26). Therefore, the commercial rapid test kit of FPV or IC test was 62.96% of sensitivity and 65.38% of PPV ratio when compared with the gold standard method such as PCR.



**Figure 1** A chart illustrating a comparison between the number of cats exhibiting gastrointestinal symptoms (Blue color) and the number of cats diagnosed with Feline parvovirus virus (FPV) infection (Orange color), recorded from November 2022 to April 2024, covering a total period of 1 year and a half. X bar represented each month of year which was collecting cat samples or the period of the experiment. Y bar represented the number of cat samples.

**Note:** The total number of cat samples (N) = 280, and the number of FPV-infected samples (N) = 40



**Figure 2** Gel electrophoresis showed FPV detection in cat samples using specific primers of the VP2 gene.

Figure 2A demonstrated the PCR product of VP2-FPV was 236 bp in size. The lanes were labeled as following:

Lane M represented the 3,000 bp DNA marker ladder, Lane 1 contained the Felocell CVR<sup>®</sup> vaccine, which served as positive control. Lanes 2 to 6 corresponded to samples C01–C05, respectively.

Lanes 7 and 8 represented sample C18, and Lane 9 was the negative control.

Figure 2B represented the PCR product of VP2-CPV2 was 529 bp. The lanes were labeled as following:

Lane M represented the 3,000 bp DNA marker ladder, Lane 1 contained the BayovacDHPPi/L<sup>®</sup> vaccine served as positive control. Lanes 2 to 6 corresponded to samples C01–C05, respectively. Lanes 7 and 8 represented sample C18, and Lane 9 was the negative control.

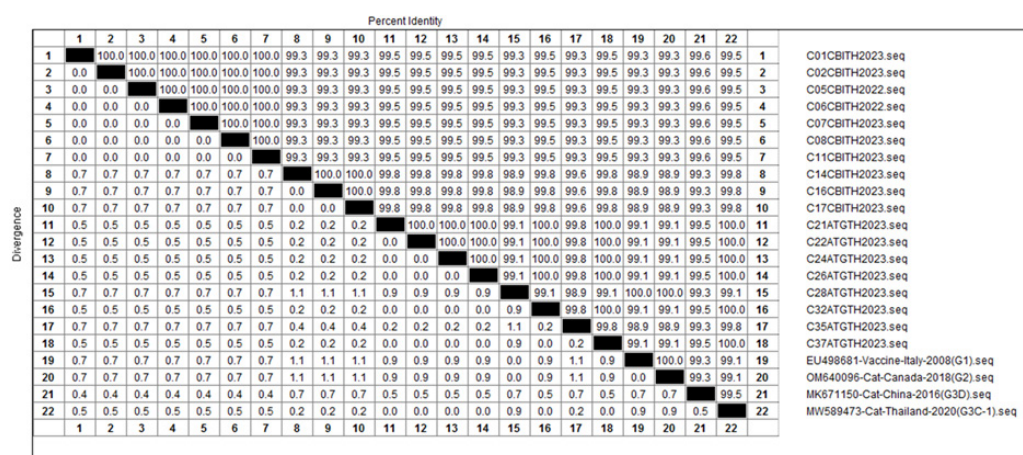
**Table 2** Comparison of diagnostic methods between a commercial rapid test kit (Immunochromatography or IC test) and Polymerase Chain Reaction (PCR) for FPV detection from 40 cats in Central and Eastern regions of Thailand.

| Province<br>/Diagnostic method | Positive<br>IC test | Negative IC<br>test | Total number of<br>sample | Positive<br>VP-2 /FPV<br>PCR | Negative VP-2<br>/FPV PCR | Total number of<br>sample |
|--------------------------------|---------------------|---------------------|---------------------------|------------------------------|---------------------------|---------------------------|
| Chonburi                       | 13                  | 3                   | 16                        | 17                           | 0                         | 17                        |
| Ayutthaya                      | 1                   | 1                   | 2                         | 1                            | 1                         | 2                         |
| Ang Thong                      | 11                  | 6                   | 17                        | 11                           | 9                         | 20                        |
| Bangkok                        | 1                   | 0                   | 1                         | 1                            | 0                         | 1                         |
| FPV-infection ratio            | 26/36               | 10/36               | 36                        | 30/40                        | 10/40                     | 40                        |

#### 4. DNA sequence analysis of the VP2 gene of Feline Parvovirus (FPV) obtained from cats infected-FPV in the central and eastern regions of Thailand, compared with reference VP2 gene of FPV/CPV-2 from GenBank database

A 236 bp of the VP2 gene of feline parvovirus (FPV), obtained from PCR-positive feline fecal samples, was amplified and purified for nucleotide sequencing. The resulting sequences were compared with reference VP2-FPV gene sequences available in the GenBank database using the MegAlign program (DNASTAR). The analysis revealed that the nucleotide sequences of all the VP2

gene fragments obtained from the samples were identical to those of four-reference FPV strains such as FPV/G1/Vaccine/ Italy/ 2008/EU498681, FPV/G3D/Cat/China/2021/MK 671150, FPV/G2/Cat/Canada/2018/OM64009 and FPV-G3C-1/Cat/Thailand/2020/MW589473). The result showed there was no nucleotide variation, nor any similarity to the VP2 gene of Canine Parvovirus (CPV). These findings suggested that feline parvovirus infections in the central and eastern regions of Thailand are still caused by the original FPV strain, or ancestral FPV viruses, rather than by variants such as CPV-2 (Figure 3).



**Figure 3** The percentage of nucleotide similarity (% nucleotide similarity) of the partial VP2 gene (236 bp) was conducted by comparison with four representative FPV strains retrieved from the GenBank database (FPV/G1/Vaccine/ Italy/ 2008/EU498681, FPV/G2/Cat/Canada/2018/OM64009, FPV/G3D/Cat/China/2021/MK 671150, FPV-G3C-1/Cat/Thailand/2020/MW589473) using the ClustalW Method within the MEGALIGN program (DNASTAR)

## Discussion

This study underscores the pivotal role of Feline Parvovirus (FPV) or Feline Panleukopenia as the primary etiological agent responsible for feline enteritis, particularly in the central and eastern regions of Thailand. The notably high FPV detection rate (75%) among the collected samples reflects the substantial prevalence of the virus within the feline population in these areas. These findings align with previous reports, which characterize FPV as a highly transmissible and globally widespread pathogen affecting domestic cats (Pacini *et al.*, 2023). Furthermore, the results confirmed that FPV continues to be the predominant parvovirus infecting domestic cats in the central and eastern parts of Thailand, with no detection of Canine Parvovirus type 2 (CPV-2) in the tested specimens. This observation contrasts with previous findings reported by Inthong *et al.* (2020), who identified FPV in canine samples collected from the Bangkok metropolitan area. Additionally, Wang *et al.* (2024) documented the co-circulation of FPV and CPV-2c as causative agents of FPV in cats from eastern Shandong Province, China, during 2021–2022. In another study, Wang *et al.* (2022) examined the prevalence of CPV-2 in 192 canine samples from southwestern China and found that 113 samples were CPV-2 positive. Among these, 28 samples (24.8%) were identified as FPV-like viruses. Collectively, these findings suggested regional variation in molecular epidemiology of FPV was recognized. Continuous molecular surveillance is therefore imperative to monitor viral genetic diversity and to detect potential emergent strains that may drive future outbreaks. In Thailand, the data of genetic diversity of FPV remain limited, hindering a comprehensive understanding of its epidemiology, including prevalence, geographic distribution, and potential outbreak sources. These data are essential for veterinarians, relevant authorities, and pet owners to develop and implement effective strategies for the prevention and control of FPV transmission in the country's feline population. In this study, fecal samples were collected from cats exhibiting clinical signs specifically indicative of disease. A total of 36 samples underwent initial screening using a rapid

immunochromatographic (IC) test prior to confirmation of FPV infection by conventional PCR. The results showed that 17 samples tested positive by both IC test and PCR, while 10 samples tested negative by IC test but were positive by PCR. When calculating the sensitivity of the IC test, the value was found to be 62.96%. From these results, it suggested that the FPV-suspected samples were collected from various veterinary clinics across different regions, without a predetermined sample size and by multiple sample collectors. The false-negative IC test results could be attributed to a low viral load in the collected samples or testing performed during the early stage of infection. However, when these samples were subjected to PCR after amplification of viral genetic material, positive results were obtained. Additionally, there were 9 samples that yielded false-positive results. It showed that testing of 9 samples were positive by IC test but negative by PCR. The positive predictive value (PPV) of the IC test was calculated to be 65.38%, indicating the probability that a positive IC test result truly reflected FPV infection when compared to PCR. This PPV suggests that some animals testing positive by IC test may not actually be infected (Ingsatit, 2024). The discrepancies in this study may be due to variations in sample collection across provinces, differences in personnel collecting the samples, and the use of different test kits in each area so these factors that may contribute to diagnostic inaccuracies.

## Conclusion

This study investigated the prevalence and genetic variation of Feline parvovirus (FPV) causing viral enteritis in cats in the central and eastern regions of Thailand. Screening for FPV infection was conducted using an immunochromatographic (IC) test, and the results were compared with those obtained from polymerase chain reaction (PCR), which served as the gold standard for diagnostic confirmation. The findings revealed that the prevalence of feline parvoviral enteritis or FPV followed a consistent seasonal trend in both regions, with the highest incidence observed during the winter months particularly in November, December, January, and February. December recorded the peak

occurrence of FPV outbreak. The IC test demonstrated a diagnostic accuracy of 65.38% when compared with PCR. Genetic analysis of the viral DNA extracted from all 30 PCR-positive fecal samples indicated that all sequences corresponded to FPV and exhibited 100% similarity with reference FPV sequences available in the GenBank database. No evidence of cross-species transmission involving canine parvovirus type 2 (CPV-2) from dogs to cats was detected. These results suggested that FPV strains circulating in the central and eastern regions of Thailand currently exhibit low genetic diversity. This information supports the continued efficacy of existing FPV vaccines. Furthermore, ongoing surveillance and genetic monitoring of FPV are essential for informing the development of effective vaccines tailored to emerging viral strains and optimizing strategies for the prevention of feline parvoviral enteritis in Thailand.

## Acknowledgement

This research was received the grant from IRD of Rajamangala University of Technology Tawan-ok, Chonburi, 20110, Thailand in 2024.

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