UNIVERSITI PUTRA MALAYSIA

MOLECULAR DETECTION OF FELINE PARVOVIRUS AND ITS RISK FACTORS AMONG PET CATS DISPLAYING GASTROINTESTINTAL CLINICAL SIGNS IN SELANGOR, MALAYSIA

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JUSTIN TAY HONG CHUAN

A project paper submitted to the Faculty of Veterinary Medicine, Universiti Putra Malaysia
In partial fulfilment of the requirement for the
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CERTIFICATION

It is hereby certified that I have read this project paper entitled "Molecular Detection of Feline Parvovirus and its Risk Factors among Pet Cat Displaying Gastrointestinal Clinical Signs in Selangor, Malaysia", by Justin Tay Hong Chuan, and in my opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Final Year Project.

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DEDICATION

This thesis is especially dedicated to:

My beloved parents (Jason Tay and Lee Su Ying)

My respected supervisor (Associate Professor Dr Farina Mustaffa Kamal)

My fellow DVM friends

ACKNOWLEDGMENT

I would like to thank Assoc Prof Dr Farina Mustaffa Kamal, Dr Syamira Syazuana Binti Zaini, Dr Juliana, Farris, and En. Adam who guided me throughout this project. Their patients, guidance and encouragement were crucial for the realisation of this final year project.

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LIST OF ABBREVIATIONS

UPM	Universiti Putra Malaysia
FYP	Final Year Project
FPV	Feline Parvovirus
PCR	Polymerase Chain Reaction
CPV	Canine Parvovirus
DNA	Deoxyribonucleic acid
FPL	Feline Panleukopenia
RBC	Red blood cells
WBC	White blood cells

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek Tahun Akhir.

Pengesanan Molekul Feline Parvovirus dan Faktor Risikonya di Kalangan Kucing Kesayangan yang Menunjukkan Tanda-Tanda Klinikal Gastrointestinal di Selangor

Oleh

Justin Tay Hong Chuan

2023

Penyelia: Prof. Madya Dr. Farina Mustaffa Kamal

Penyalia sampingan: Dr. Syamira Syazuana Bt Zaini

Parvovirus felin adalah virus DNA bebenang tunggal yang sangat mudah merebak dan menyebabkan panleukopenia felin, juga dikenali sebagai enteritis berjangkit kucing. Panleukopenia felin adalah penyakit serius yang berpotensi menyebabkan kematian dan kesan kepada kucing dari semua peringkat umur. Kajian ini dijalankan dengan tujuan untuk menentukan kadar pengesanan molekular parvovirus felin dan menilai faktor risiko yang menyumbang kepada penularan di kalangan kucing kesayangan di Selangor, Malaysia. Oleh kerana tidak ada kajian terdahulu yang baru dilakukan berkenaan parvovirus felin di Malaysia, hasil kajian ini berfungsi sebagai data asas untuk penyelidikan pada masa hadapan. Sejumlah 45 sampel najis dari kucing kesayangan yang menunjukkan petanda cirit-birit dan/atau muntah dikumpulkan dari klinik haiwan kesayangan di semua sembilan daerah dalam Selangor. DNA diekstrak dari setiap sampel tinja menggunakan kit pengekstrakan DNA dan kemudian dijalankan melalui reaksi rantai polimerase (PCR) dan elektroforesis gel agarosa. Gel yang diproses dilihat di bawah mesin pengimbas GeneSnap untuk keputusan. Analisis deskriptif digunakan untuk menentukan prevalensi molekul parvovirus felin. Hubungkait signifikan antara faktor risiko dan jangkitan feline parvovirus ditentukan menggunakan ujian Fisher's Exact (GraphPad Prism 9). Secara keseluruhan, 28 (62.2%) daripada 45 sampel diuji positif untuk FPV, di mana daerah dengan kadar prevalensi tertinggi adalah Petaling (83%), Gombak (80%), Sepang (80%) dan Hulu Langat (67%). Beberapa faktor risiko yang menunjukkan hubungan yang signifikan dengan status positif parvovirus felin termasuk umur ($P<0.0001$), pengurusan luar ($P=0.0017$), dan status vaksinasi. Dua status vaksinasi dengan hubungan yang signifikan adalah tidak divaksin dan tidak lengkap divaksin ($P<0.0001$). Kesimpulannya, terdapat kadar pengesanan molekular yang tinggi bagi parvovirus felin di Selangor. Kadar pengesanan molekular yang tinggi mengesyorkan tindakan pencegahan segera melalui rutinamalan vaksinasi untuk mengawal penyebaran virus ini. Virus ini menyebabkan kematian kerana tidak terdapat rawatan khusus untuk panleukopenia felin, hanya penjagaan sokongan sahaja.

Kata Kunci: Pengesanan molekular; Parvovirus felin; faktor risiko; PCR

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Final Year Project.

Molecular Detection of Feline Parvovirus and its Risk Factors among Pet Cats Displaying Gastrointestinal Clinical Signs in Selangor, Malaysia

by

Justin Tay Hong Chuan

2023

Supervisor: Associate Professor Dr. Farina Mustaffa Kamal

Co-Supervisor: Dr. Syamira Syazuana Bt Zaini

Feline parvovirus is a highly contagious, single-stranded DNA virus that causes feline panleukopenia, also known as feline infectious enteritis. Feline panleukopenia is a serious, potentially fatal disease that affects cats of all ages. This study was conducted with the intent to determine the molecular detection rate of feline parvovirus and assess the risk factors that contribute to its transmission among pet cats in Selangor, Malaysia. Since no previous recent studies were done regarding feline parvovirus in Malaysia, the result of this study serves as a baseline database for future research. A total of 45 faecal samples from pet cats exhibiting signs of diarrhoea and/or vomiting were collected from small animal clinics in all nine districts within Selangor. DNA was extracted from each faecal sample using a DNA extraction kit and then subjected to PCR and agarose gel electrophoresis. Processed gel was viewed under a GeneSnap viewer machine to determine the result. Descriptive analysis was used to determine the molecular prevalence of feline parvovirus. The significance of association between risk factors and feline parvovirus infection was determined using Fisher's Exact Test. Overall, 28 (62.2%) among 45 samples tested positive for FPV, where the districts with the highest prevalence rate were Petaling (83%), Gombak (80%), Sepang (80%) and Hulu Langat (67%). Several risk factors that indicate significant association with feline parvovirus positivity, include age (P<0.0001), outdoor management (P=0.0017) and vaccination status. The two vaccination statuses with significant association were unvaccinated and incompletely vaccinated (P<0.0001). In conclusion, there was high molecular detection rate of feline parvovirus in Selangor. The high molecular detection rate warrants immediate preventive action through routine core vaccination practice to curb the spread of this virus. This virus is fatal as there is currently no treatment for feline panleukopenia but only supportive care.

Keywords: Molecular detection; feline parvovirus;risk factors; PCR

Chapter 1.0

INTRODUCTION

1.1 Overview of FPV

Feline parvovirus, more commonly known as feline panleukopenia virus (FPV), stands as a pervasive and highly contagious viral disease that exerts its impact on domestic and wild cats across the globe. This virus falls within the *Parvoviridae* family, a group that encompasses various viral agents responsible for causing diseases in a wide array of animal species, including dogs, pigs, and even humans. It is characterized by its small size, non-enveloped, and single-stranded DNA genome. A remarkable feature of FPV is its tenacity in the environment, as it can remain viable for several months. Adding to its hardy nature, FPV exhibits resistance to many common disinfectants, thus facilitating its swift transmission through contaminated surfaces, faecal matter, urine, and other bodily secretions of infected cats (Sykes, 2014). Clinical signs and symptoms of FPV infection vary in intensity, influenced by factors such as the age, immune status, and overall health of the affected cat. Common clinical manifestations include vomiting, diarrhoea, fever, anorexia, lethargy, and dehydration. In more severe cases, afflicted cats may display neurological symptoms like ataxia, seizures, and tremors. Among all feline age groups, kittens under six months are particularly vulnerable to FPV's devastating effects (Sykes, 2014). Moreover, pregnant cats infected with FPV can transmit the virus to their offspring, leading to severe consequences such as foetal death, stillbirths, or the development of cerebellar hypoplasia, a condition causing profound neurological deficits in affected kittens (Sykes, 2014). Diagnosing FPV infection involves a multifaceted approach combining clinical observations, historical context, and laboratory examinations. PCR and enzyme-linked immunosorbent assay (ELISA) tests conducted on faecal, or blood samples provide the means to confirm the presence of the virus (Sykes, 2014). As for treatment, managing FPV infections primarily entails supportive care, including essential interventions like fluid therapy, electrolyte rebalancing, and nutritional supplementation. In cases where secondary bacterial infections pose a risk, veterinarians may prescribe antibiotics. Notably, antiviral drugs remain ineffective against FPV. Preventing FPV infection assumes critical importance, with vaccination representing the foremost strategy for safeguarding the feline population against this debilitating disease (Truyen, 2009). Many regions advocate for core vaccines, which typically include a combination of the rabies vaccine and a comprehensive FVRCP (Feline Viral Rhinotracheitis, Calicivirus, and Panleukopenia) vaccine shot (Department of Veterinary Services, 2014). This combined vaccine confers robust protection against a spectrum of feline diseases, including FPV, thus ensuring the overall health and well-being of our feline companions. Beyond vaccination, it is imperative to emphasize the significance of maintaining stringent hygiene practices in environments where cats reside or interact. Proper disinfection protocols can help mitigate the risk of FPV transmission. Quarantine measures should be implemented to isolate infected cats and prevent the spread of the virus to healthy individuals. Education and awareness campaigns among cat owners and veterinarians play a crucial role in early detection and management of FPV outbreaks.

1.2 Study Justification

This research is critical as previous studies on the molecular detection of feline parvovirus had not been done in Malaysia. Hence, this study will serve as a crucial baseline data for future studies and research on FPV in Malaysia. Other recent studies conducted in neighbouring countries have also shown above average serological and molecular detection of FPV, namely Thailand with a 58% serological detection rate (Kamonpan et al., 2019) and Vietnam with a 54% molecular detection rate (Nakamura et al., 2017). The data provided in this study can be used to inform and educate the public about this disease.

1.3 Hypothesis of the study

H_0: There is no molecular detecction of FPV among pet cats displaying gastrointestinal clinical signs within Selangor.

H_A: There is molecular detection of FPV among pet cats displaying gastrointestinal clinical signs within Selangor.

H_0: There is no association between risk factors and its contribution towards the transmission of FPV.

H_A: There is association between risk factors and its contribution towards the transmission of FPV.

1.4 Objectives of the study

The objectives of this study were:

1. To detect FPV among pet cats displaying gastrointestinal clinical signs within Selangor using conventional PCR method and agarose gel electrophoresis.

2. To assess the association of risk factors and their contribution to the transmission of FPV.

Chapter 2.0

LITERATURE REVIEW

2.1 Overview of Feline Parvovirus

Feline parvovirus (FPV) is a highly contagious, single-stranded DNA virus that causes feline panleukopenia (FPL), also known as feline infectious enteritis (FIE). FPL is a serious, potentially fatal disease that affects cats of all ages, but it is most common in kittens between three and five months old (Tasker & Sparkes, 2007). FPV is a single-stranded DNA virus that is closely related to canine parvovirus (Tasker & Sparkes, 2007). FPV is a member of the *Parvoviridae* family, which also includes canine parvovirus (CPV). FPV and CPV are closely related viruses, but they are not cross-protective. This means that vaccination against FPV does not protect cats from CPV infection, and *vice versa*. FPV is transmitted through contact with infected faeces, saliva, or vomit, or through contact with contaminated objects, such as food bowls, bedding, or toys. The virus can survive in the environment for several months, making it difficult to eradicate (Gaskell & Povey, 2013). FPV is a very stable virus that can survive in the environment for several months. It is resistant to many common disinfectants, such as soap and water, and can be transmitted through direct contact with infected cats, their faeces, or contaminated objects (American Association of Feline Practitioners, 2009). FPV is a genetically diverse virus, with multiple strains circulating worldwide. The genetic diversity of FPV is mainly due to point mutations and recombination events. FPV strains can be classified into two main groups: FPV type 1 (FPV-1) and FPV type 2

(FPV-2). FPV-1 is the most common type of FPV found in cats. FPV-2 is less common, and it is thought to be a recombinant virus between FPV-1 and CPV-2. In addition to the two main types, there are also several FPV variants that have been identified. These variants are typically classified based on their genetic similarity to known FPV strains (Decaro et al., 2011). Molecular epidemiological studies have shown that FPV strains are widely distributed around the world (Decaro et al., 2011). However, the prevalence of different FPV strains varies by region. For example, FPV-1 is the most common type of FPV found in North America and Europe, while FPV-2 is more common in Asia. Molecular epidemiological studies have also shown that FPV strains can evolve rapidly (Tasker & Sparkes, 2007). This is due to the high mutation rate of the FPV genome. The rapid evolution of FPV strains can make it difficult to develop effective vaccines and control strategies.

2.2 Pathogenesis and Clinical Manifestations of Feline Panleukopenia

Clinical signs of FPL typically develop 3-5 days after infection and can include lethargy, anorexia, vomiting, diarrhoea, fever, dehydration, abdominal pain, weight loss, pale gums, depression, and collapse (Stuetzer, Hartmann, & Truyen, 2014). In severe cases, FPL can be fatal. Death is usually caused by dehydration, sepsis, or shock (Tasker & Sparkes, 2007). When a cat is infected with FPV, the virus enters the body through the oral or nasal route. After infection, FPV replicates rapidly in rapidly dividing cells, such as those in the bone marrow, intestinal lining, and lymphoid tissue (Gaskell & Povey, 2013). Destruction of lymphoid tissue results in a decrease in white blood cells production and population, which makes cats more susceptible to infection. This destruction of white blood cells leads to the clinical signs of FPL, such as leukemoid, diarrhoea, and vomiting (Tasker & Sparkes, 2007). FPV can also damage the intestinal lining, leading to diarrhoea, vomiting, and dehydration (Stuetzer, Hartmann, & Truyen, 2014).

2.3 VP-2 Gene and Emergence of New FPV Variants

The VP-2 gene, encoding the major capsid protein of FPV, plays a crucial role in the virus transmission and pathogenesis (Akkutay-Yoldar et al., 2019). Located on the FPV genome, the VP-2 gene comprises approximately 1,700 nucleotides. The resulting VP-2 protein forms the icosahedral capsid shell, protecting the viral genome and facilitating viral entry into host cells. Additionally, the VP-2 protein is responsible for hemagglutination, the virus's ability to agglutinate red blood cells (Decaro et al., 2008). It contains specific binding sites enabling FPV to attach to and enter host cells, interacting with species-specific binding receptors like the transferrin receptor (TfR) present on susceptible cell surfaces. Upon binding to TfR, FPV undergoes internalization via endocytosis (Tang et al., 2022). Pan et al. (2013) notes that the genetic diversity of FPV is evident in its VP2 protein. VP2 sequences from different FPV strains can vary up to 10%, resulting in significant differences in virulence and immunogenicity. Genetic variations in the VP-2 gene can also impact the host range and antigenicity of FPV. Some FPV variants have been shown to infect other carnivores, such as raccoons and skunks (Greene et al., 2014). A study by Richards A. Squires in 2020 reveals that recombination between FPV and CPV has led to the emergence of CPV type 2, with three variants: CPV-2a, CPV-2b, and CPV-2c. All three variants can clinically infect both canines and felines, with CPV-2c causing asymptomatic infections in felines (Nakamura et al., 2021). Furthermore, mutations in the VP-2 gene can alter the virus's ability to evade the host immune system, potentially leading to vaccine escape (Decaro et al., 2015).

2.4 Methods of Detection of FPV

Molecular detection is the most sensitive and specific methods for diagnosing FPV infection. This method can be used to detect FPV DNA in a variety of samples, including faeces, blood, and tissues (Adieb Awad, Khalil, & Attallah, 2018). There are a few common molecular detection methods for FPV. First, PCR which is a technique that amplifies a specific region of DNA. FPV PCR assays are designed to amplify a region of the FPV genome that is unique to FPV. This means that FPV PCR assays can be used to distinguish FPV from other viruses, such as canine parvovirus (Gaskell & Povey, 2013). Second, real-time PCR (qPCR) which is a type of PCR that allows for the quantification of DNA in real time. This means that qPCR assays can be used to determine the amount of FPV DNA in a sample. qPCR assays are more sensitive than conventional PCR assays and can be used to detect FPV DNA in samples with low viral loads (Stuetzer, Hartmann, & Truyen, 2014). Third, loop-mediated isothermal amplification (LAMP) which is a technique that amplifies DNA without the need for thermal cycling. LAMP assays are simpler and faster to perform than PCR assays and can be used to detect FPV DNA in field settings (Decaro et al., 2011). Other molecular detection methods for FPV include reverse transcription PCR (RT-PCR) which is a technique that can be used to detect FPV RNA transcripts. FPV RT-PCR assays are useful for detecting FPV infection in early stages of disease when the virus is replicating rapidly. It also includes *in-situ* hybridization (ISH) which is a technique that can be used to detect FPV DNA or RNA in tissue samples. ISH assays are useful for identifying FPV-infected cells in tissues. Serological detection of FPV on faecal samples is the measurement of antibodies to FPV in faeces. Serological tests can be used to diagnose FPV infection and to determine whether a cat has been vaccinated against FPV. There are several challenges associated with the serological detection of FPV on faecal samples. First, the concentration of antibodies in faeces is typically lower than in blood. This means that serological tests for FPV on faecal samples need to be more sensitive than those for blood. Second, faeces can contain interfering substances, such as bacteria and other viruses, which can interfere with the serological test. It is important to pretreat faecal samples to remove these interfering substances before performing a serological test (Gaskell & Povey, 2013). There are several different methods that can be used for the serological detection of FPV on faecal samples (Decaro et al., 2011). The most common methods are ELISA (enzyme-linked immunosorbent assay) and HI (haemagglutination inhibition). ELISA is a technique that uses antibodies to detect antigens or antibodies in a sample. FPV ELISA tests can be used to detect FPV antibodies in faeces. HI is a technique that measures the ability of antibodies to inhibit the agglutination of red blood cells by FPV. HI tests can be used to detect FPV antibodies in faeces, but they are less sensitive than ELISA tests. Other methods that can be used for the serological detection of FPV on faecal samples include virus neutralisation test (VNT) which measures the ability of antibodies to neutralise FPV infectivity. VNT is the most sensitive serological test for FPV, but it is also the most time-consuming and expensive to perform. Indirect immunofluorescence assay (IFA) uses antibodies conjugated to fluorescent dyes to detect FPV antigens in cells. IFA can be used to detect FPV antigens in faecal samples, but it is less sensitive and specific than ELISA or HI. Molecular detection methods are more sensitive and specific than serological methods for detecting FPV in faecal samples. This is because molecular detection methods can detect FPV DNA directly, while serological methods can only detect antibodies to FPV (Tasker & Sparkes, 2007).

2.5 Prevalence of FPV

Feline parvovirus (FPV) is a common disease in Southeast Asia, with prevalence rates ranging from 11.1% to 58.73%. This is likely due to several factors, including the high density of cats in the region, the lack of widespread vaccination, and the poor sanitation conditions in some areas. FPV is widespread throughout the world. Several studies have been conducted to investigate the prevalence of FPV in Southeast Asian countries, with varying results. A study conducted in Thailand in 2020 found that the overall seroprevalence of FPV in cats was 58.73% (Kamonpan et al., 2019). A study conducted in Vietnam in 2017 by Nakamura et al. found that the molecular detection rate of FPV in cats was 54%. A study conducted in Indonesia in 2016 found that the prevalence of FPV in cats was 17.5% (Supriyadi et al., 2016). These studies suggest that the seroprevalence of FPV in Southeast Asia is relatively high. However, it is important to note that the prevalence rates reported in these studies may vary depending on the study population, the sampling methods used, and the diagnostic tests used. Despite the variation in prevalence rates, it is clear that FPV is a common disease in Southeast Asia. This is likely due to several factors, including the high density of cats in the region, the lack of widespread vaccination, and the poor sanitation conditions in some areas.

2.6 Prevention and Treatment of FPL (Feline Panleukopenia)

Vaccination is the most effective way to prevent FPV infection and FPL. All cats should be vaccinated against FPV, regardless of their age or lifestyle. There are two types of FPV vaccines available, inactivated and modified live. Inactivated vaccines contain killed FPV particles and are safer than modified live vaccines, but they may not be as effective. Modified live vaccines contain a weakened form of FPV and are more effective than inactivated vaccines, but they may cause mild side effects in some cats (Gaskell & Povey, 2013; Tasker & Sparkes, 2007). Kittens should receive their first FPV vaccine at 6-8 weeks of age, followed by a second booster vaccine 2-4 weeks later. Kittens should receive a third booster vaccine at 16-20 weeks of age. Adult cats should be vaccinated against FPV every 1-3 years (World Small Animal Veterinary Association, 2007). In addition to vaccination, there are several other things that can be done to help prevent FPV infection, including keeping cats indoors, avoiding contact with unvaccinated cats, disinfecting areas where unvaccinated cats have been and washing hands thoroughly after handling unvaccinated cats. The treatment of FPV involves a comprehensive approach aimed at managing the clinical manifestations and supporting the affected feline patients. The primary focus is on addressing the severe gastrointestinal and hematopoietic manifestations associated with FPV infection. Supportive care is crucial, encompassing fluid therapy to correct dehydration and electrolyte imbalances, as FPV often induces severe vomiting and diarrhoea, leading to fluid loss. Additionally, anti-emetics may be administered to alleviate nausea and vomiting. Broad-spectrum antibiotics are often prescribed to prevent secondary bacterial infections resulting from the immunosuppressive effects of FPV. Monitoring and managing the feline patient's nutritional status are essential components of the treatment plan, with nutritional support provided through enteral feeding or parenteral nutrition if necessary. Antiviral medications, such as feline interferon omega, have been explored for their potential benefits in managing FPV infections. However, further research is needed to establish their efficacy conclusively. Veterinary practitioners play a pivotal role in tailoring treatment protocols based on the individual patient's clinical condition, thus optimizing outcomes in the face of this challenging viral infection (Smith et al., 2021; Truyen 2009).

Chapter 3.0

MATERIALS AND METHODS

3.1 Animal and Study Design

This study has acquired the necessary approval from Universiti Putra Malaysia's Institutional Animal Care and Use Committee (IACUC) with the approval number of U030/2023. The study was conducted on all nine districts around Selangor, namely Gombak, Hulu Selangor, Hulu Langat, Petaling, Kuala Langat, Sabak Bernam, Kuala Selangor, Sepang and Klang where a total of 54 samples from cats exhibiting signs of diarrhoea and or vomiting. The sampling method used for faecal samples were convenient sampling whereas stratified sampling method was used to select the participating small animal clinics. Animed Veterinary Medical Center was chosen for Hulu Langat district, Klinik Haiwan Hijra was chosen for Kuala Selangor, St. Angel's Veterinary Hospital was chosen for Petaling, two franchises of Taqwa Veterinary Clinic was chosen for Klang and Kuala Langat, Klinik Haiwan Nurmi was chosen for Gombak, Merci Veterinary Clinic was chosen for Sepang, Batang Kali Veterinary Clinic was chosen for Hulu Selangor and Sabak Bernam Veterinary Petcare was chosen for Sabak Bernam. Faecal samples are the preferred specimen for feline parvovirus (FPV) detection because the virus was shed in high concentrations in faces from infected cats (Jacobson et al., 2021). Faecal samples from cats exhibiting signs of vomiting and/or diarrhoea were collected as these are the hallmark signs of Feline Panleukopenia (FPL) caused by Feline Parvovirus (FPV) (Tasker & Sparkes, 2007). Instruction for sample collection were sent to the participating clinics to facilitate proper sample collection and prevent contamination. An online questionnaire was sent along with the instructions to participating clinics to allow further assessment of the risk factors (Appendix 1). The questions were divided into three sections namely patient information, patient clinical history and vaccination history. The first section includes breed, gender, household management, and behavioural management of the cats. The second section includes primary clinical signs, presence of concurrent disease, duration of clinical signs, previous exposure to FPV, and if the cats were tested for FPV using test kits of the clinic's preference. The third section includes vaccination status of the cats. Faecal samples collected from each clinic were only stored for a maximum of 3 days at between 1 °C to 10 °C before collection to maintain molecular integrity (Jacobson et al., 2021).

3.2 Sample Collection

A total of ten grams of faecal sample from each cat is collected using latex gloves. The samples were collected after excretion, from the bedding provided by participating clinics. A new latex glove is required every time during sampling to prevent cross contamination. Each clinic was instructed to collect ten grams of sample from six different cats for a total of 60 g of faecal sample per clinic. The faecal samples were stored in urine cups and put in the freezer or chiller; whichever facility was available at the clinic. The urine cups were labelled with the case number of the patient to ease history collection or clarification if necessary. The faecal samples were then collected and transported to the Virology Lab in the Faculty of Veterinary Medicine to be stored in the -30 °C freezer.

3.3 DNA Extraction

The DNA extraction of the faecal samples was performed using the QIAGEN DNeasy® Blood & Tissue Kit (Qiagen, Malaysia). The manufacturer's instructions were followed during the DNA extraction process. Stored faecal samples were retrieved from the freezer and left to thaw. One gram of faeces was taken and weighed, then added to three millilitres of phosphate -buffered saline (PBS) in a conical collection tube. The mixture was vortexed and centrifuged at 3000 revolutions per minute (rpm) for 10 minutes. Two hundred microliters of sample supernatant from the conical collection tube were resuspended into a two ml microcentrifuge tube. A total of 20 microliters of proteinase K and 200 microliters of Buffer AL were added to the two-millilitre microcentrifuge tube and mixed thoroughly via vortex. The sample was then incubated at 56 °C for 10 minutes using a water bath. Two hundred microliters of ethanol were added to the sample and mixed using a vortex. The mixture in the microcentrifuge tube was pipetted into a DNeasy spin column placed in a two ml collection tube. The spin column was centrifuged at 8000 rpm for one minute, and the flow-through was discarded. A total of 500 microliters of Buffer AW1 were pipetted into the spin column, then centrifuged at 8000 rpm for one minute, and the flow-through was discarded. Then, 500 microliters of Buffer AW2 were pipetted into the spin column, centrifuged at 14,000 rpm for three minutes, and the flow-through was discarded. The spin column was transferred to a new two ml microcentrifuge tube. The extracted DNA was then eluted by adding 200 μl of Buffer AE to the centre of the spin column membrane. The sample was incubated at room temperature for one minute, then centrifuged at 8000 revolutions per minute for one minute. The spin column was discarded, and the extracted DNA was stored in a -30 °C freezer.

3.4 PCR and Agarose Gel Electrophoresis

A PCR master mix was prepared according to the manufacturer's instructions. For each sample, 12.5 μl of MyTAQ Redmix, 0.5 μl of 10 μM forward primer (5' – ACAAGATAAAAGACGTGGTGTAACTCAA-3') (Decaro et al., 2015), 0.5 μl of 10 μM reverse primer (5' – CAACCTCAGCTGGTCTCATAATAGT-3') (Decaro et al., 2015), and 7.5 μl of ultra-pure water were added and mixed in a microcentrifuge tube. The ratio of the master mix was adjusted to the number of samples that were available for processing at the time. Then, 21 μl of the PCR mix were transferred to each PCR tube. Four microlitres of sample DNA were added to the PCR tube. The PCR tubes were then inserted into the BioRad PCR thermocycler (Bio-Rad, USA). The protocol used consisted of initial activation at 95 °C for 10 minutes for one cycle, followed by denaturation at 95 °C for 30 seconds for 45 cycles, primer-annealing at 52 °C for 30 seconds for 1 cycle, and spinal extension at 60 °C for one minute for one cycle. A 2% agarose gel was prepared using 0.4 g of agarose gel powder and 20 millilitres of Tris-acetate-EDTA (TAE) solution.

The mixture was heated in a microwave for 1 minute. A total of 1.1 μl of RedSafe® solution were added to visualize the DNA bands after electrophoresis. Agarose gel solution was poured into a cast and allowed to cool. The agarose gel was then placed in an electrophoresis tank filled with TAE bath solution. DNA ladder was added to the first well. The positive control used was a feline parvovirus vaccine was loaded into the second well, and the negative control which was ultrapure water was into the last well to prevent contamination. Samples were added to the agarose gel starting from the third well. Electrophoresis was run at 120 volts and 350 milliamperes for 25 min. The gel was removed from the tank and placed into the Snap Gene viewer machine (Appendix 3, Appendix 4, Appendix 5). Snap Gene software (Dotmatics, USA) Transilluminator was used to visualize the DNA bands to determine the results of the DNA samples. The final PCR product was 82 base pairs.

3.5 Statistical Analysis

All data collected were presented as positive or negative. The first objective of this study was to determine if there was molecular detection of feline parvovirus among the faecal samples collected. Hence, descriptive analyses were used to describe the data. The second objective of the study was to determine if there were significant association between six risk factors and its contribution towards the transmission of FPV. Fisher's exact test would be used to calculate the significance of each category of risk factor. Statistical analysis was done on GraphPad Prism 9 software (Dotmatics, USA)

(Appendix 6). The confidence interval used for Fisher's exact test was 95% and P-value < 0.05 was considered statistically significant.

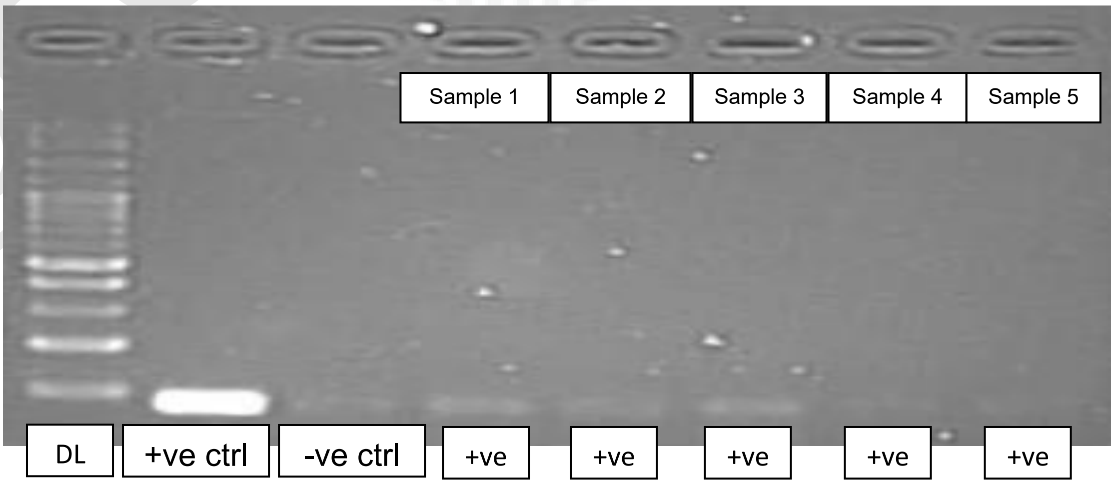
Chapter 4.0

RESULTS

4.1 PCR and Agarose Gel Electrophoresis Results

Agarose gels that underwent electrophoresis were read using GeneSnap Transilluminator (GeneSnap, USA). The fixated functional samples were loaded in the order of first well containing DNA Ladder (DL), second well containing positive control (+ve ctrl), third well containing negative control (-ve ctrl). The results were interpreted with presence of white band in sample wells indicating positive detection of FPV molecules, absence of white band indicating negative detection.

Figure 1: Agarose gel processed on 5th September 2023. Results were viewed under GeneSnap Transilluminator. Samples processed were (from third column, left to right) P1, P2, HL1, HL2 and SB3. This agarose gel showed that five out of five samples were positive.



4.2 Molecular Detection Rate of FPV from Faecal Samples Collected

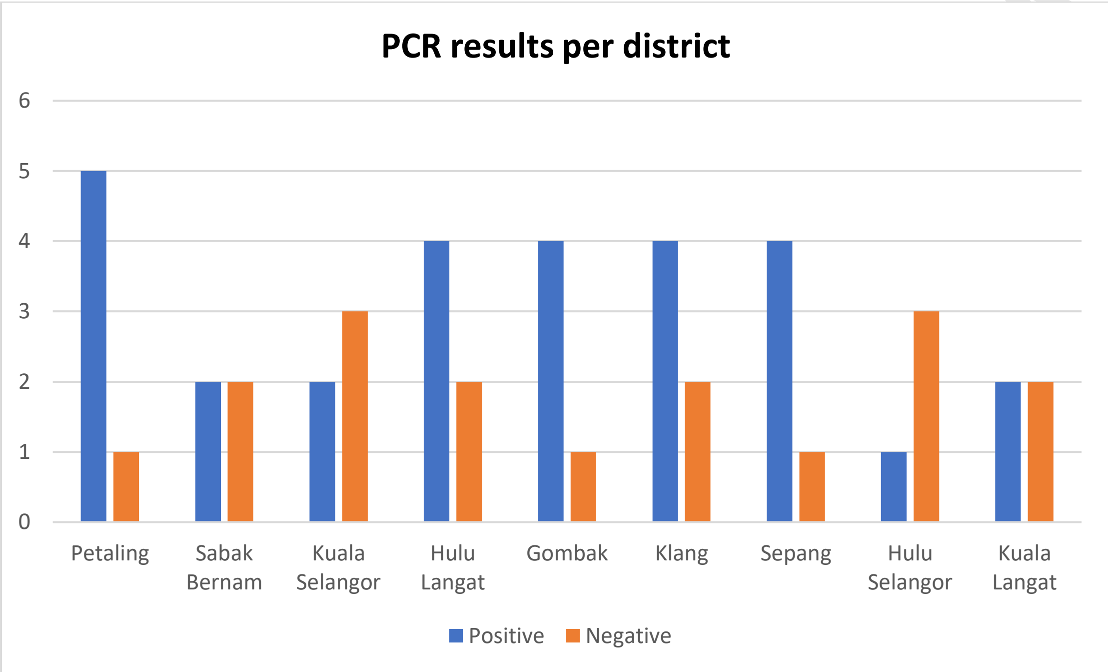
The results of PCR amplification of VP-2 gene found in blood samples from patients as in Table 1 which were eliciting signs of diarrhoea and/or vomiting, arranged from the highest to lowest detection rate were Petaling (83.33%), Gombak (80.00%), Sepang (80.00%), Hulu Langat, (66.67%), Klang (66.67%), Sabak Bernam (50.00%), Kuala Langat (50.00%), Kuala Selangor (40.00%) and Hulu Selangor (25.00%).

Table 1 PCR results of faecal samples from each district

District	Positive (n)	Negative (n)	Detection rate (%)
Petaling	5	1	83.33
Sabak Bernam	2	2	50.00
Kuala Selangor	2	3	40.00
Hulu Langat	4	2	66.67
Gombak	4	1	80.00
Klang	4	2	66.67
Sepang	4	1	80.00
Hulu Selangor	1	3	25.00
Kuala Langat	2	2	50.00

The bar graph below in Figure 2 shows that Petaling, Hulu Langat, Gombak, Klang, and Sepang had more positive samples compared to negative samples. Sabak Bernam and Kuala Langat had equal positive and negative samples. Kuala Selangor and Hulu Selangor had more negative samples compared to positive samples.

Figure 2: PCR results of faecal samples from each district



4.3 Risk Factor Association with Transmission of FPV

Faecal samples collected from each small animal clinic were categorized based on the respective risk factors experienced by the cats as tabulated in Table 2. Age proven to be a significant risk factor with a P-value of less than 0.0001 and a relative risk of 2.87 for cats that were less than six months of age. Cats that were allowed to express outdoor roaming behavior were significantly associated with FPV infection shown by P-value of less than 0.0017 and a relative risk of 2.44. Unvaccinated cats and incompletely vaccinated cats were also shown to be significant risk factors for FPV infection with a P-value of less than 0.0001 and a relative risk of 14.12 and 11.05, respectively.

Table 2 Association significance and relative risk of risk factors

Risk Factors		Numbers tested		P-value	Relative Risk
		Positive n (%)	Negative n (%)		
Age	< 6 months	21 (91.30)	2 (8.70)	< 0.0001	2.87
	> 6 months	7 (31.82)	15 (68.18)		
Gender	Male	22 (70.97)	9 (29.03)	0.1007	1.66
	Female	6 (42.86)	8 (57.14)		
Household management	Single Cat	5 (41.67)	7 (58.33)	0.1626	1.67
	Multiple cats/species	23 (69.70)	10 (30.30)		
Behavioural management	Indoor	6 (33.33)	12 (66.67)	0.0017	2.44
	Outdoor	22 (81.48)	5 (29.41)		
Previous exposure	Yes	6 (66.67)	3 (33.33)	> 0.9999	1.14
	No	14 (58.33)	10 (41.67)		
Vaccination status	Unvaccinated	16 (94.12)	1 (5.88)	< 0.0001	14.12
	Up to date	1 (6.67)	14 (93.33)		
	Incomplete	13 (76.47)	4 (23.53)		11.05

Chapter 5.0

DISCUSSION

The results of the molecular detection of FPV among pet cats displaying gastrointestinal clinical signs within Selangor showed that there was a 62.2% detection rate of FPV among pet cats from all nine districts in Selangor. This is the first study reporting the molecular detection rate of FPV in Selangor. This study also showed the districts with the highest molecular detection rate of FPV were Petaling (87%), Sepang (80%), Gombak (80%) and Hulu Langat (67%). The mentioned districts are amongst the most highly populated districts in Selangor with an average of 5.2 million human inhabitants (Statista Research Department, 2013). It is known that FPV spreads easily through infected faecal material and bodily fluids such as saliva, urine and blood. The mentioned districts with a high population density also entail higher numbers of owned pets. This in turn indicates high possibility of orofaecal transmission in the area due to high population density of stray and pet cats. High exposure to infected faecal material and infected cats increases the risk of FPV transmission to healthy cats which increases the overall infection rate of FPV in these districts. A study done by Zhang et al. in 2023 states that the seroprevalence of FPV was significantly higher in cats from urban areas (23.2 %) than in cats from rural areas (16.3%) (Zhang et al., 2023).

During this study, six risk factors were analyzed for their significance in association with FPV transmission, which were age, gender, behavioral management, household management, previous exposure to FPV, their vaccination status. Age, behavioural management and vaccination status of the cat were shown to be significant risk factors contributing to the infection rate of FPV.

According to a study done by Hartmann & Stuetzer in 2014, 57% of cats affected by FPV were younger than 6 months of age, indicating that young cats could be more susceptible to FPV than older cats (Hartmann & Stuetzer, 2014). Young kittens, particularly those less than six months old, are more susceptible to feline panleukopenia virus (FPV) infection due to their immature immune systems and the rapid growth and division of cells in their bodies. According to Squires, FPV is more likely to target actively dividing cells in the host which makes growing kittens a more susceptible target (Squires, 2018). Young kittens are born without active or passive immunity towards the virus which further increases their susceptibility to infection. The infection window is within the range of 6 months which is the time required for the cat to develop a proper and mature immune system. A study done by Zhang et al., in 2023 stated that the seroprevalence of FPV was highest in kittens (< 1 year old) at 28.1%, followed by adult cats (1 – 6 years old) at 19.8%, and senior cats (> 6 years old) at 11.7% (Zhang et al., 2023).

Throughout this study, it was seen that roaming or semi-roaming cats were 2.4 times more likely to be infected with FPV compared to indoor cats. FPV is a highly contagious and hardy virus that can survive in the environment for months to years due to its nature of non-enveloped virus (Struezter & Hartmann, 2014). FPV is also prevalent in stray cats that may be clinically or asymptomatically shedding the virus. When cats are allowed to roam outdoors, they are very likely to encounter infected cats or contaminated surfaces, increasing their risk of infection. A study done by Bennett et al., in 2013 tallies with this study’s results which states that roaming cats were 2.4 times more likely to be infected with FPV than indoor cats due to their increased exposure to the virus and unvaccinated cats (Bennett et al., 2013).

The final significant risk factor were status of vaccination. According to Sykes (2013), feline panleukopenia caused by FPV is most likely to infect cats younger than 1 year of age, predominantly observed to infect unvaccinated and incompletely vaccinated cats. Cats without protection of immunity from vaccinations are exposed and vulnerable to all kinds of viral diseases but not limited to FPV (Skyes, 2013).

Chapter 6.0

CONCLUSION & RECOMMENDATION

This preliminary study has recorded an overall molecular detection rate of 62.2% for FPV among pet cats displaying gastrointestinal clinical signs in Selangor, Malaysia. There were three significant risk factors associated with the transmission of FPV in this study which are cats less than 6 months of age, roaming cats and cats that are unvaccinated and incompletely vaccinated.

For future studies, it is recommended to increase the sample size by recruiting more participating clinics. A higher sample size provides a more accurate depiction of molecular detection rate. Next, the PCR sample products should also be sent for detailed DNA sequencing to further analyze the genome. Such processes include whole genome sequencing and real-time PCR. DNA sequencing also allows for quantification of DNA sample load which allows us to determine the severity of the infection and assist in staging the patient. Finally, serological testing such as ELISA test to screen for FPV infection to determine the serological prevalence of FPV.

Chapter 7.0

REFERENCE:

Agbandje, M., Parrish, C. R., Sgro, J. V., & Partidos, M. (1993). Crystal structure of the major capsid protein of feline parvovirus. Journal of molecular biology, 233(4), 783-796.

American Association of Feline Practitioners. Vaccination Guidelines for the Prevention of Infectious Diseases in Cats. Journal of the American Association of Feline Practitioners, 2009; 39(1):12-19.

Adieb Awad, R., Khalil, W. K. B., & Attallah, A. (2018). Feline panleukopenia viral infection in cats: Application of some molecular methods used for its diagnosis. Veterinary world, 11(8), 1049.

Decaro, N., Mariani, F., Campolo, M., & Tollis, M. (2015). Genetic variability of VP2 gene in feline parvovirus isolates from southern Italy. Journal of veterinary medicine and animal health, 57(1), 67-72.

Greene, C. E., Curtis, K. M., & Parrish, C. R. (2014). Evidence for recombination between feline parvovirus and raccoon parvovirus in the United States. Virology journal, 2(1), 12.

Hamir, M. S. B., Yusoff, K., Omar, A. R., & Ibrahim, J. (2015). Prevalence of feline parvovirus infection in cats in Kelantan, Malaysia. Journal of veterinary science, 16(4), 529.

Haryanto, A., & Raj, V. P. (2020). Clinical study and rapid detection of feline parvovirus in suspected cats by polymerase chain reaction method. Indonesian Journal of Veterinary Sciences, 1(1).

Mochizuki, M., Ohshima, T., Une, Y., & Yachi, A. (2008). Recombination Between Vaccine and Field Strains of Canine Parvovirus is Revealed by Isolation of Virus in Canine and Feline Cell Cultures. Journal of Veterinary Medical Science, 70(12), 1305–1314. https://doi.org/10.1292/jvms.70.1305

Nguyen, T. L., Le, T. T., Do, T. H., Nguyen, D. M., Nguyen, Q. T., Bach, L. T., ... & Nguyen, T. L. T. (2017). Prevalence of feline parvovirus and feline calicivirus in cats in Hanoi, Vietnam. Tropical Animal Health and Production, 49(1), 155-159.

Parrish, C. R. (2009). Parvoviruses. In Encyclopedia of Virology (pp. 237-240). Academic Press.

Parker, J. (2016). Feline parvovirus infection. In Small Animal Infectious Diseases (pp. 109-114). Elsevier Health Sciences.

Willcocks, B. L., Brown, T. D., & Nash, M. J. (1996). Cloning and expression of the feline parvovirus transferrin receptor-binding domain. Journal of virology, 70(9), 5782-5789.

Stuetzer, B., & Hartmann, K. (2014). Feline parvovirus infection and associated diseases. Veterinary Journal, 201(2), 150–155. https://doi.org/10.1016/j.tvjl.2014.05.027

Supriyadi, H., Istijono, S., & Utomo, P. (2016). Prevalence of feline parvovirus in cats in Yogyakarta, Indonesia. Indonesian Journal of Veterinary Medicine, 13(1), 1-5.

Truyen, U., Addie, D., Belák, S., Boucraut-Baralon, C., Egberink, H., Frymus, T., Gruffydd-Jones, T., Hartmann, K., Hosie, M. J., Lloret, A., Lutz, H., Marsilio, F., Pennisi, M. G., Radford, A., Thiry, É., & Horzinek, M. C. (2009). Feline Panleukopenia: ABCD Guidelines on Prevention and Management. Journal of Feline Medicine and Surgery, 11(7), 538–546. https://doi.org/10.1016/j.jfms.2009.05.002

Tasker, S., & Sparkes, A. H. (2007). Feline panleukopenia. Veterinary clinics of North America: Small animal practice, 37(6), 1055-1084.

Tang, Y., Tang, N., Zhu, J., Wang, M., Liu, Y., & Lyu, Y. (2022). Molecular characteristics and genetic evolutionary analyses of circulating parvoviruses derived from cats in Beijing. BMC Veterinary Research, 18(1). https://doi.org/10.1186/s12917-022-03281-w

Ohshima, T., & Mochizuki, M. (2009). Evidence for recombination between feline panleukopenia virus and canine parvovirus type 2. Journal of Veterinary Medical Science, 71(4), 403–408. https://doi.org/10.1292/jvms.71.403

Xue, H., Hu, C., Ma, H., Song, Y., Zhu, K., Fu, J., Mu, B., & Gao, X. (2023). Isolation of feline panleukopenia virus from Yanji of China and molecular epidemiology from 2021 to 2022. Journal of Veterinary Science, 24(2). https://doi.org/10.4142/jvs.22197

Chapter 8.0

Appendices

Appendix 1:

Questionnaire for History Taking

This questionnaire is designed to collect more information regarding the cats where feaces were collected from. The information collect is critical to **assess the risk factors for cats contracting this disease**.

Personal information regarding the client is not included, **all information acquired via this questionnaire will be used for academic purposes only**.

Please **fill in this google form once for each feacal sample collected**. Information provided should be from the cat where the faecal sample is collected from.

tayhongchuan@gmail.com Switch accounts

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Google Forms

Brief Introduction

Feline panleukopenia virus (FPV) is a viral disease that infects domestic and wild cats worldwide. It is a member of the Parvoviridae family, which includes other viruses that cause diseases in different animal species.

Diagnosis of FPV infection is based on clinical signs, history, and laboratory tests. The virus can be detected using polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) tests on faecal or blood samples.

To date, research on molecular prevalence of FPV has not been conducted in Malaysia. This project is important as it serves as a baseline database for future studies and research on FPV in Malaysia.

Objective of this study:

1. To detect FPV among pet cats within Selangor using conventional polymerase chain reaction (PCR) method.
2. To assess the risk factors that contribute to the transmission of feline parvovirus among pet cats within Selangor.

Faecal sample number (Clinical Case Number ID) *

Your answer

Breed

- ◯ DSH
- ◯ LSH
- ◯ Option 3

Gender *

- ◯ Male
- ◯ Female
- ◯ Castrated Male
- ◯ Spayed Female

Household management *

- ◯ Single cat
- ◯ Multiple cats
- ◯ Multiple species (e.g. dogs and cats in the same house)

Behavioural management *

- ◯ Indoor cat
- ◯ Semi-roaming cat
- ◯ Outdoor cat (roaming)

Patient Clinical History

Primary clinical signs (Can choose more than one) *

- [ ] Vomiting
- [ ] Diarrhoea
- [ ] Weakness
- [ ] No appetite
- [ ] Fever
- [ ] Abdominal pain
- [ ] Swollen lymph nodes
- [ ] Pale mucous membrane
- [ ] Other: ____________

Concurrent diseases (if available)

Your answer

Duration of the clinical signs *

- ○ 1- 7 days
- ○ 8 - 14 days

Previous exposure/outbreak of Feline Parvovirus within the household *

- ○ Yes
- ○ No
- ○ Not known

Testing using **Point of Care Test Kit (if tested)** *

- ○ Positive
- ○ Negative
- ○ Did not Test

Brand of Test kit used (if applicable)

- ○ Anigen
- ○ RapiGen
- ○ SensPERT

Vaccination History

Vaccination status *

- ○ Complete vaccination (including booster doses)
- ○ Only 1st dose
- ○ Only 1st and 2nd dose
- ○ 1st and 2nd doses but lapse in booster doses (annually)
- ○ Unvaccinated (No vaccines at all)

History taking questionnaire via Google Form that was sent to participating clinics

Appendix 2:

Questionnaire for History Taking (Responses)

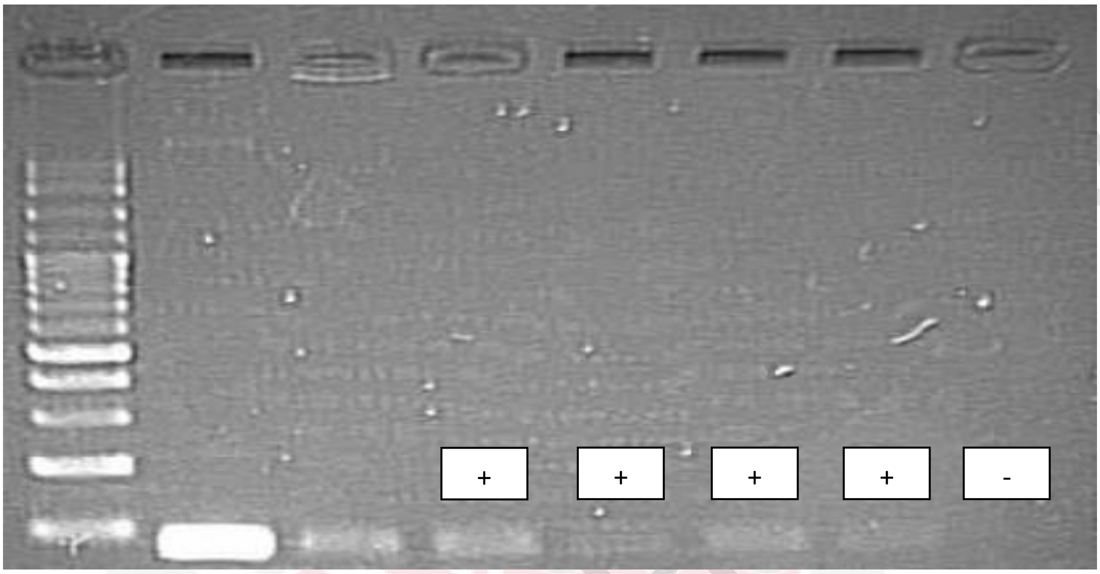
File Edit View Insert Format Data Tools Extensions Help

	ID	Faecal sample number (Clinical Case Number ID)	ID	PCR Result	Age	Breed	Gender	Household management	Behavioural management	Primary clinical signs (Can choose more than one)	Concurrent diseases (if available)
2	PETALING	BDPP7-22	1	POSITIVE	<6 mo	DSH	Female	Multiple species (e.g. dogs and cats in the same house)	Outdoor cat (roaming)	Diarrhoea, Conjuctivitis	Bilateral conjuctivitis
3		BDPP7-28	2	POSITIVE	6 mo - 1 year	MAINE COON	Male	Multiple cats	Outdoor cat (roaming)	Diarrhoea, No appetite, Abdominal pain	
4		TU8-34	3	POSITIVE	> 1 year	DSH	Male	Multiple species (e.g. dogs and cats in the same house)	Semi-roaming cat	Diarrhoea, No appetite, Fever	
5		RTP13-1	4	POSITIVE	<6 mo	BSH	Male	Multiple species (e.g. dogs and cats in the same house)	Indoor Cat	Diarrhoea, Fever, Conjuctivitis	
6		BDPP36-76	5	POSITIVE	<6 mo	DSH	Male	Single Cat	Semi-roaming cat	Diarrhoea, Fever, Weakness	
7		JD3-61	6	NEGATIVE	> 1 year	DSH	Female	Multiple species (e.g. dogs and cats in the same house)	Outdoor cat (roaming)	Diarrhoea	
8	Sabak Bernam	5438	1	POSITIVE	6 mo - 1 year	DSH	Male	Multiple cats	Semi-roaming cat	Diarrhoea, No appetite, Fever, Stomatitis, ulceration at pharynx, salivation	
9		2106	2	POSITIVE	<6 mo	DSH	Male	Multiple species (e.g. dogs and cats in the same house)	Outdoor cat (roaming)	Diarrhoea, Abdominal pain, Pale mucous membrane	Chronic fleas infestation
10		417	3	NEGATIVE	> 1 year	DSH	Male	Multiple cats	Outdoor cat (roaming)	Diarrhoea	
11		691	4	NEGATIVE	6 mo - 1 year	DSH	Male	Multiple cats	Outdoor cat (roaming)	Diarrhoea	
12	Kuala Selangor	FD1654	1	POSITIVE	<6 mo	SIAMESE	Male	Multiple cats	Indoor Cat	Diarrhoea, Fever, Hypersalivation, Weakness	
13		FD6281	2	NEGATIVE	<6 mo	DSH	Female	Single Cat	Semi-roaming cat	Diarrhoea	
14		FD62-87	3	POSITIVE	<6 mo	DSH	Male	Multiple species (e.g. dogs and cats in the same house)	Semi-roaming cat	Diarrhoea, Fever, Weakness	
15		FS385	4	NEGATIVE	6 mo - 1 year	BSH	Male	Multiple cats	Indoor Cat	Diarrhoea, Fever	
16		FS608	5	NEGATIVE	> 1 year	DSH	Female	Single Cat	Outdoor cat (roaming)	Diarrhoea, No appetite, Fever	Chronic fleas infestation
17	HULU LANGAT	B756	1	POSITIVE	<6 mo	DSH	Male	Multiple cats	Outdoor cat (roaming)	Diarrhoea, Fever	Chronic fleas infestation
18		B4050	2	POSITIVE	<6 mo	DSH	Male	Multiple species (e.g. dogs and cats in the same house)	Outdoor cat (roaming)	Diarrhoea	
19		B6735	3	NEGATIVE	> 1 year	DSH	Female	Single Cat	Indoor Cat	Diarrhoea, Abdominal pain	AKI
20		B6057	4	POSITIVE	<6 mo	RAGDOLL	Male	Multiple cats	Indoor Cat	Diarrhoea, Fever	
21		134-92	5	POSITIVE	6 mo - 1 year	DSH	Male	Single Cat	Semi-roaming cat	Diarrhoea, Fever, No appetite	
22		98-29	6	NEGATIVE	6 mo - 1 year	SCOTTISH FOLD	Male	Single Cat	Indoor Cat	Diarrhoea, No appetite, Abdominal pain	
23	GOMBAK	23-8-0440	1	POSITIVE	<6 mo	DSH	Female	Multiple species (e.g. dogs and cats in the same house)	Outdoor cat (roaming)	Diarrhoea, No appetite, Abdominal pain	
24		23-8-0349	2	POSITIVE	<6 mo	DSH	Female	Multiple species (e.g. dogs and cats in the same house)	Outdoor cat (roaming)	Diarrhoea, No appetite, Abdominal pain	
25		23-8-0721	3	POSITIVE	<6 mo	DSH	Male	Multiple species (e.g. dogs and cats in the same house)	Semi-roaming cat	Diarrhoea, Abdominal pain	
26		23-8-0251	4	POSITIVE	6 mo - 1 year	MUNCHKIN	Male	Multiple cats	Indoor Cat	Diarrhoea, No appetite	
27		23-8-0683	5	NEGATIVE	> 1 year	BSH	Male	Single Cat	Indoor Cat	Diarrhoea, Abdominal pain	AKI
28	KLANG	FF4091	1	POSITIVE	<6 mo	DSH	Female	Single Cat	Semi-roaming cat	Diarrhoea, No appetite, Abdominal pain	
29		FM3651	2	POSITIVE	<6 mo	DSH	Male	Multiple cats	Outdoor cat (roaming)	Diarrhoea, Fever, Abdominal pain	Chronic fleas infestation
30		FM698	3	NEGATIVE	6 mo - 1 year	SCOTTISH FOLD	Male	Multiple species (e.g. dogs and cats in the same house)	Indoor Cat	Diarrhoea, Fever	
31		FM561	4	POSITIVE	<6 mo	DSH	Male	Multiple species (e.g. dogs and cats in the same house)	Outdoor cat (roaming)	Diarrhoea, Conjuctivitis, Weakness	Chronic fleas infestation
32		FM6849	5	POSITIVE	6 mo - 1 year	DSH	Male	Single Cat	Semi-roaming cat	Diarrhoea, Abdominal pain, Weakness	
33		FM7936	6	NEGATIVE	6 mo - 1 year	BSH	Male	Multiple cats	Indoor Cat	Diarrhoea	
34	SEPANG	WP5379	1	POSITIVE	<6 mo	BSH	Male	Multiple species (e.g. dogs and cats in the same house)	Indoor Cat	Diarrhoea, Conjuctivitis	
35		OP6381	2	POSITIVE	<6 mo	SIAMESE	Female	Single Cat	Indoor Cat	Diarrhoea, Fever	
36		WP4278	3	POSITIVE	<6 mo	DSH	Male	Multiple cats	Semi-roaming cat	Diarrhoea, Weakness	
37		WP5834	4	POSITIVE	> 1 year	DSH	Male	Multiple species (e.g. dogs and cats in the same house)	Semi-roaming cat	Diarrhoea, No appetite, Fever	
38		WP6395	5	NEGATIVE	6 mo - 1 year	BSH	Female	Multiple species (e.g. dogs and cats in the same house)	Indoor Cat	Diarrhoea	
39	HULU SELANGOR	8081	1	POSITIVE	<6 mo	DSH	Male	Multiple cats	Semi-roaming cat	Diarrhoea, No appetite, Fever	
40		8010	2	NEGATIVE	> 1 year	SIAMESE	Male	Multiple cats	Indoor Cat	Diarrhoea, Weakness	
41		8021	3	NEGATIVE	6 mo - 1 year	DSH	Female	Single Cat	Indoor Cat	Diarrhoea, Fever	
42		8035	4	NEGATIVE	6 mo - 1 year	BSH	Female	Multiple cats	Indoor Cat	Diarrhoea	
43	KUALA LANGAT	769	1	NEGATIVE	<6 mo	MAINE COON	Female	Single Cat	Indoor Cat	Diarrhoea, No appetite, Fever	
44		773	2	POSITIVE	<6 mo	DSH	Female	Multiple species (e.g. dogs and cats in the same house)	Semi-roaming cat	Diarrhoea, Weakness	
45		745	3	NEGATIVE	6 mo - 1 year	BSH	Male	Multiple species (e.g. dogs and cats in the same house)	Indoor Cat	Diarrhoea	
46		755	4	POSITIVE	<6 mo	DSH	Male	Multiple cats	Semi-roaming cat	Diarrhoea, Weakness	

	Duration of the clinical signs	Previous exposure/outbreak of Feline Parvovirus within the household	Testing using **Point of Care Test Kit (if tested)**	Brand of Test kit used (if applicable)	Vaccination status
2	1- 7 days	Not known	Did not Test	-	Unvaccinated (No vaccines at all)
3	1- 7 days	No	Positive	Anigen	Only 1st dose
4	1- 7 days	No	Positive	Anigen	1st and 2nd doses but lapse in booster doses (annually)
5	1- 7 days	No	Positive	SensPert	Unvaccinated (No vaccines at all)
6	1- 7 days	Yes	Positive	Anigen	Unvaccinated (No vaccines at all)
7	8 - 14 days	Not known	Negative	RapiGEN	Up to date
8	1- 7 days	Yes	Positive	Anigen	Unvaccinated (No vaccines at all)
9	1- 7 days	Not known	Did not Test	-	Unvaccinated (No vaccines at all)
10	8 - 14 days	No	Negative	SensPert	Up to date
11	1- 7 days	No	Negative	Anigen	Up to date
12	1- 7 days	No	Positive	Anigen	Unvaccinated (No vaccines at all)
13	8 - 14 days	Not known	Did not Test	-	1st and 2nd doses but lapse in booster doses (annually)
14	1- 7 days	Yes	Positive	Anigen	1st and 2nd doses but lapse in booster doses (annually)
15	8 - 14 days	No	Negative	Anigen	Only 1st dose
16	1- 7 days	Not known	Did not Test	-	Up to date
17	1- 7 days	Yes	Did not Test	-	Unvaccinated (No vaccines at all)
18	1- 7 days	No	Positive	Anigen	Unvaccinated (No vaccines at all)
19	8 - 14 days	Yes	Did not Test	-	Up to date
20	1- 7 days	No	Positive	SensPert	Only 1st dose
21	1- 7 days	Yes	Did not Test	-	1st and 2nd doses but lapse in booster doses (annually)
22	8 - 14 days	No	Negative	Anigen	Up to date
23	1- 7 days	No	Did not Test	-	Only 1st dose
24	8 - 14 days	Yes	Did not Test	-	Only 1st dose
25	1- 7 days	Not known	Positive	Anigen	Unvaccinated (No vaccines at all)
26	1- 7 days	No	Positive	Anigen	1st and 2nd doses but lapse in booster doses (annually)
27	8 - 14 days	No	Negative	Anigen	Up to date
28	1- 7 days	No	Did not Test	-	Only 1st dose
29	1- 7 days	Not known	Positive	Anigen	Unvaccinated (No vaccines at all)
30	1- 7 days	No	Negative	Anigen	1st and 2nd doses but lapse in booster doses (annually)
31	1- 7 days	No	Positive	RapiGEN	Unvaccinated (No vaccines at all)
32	1- 7 days	Not known	Positive	SensPert	1st and 2nd doses but lapse in booster doses (annually)
33	8 - 14 days	Not known	Did not Test	-	Up to date
34	1- 7 days	No	Positive		Only 1st dose
35	1- 7 days	No	Positive	Anigen	Unvaccinated (No vaccines at all)
36	1- 7 days	Not known	Did not Test	-	Unvaccinated (No vaccines at all)
37	1- 7 days	No	Positive	Anigen	1st and 2nd doses but lapse in booster doses (annually)
38	1- 7 days	Not known	Did not Test	-	Up to date
39	8 - 14 days	No	Did not Test	-	Unvaccinated (No vaccines at all)
40	8 - 14 days	Not known	Negative	Anigen	Only 1st dose
41	1- 7 days	No	Did not Test	-	Up to date
42	1- 7 days	Yes	Negative	Anigen	Up to date
43	1- 7 days	No	Negative	Anigen	Up to date
44	1- 7 days	No	Positive	Anigen	Unvaccinated (No vaccines at all)
45	8 - 14 days	No	Did not Test	-	Up to date
46	1- 7 days	Yes	Did not Test	-	1st and 2nd doses but lapse in booster doses (annually)

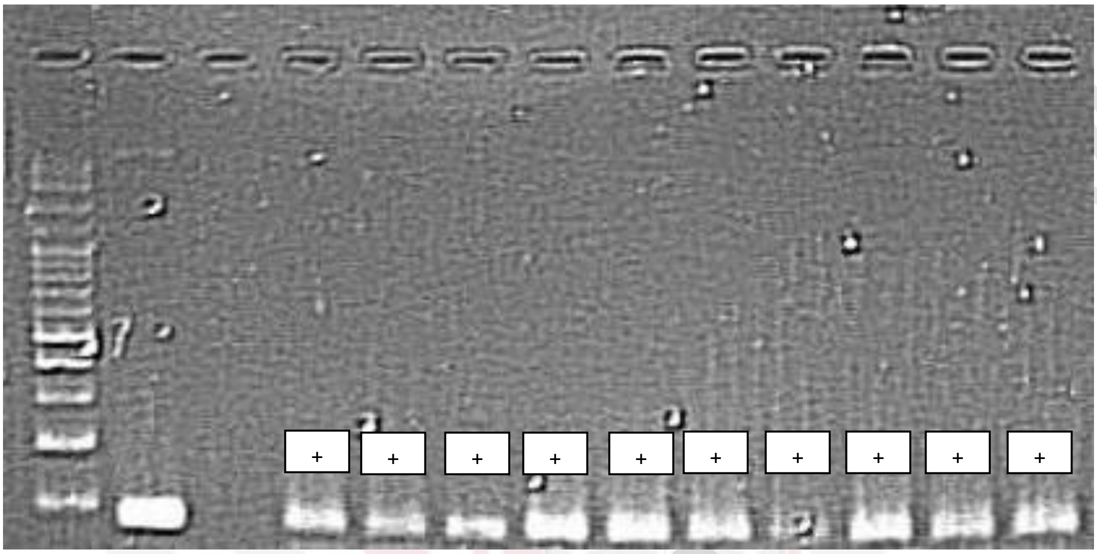
Raw data collected via Google Form questionnaire

Appendix 3:



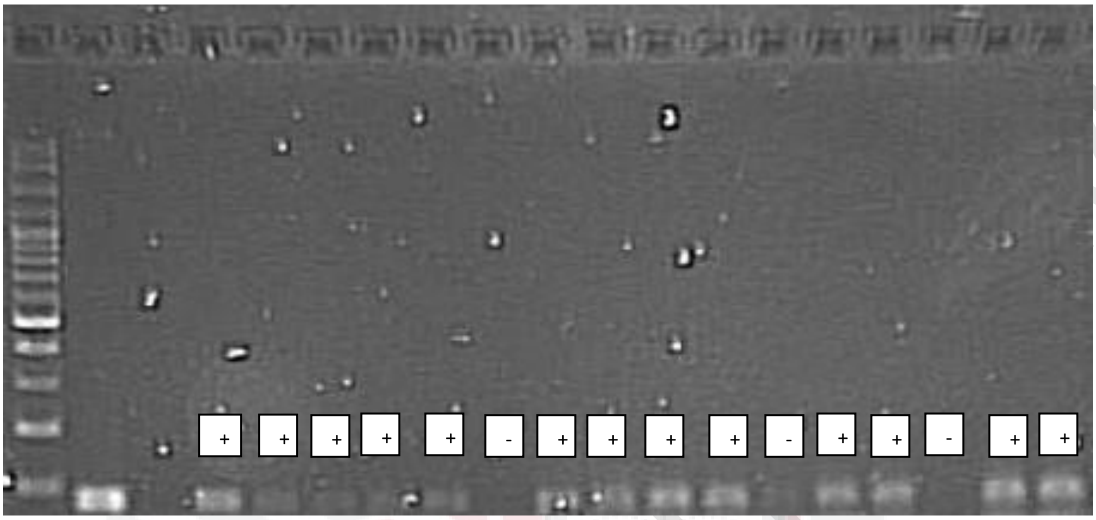
Agarose gel processed on 7th September 2023. Samples processed were (from third column, left to right) P3, P4, HL3, HL4 and SB4. This agarose gel showed that four out of five samples were positive.

Appendix 4:



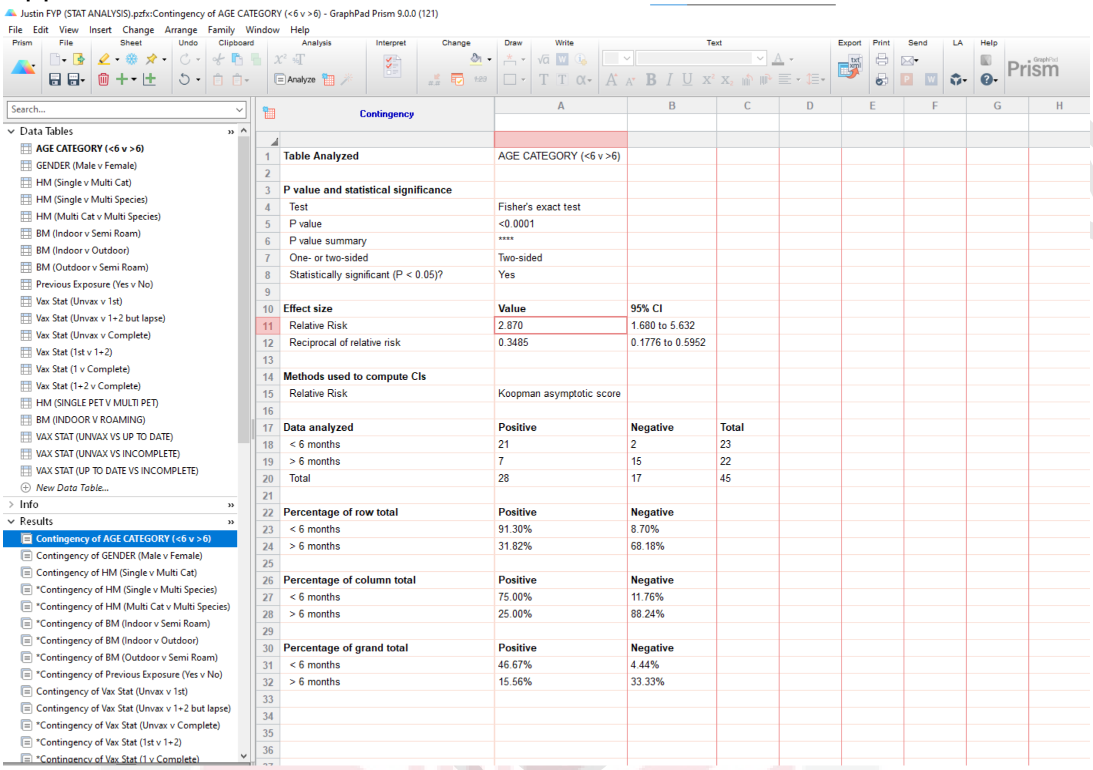
Agarose gel processed on 8th September 2023. Samples processed were (from fourth column, left to right) G1, G2, G3, G4, K1, K2, K3, K4, S1 and S2. This agarose gel showed that 10 out of 10 samples were positive.

Appendix 5:



Agarose gel processed on 14th September 2023. Samples processed were (from fourth column, left to right) HS1, HS2, HS3, KL1, KL2, KL3, KL4, K5, K6, P5, G5, KS3, S3 and S4. This agarose gel showed that 13 out of 16 samples were positive.

Appendix 6:



		A	B	C
1	Table Analyzed	AGE CATEGORY (<6 v >6)		
2				
3	P value and statistical significance			
4	Test	Fisher's exact test		
5	P value	<0.0001		
6	P value summary	****		
7	One- or two-sided	Two-sided		
8	Statistically significant (P < 0.05)?	Yes		
9				
10	Effect size	Value	95% CI	
11	Relative Risk	2.870	1.680 to 5.632	
12	Reciprocal of relative risk	0.3485	0.1776 to 0.5952	
13				
14	Methods used to compute CIs			
15	Relative Risk	Koopman asymptotic score		
16				
17	Data analyzed	Positive	Negative	Total
18	< 6 months	21	2	23
19	> 6 months	7	15	22
20	Total	28	17	45
21				
22	Percentage of row total	Positive	Negative	
23	< 6 months	91.30%	8.70%	
24	> 6 months	31.82%	68.18%	
25				
26	Percentage of column total	Positive	Negative	
27	< 6 months	75.00%	11.76%	
28	> 6 months	25.00%	88.24%	
29				
30	Percentage of grand total	Positive	Negative	
31	< 6 months	46.67%	4.44%	
32	> 6 months	15.56%	33.33%	

Statistical analysis done using GraphPad Prism 9 (Dotmatics, USA)