COMPARISON OF FELINE CORONAVIRUS STRAINS DETECTED IN POSITIVE ASYMPTOMATIC AND SYMPTOMATIC CATS IN MICROREGIONS OF KELANTAN.

BY

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D20A0057

A RESEARCH PAPER SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF VETERINARY MEDICINE

FACULTY OF VETERINARY MEDICINE

UNIVERSITY MALAYSIA KELANTAN

2024

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ACKNOWLEGEMENTS

I present my warmest gratitude to the individuals involved in making this research possible.

PROF. MADYA DR. ERKIHUN AKLILU WOLDEGIORGIS

PROF. DR. MAIZAN BINTI MOHAMED

DR BRENDA SABRINA GILBERT

DR NUR ATIKAH BINTI HASHIM

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DEDICATIONS

I would like to first extend my humble gratitude to my Almighty God, Allah S.W.T for intertwining my fate with the wonderful people which are the ones who gave birth to me first. Mr. Abdul Halim Bin Abdul Hamid, my hero and bread winner, as well as to my compassionate mother Mrs. Che Zaniyah Binti Hassan who had carried me within her womb for 9 months. My God and these Individuals are the reason that I can get up every day to pursue my dream and finish this research project. Next, I without prejudice extend my gratitude to my eldest sister who has been such a great friend and has been supporting me through my journey. This also goes to my younger siblings who always believe in my capabilities and have been such best friends. Not to forget my lovely pet which has always been there to make me happy every time I come back from a tiring day of work, Patah Amin.

However, this study would have never come to fruition without the great minds of Associate Professor Dr Erkihun Aklilu Woldegeorgis and Associate Professor Dr Maizan Binti Mohammed which have guided and provided very wonderful ideas to kickstart my research journey. My thanks also go to Dr Brenda Sabrina Gilbert who has assisted me so much in finding samples and helping me during my constraints during the research project. Then Dr Atikah Binti Hashim the respected Veterinary Officer of Hospital Pengajar Veterinar University Kelantan whom had trained me in blood collection and helped with sampling. Additionally I would provide one of the highest gratitude to Ms Nani Izreen Bt Mohd Sani for all the dedication and hours of assisting, bearing patience in teaching me laboratory work in Virology lab UVDC, UMK.

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Finally, my batchmates of DVM UMK 20/25 who are always there to help brighten my day and correct my mistakes. They all mean a lot to me and I dedicate this work in their names.

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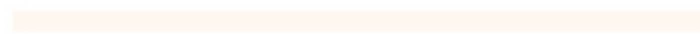

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ABSTRACT

This thesis is presented to the Faculty of Veterinary Medicine Universiti Malaysia Kelantan under the subject code DVT 55204 Research Project

This study explores the differences of the genetic diversity of the viral RNA of Feline Coronavirus which is collected from local cats of some region in Kelantan whether the cats are healthy or exerting symptoms reminiscent of Feline Infectious Peritonitis and to be compared with Feline Infectious Peritonitis viral which was sequenced from previous research. The genetic diversity stems from the two theories which had been proposed to justify the mutagenic virus of Feline Coronavirus. The theories are "Internal mutation theory" and "High-Low virulent theory". Feline Coronavirus are very ubiquitous in nature and can be isolated from the gastrointestinal tract of some felids or sometimes even the blood. There have been multiple studies which explore the patient signalment and pedigree of cats which are suggestive of pathological development for the fatal Feline infectious peritonitis virus. There was no genetic diversity observed from the sample population based on Nested Polymerase Chain Reaction. It was reported that 3/20 blood samples were positive and 14/24 rectal fluids samples were found positive when performing the molecular detection using the same primers.

Key Words: "FcoV , FCCoV, Alpha Coronavirus, FIP, RNA"

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ABSTRAK

Abstrak ini adalah dibentang kepada Fakulti Perubatan Veterinar Universiti Malasyia Kelantan dalam keperluan sebahangai subjek/kursus DVT 55204

Penyelidikan ini membincangkan perbezaan genetik RNA virus Feline Coronavirus di beberapa kawasan dalam Kelantan, Malaysia. Khususnya di Kota Bharu Kelantan. Sampel diambil daripada kucing yang sihat atau kucing bersimptom seperti yang dihadapi oleh pesakit kucing yang menhidap penyakit “Feline Infectious Peritonitis”. Terdapat dua teori yang menyatakan bahawa kelainan genetik FCoV adalah disebabkan “Internal Mutation Theory” atau “High-Low Virulent theory”. FCoV boleh ditemukan daripada sistem usus kecil, besar dan juga dalam darah haiwan demikian. Kebanyakan penyelidikan lain memberi tumpuan kepada faktor lain seperti latar belakang pesakit kucing dan bukan keadaan kesihatan semasa pesakit yang mengakibatkan Feline Infectious Peritonitis. Hasil penyelidikan ini mendapati bahawa 3/25 sampel darah adalah positif manakala 14/25 sampel usus-rektum. Hal ini menyatakan bahawa tiada perbezaan genetik secara umum daripada diagnostik “Nested PCR”.

Kata Kunci: “FcoV , FCoV, Alpha Coronavirus, FIP, RNA”

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CHAPTER 1

1.0 Introduction

Feline Coronavirus (FCoV) is a prevalent pathogen among domestic cats particularly in Malaysia, often presenting as either an asymptomatic infection or manifesting in the more severe Feline Infectious Peritonitis (FIP). While numerous studies have focused on understanding the virology and epidemiology of FCoV or FECov mutate into FIP in other countries the emergence of novel virulent strains in Kelantan, Malaysia remain unrecognized. This is because following numerous studies the genetic sequence may vary from country to country and region to region. This poses the question of what actors affect the mutation and development of Feline Infectious Peritonitis. Additionally, the use of the same primers in identifying the differences in genetic sequence of FCoV and FIPV in Kelantan is also yet to be evaluated. In this study the health status of animals where some are exhibiting signs of infectious disease and healthy cats has been isolated to suggest whether the internal mutation theory or high-low virulent theory is dominant. This study would help to determine whether the FCoV strains in Kelantan vary greatly from the FIPV in Kelantan and whether the animal which exhibit primary clinical signs from respiratory tract infections, gastrointestinal and neurological disease would affect the FCoV strain if it is present.

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1.2 Problem Statements

Feline Coronavirus (FCoV) poses a significant threat to domestic cat populations worldwide, with the potential to cause severe and often fatal disease manifestations, such as Feline Infectious Peritonitis (FIP). This disease remains poorly understood as it is highly mutagenic. The genetic variety has been studied on signalments such as management, household, breed, age and sex. However, these studies have been done abroad, and it is shown by Veloso et al. (2021) that the genetic sequence could vary according to regions and even microregions. The Coronavirus that was found in cats show some relations with other pathogenic coronavirus like Canine Coronavirus, Transmissible Gastroenteritis Virus and even SARS-Cov2 which caused the COVID 19 pandemic as mentioned by the same author. The primary genetic mutation point is the 3c gene which is an essential gene that is for viral replication. Commonly for molecular detection the least mutagenic gene would be used for reverse transcription, but is it well known that there would not be any homogeneity if there is a mutation? Other than that, the problem of the efficacy of common genetic loci used for reverse transcription that may exist in the Kelantan state, particularly Kota Bharu must be compared with the more FIPV to determine the efficacy of diagnosing the patient as to avoid unnecessary expenditure to treat feline patients.

1.3 Research questions

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1. *Would the genetic data of the cats from this study differ from FIP gene sequence if using the same primer for transcription?*
2. *Is there any difference in the gene sequences of FCoV strains from symptomatic and asymptomatic felines and would it be shown through different base pairs?*

1.4 Objective

To compare the gene sequences of FCoV strains from symptomatic and asymptomatic feline patients to the sequence of an FIP virus.

1.5 Alternate Hypotheses

The patients which exhibit clinical signs of FIP would be positive and the asymptomatic patients which are positive would show a different FCoV genetic sequence using the same primer as well as different to the FIP genetic sequence from previous studies with the same primer used for RT PCR.

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1.6 Null Hypothesis

There are no differences in the genetic sequence of asymptomatically positive and symptomatic patients to FIP genetic sequences with the same primer used for transcription.

CHAPTER 2

2.0 Literature review

2.1 Evaluation of Feline Coronavirus Viraemia in Clinically Healthy and Ill Cats with Feline Infectious Peritonitis

Based on a pilot study done in Selangor 67.5% of the total of healthy cats display viremia for FCoV. After the initial screening for FCoV genomic RNA, the blood samples from the healthy cats were evaluated to detect viral mRNA. Feline Coronavirus (FCoV) includes both virulent and avirulent biotypes. Both types can enter a cat's bloodstream. Although only the virulent biotypes infect and replicate within monocytes and macrophages, leading to Feline Infectious Peritonitis (FIP), a fatal disease. This study screened FCoV viremia in 50 cats which are 40 healthy and 10 suspected of having FIP. Genomic RNA was found and detected using the RT-PCR assay, followed by a duplex RT-PCR to detect the viral mRNA. In healthy cats, it was found in 67.5% and 15% of samples, respectively. This would indicate that the virus can replicate in some asymptomatic cats which would suggest that FCoV viremia does not always lead to FIP. It is however possible that the avirulent virus replicates at low levels in the blood or that the virulent

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virus is present at an early stage of FIP without clinical signs. In FIP-suspected cases, all ill cats tested positive for both FCoV and replicating viral mRNA, showing active replication and high levels of the virus detectable by both assays. The duplex RT-PCR assay was more specific for diagnosing FIP compared to the general RT-PCR screening. However, RT-PCR results should be considered alongside other clinical symptoms. Sharif *et al.,* (2011).

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2.2 Internal Mutation Theory and Circulating High Virulent-Low virulent

The exact nature of the relationship between the two FCoV pathotypes is crucial for a deeper understanding of FCoV diseases in cats. Two significant hypotheses have been proposed. The widely accepted "internal mutation theory" suggests that FIP occurs when a cat encounters FCoV variants that have mutated within the host, enabling them to spread from the gut (the primary infection site) by effectively replicating within macrophages. However, stable genetic differences that explain the distinct pathogenicity of FECoV and FIPV have yet to be identified. Hora *et al.,* (2013). The alternative "circulating high virulent-low virulent" FCoV hypothesis proposes that both pathogenic and benign lineages of FECoV exist within a cat population. According to this hypothesis, disease develops only in cats infected by virulent strains transmitted from other infected cats. This hypothesis, which is less popular than the "internal mutation theory," suggests that distinct "high virulent-low virulence" FCoVs are responsible for FIP pathogenesis. However, since FIP occurs sporadically and outbreaks in domestic cat populations are rare, there has been limited epidemiological support for this hypothesis. Healey *et al.,* (2022).

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2.3 The M gene.

In the same study conducted by Hora et al., (2013) we can isolate a particular gene named the M gene. RT-PCR Detection of FCoV mRNA in the Membrane Gene: FCoV mRNA was detected in at least one sample from each of the 10 cats with FIP. Out of 190 individual samples, 77 (40.53%) tested positive for the M gene. The most frequent sites of FCoV replication were the abdominal effusions (5/6), mesenteric lymph nodes (7/10), large intestines (7/10), lungs (6/10), thoracic effusions (4/8), kidneys (10/20), and aqueous humor (8/20). Additionally, 3 out of 5 fecal samples from cats without FIP were positive for FCoV M gene mRNA. Hora et al., (2013).

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2.4 The Spike protein in Type I and Type II FCov

A study by Xia et al., was done at various pet hospitals in Liaoning Province, China, from October 2017 to May 2019. The researchers examined 39 samples. From those samples are Ascites samples from 31 cats with suspected FIP and feces from 8 healthy cats were collected. The adaptive evolution of FCoV, focusing on selective pressure, was analyzed using PAML and Datamonkey for key proteins involved in viral entry, replication, and virulence. This study aimed to enhance understanding of FCoV's adaptive evolution, potentially shedding light on their pathogenic mechanisms. The finding was 3 positives for FCoV from those samples taken. Other coronaviruses such as SARS and MERS are almost like FIPV infections, to which they are globally distributed and can lead to fatal diseases that pose a significant health risk to every animal. The more prevalent FECV typically causes mild or asymptomatic enteric infections in cats. Xia et al. 2019 discovered that FIPV and FECV are classified into types I and II based on differences in the S gene's nucleotide sequence. These codes for the spike protein. While most natural feline coronavirus infections are type I FCoV, it is difficult to be cultured and unlike type II FCoV that can proliferate in various cell lines. The pathogenesis of FIP remains unclear even until this day. However, it is believed that the viral Quasi species resulting from RNA replication errors are the factors that overwhelm the weak immune system of a cat leading to FIP. The spike (S) glycoprotein is crucial for receptor binding and viral entry. Like the S gene, other important genes in the FCoV genome include nsp12 (RNA-dependent RNA

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polymerase), nsp13 (helicase), and nsp14 (exoribonuclease), essential for genome replication. The N gene is usually used for phylogenetic analysis, and it encodes the nucleocapsid. The 7b gene, located downstream of the N gene, is a significant marker of virulence. Xia *et al.,* (2020)

Type I FCoV is common globally, while type II FCoV infections range from 2-30% prevalence. Type I FCoV is prevalent in Europe, Japan, Australia, Korea, and the USA. Where Japan and Taiwan reported more FIPV cases associated with type II FCoV which writes down geographical variations in FCoV serotypes. In China, almost all isolated FCoV strains are type I. The study expanded FCoV sampling in China and examined the selective pressures on FCoV genes from different global regions.

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2.5 Feasible diagnostic methods in FIP.

The same study of [2.5] reported A/G ratio is different from the ideal critical value [29–35]. FIP is suspected when at least four of the following criteria are observed: abnormal clinical signs (such as abdominal enlargement), changes in hematological and biochemical profiles, diagnostic imaging (X-rays or ultrasound) showing fluid accumulation in the thoracic and/or abdominal cavities, a positive PCR result (detecting FCoV), a positive Rivalta's test, and the presence of FIP antigen in affected tissues confirmed by histopathology. Tuanthap *et al.,*(2021).

2.6 Viral RNA and Reverse Transcription.

The RNA can be extracted from 140 µl of effusion or feces suspension with the QIAamp viral RNA mini kit (Qiagen, Shenyang, China), Xia et al., (2020). Mentioned that following the manufacturer's instructions, and stored at -80° C. Extracted RNA was used as the template for cDNA synthesis with PrimeScript™ II 1st Strand cDNA Synthesis Kit (Takara, China) with random hexamers, following the manufacturer's instructions in their particular study in China.

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2.7 Molecular differences between FIPV and FECV outside of Malaysia

Over the past decades, extensive research has focused on identifying mutations responsible for the biotype switch in feline coronaviruses (FCoVs) linked to feline infectious peritonitis (FIP). According to Tekes and Thiel (2016), mutations in accessory genes and the S gene were associated with the development of FIP. A focus has been on the accessory gene 3c. Studies related have revealed that while FECVs typically have an intact 3c gene, over two-thirds of FIPV-derived 3c sequences have mutations or deletions and point mutations that prevent the translation of a full-length protein. Initially, these 3c mutations were considered virulence markers that help indicate FIPV. However, based on the author, newer studies confirmed these observations, showing high mutations in the majority of FIPV isolates which would suggest that an intact 3c gene is essential for viral replication in the gut but not for the systemic replication of FIPVs. Recently it was believed that 3c mutations are a consequence of systemic spread and enhanced replication of FIPVs, though they may also contribute to increased viral fitness in monocytes/macrophages, thereby aiding in the development of FIP.

Mutations in the 7a gene are currently not considered significant for the biotype switch. Although deletions in the 7b gene were indicated to play a role in FIP development from

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FECV, some analyses of other research revealed that such deletions primarily occur during cell culture adaptation, and they found that it was associated with loss of virulence. The presence of 7b deletions in naturally occurring FECVs contradicts a significant role for 7b mutations in FIP development.

In addition to that, other research mentioned by the author on FIP pathogenesis has shifted towards investigating the S gene that encodes the spike protein and that mutations of these genes contribute to virulence. This virulence is crucial for receptor binding and virus entry. Since the transition from FECV to FIPV involves a switch in target cell tropism, mutations in the S gene, alone or in combination with changes in other genes, may contribute to this biotype switch. There were 11 FECV and 11 FIPV full-length genome sequences that identified two-point mutations in the S gene that can distinguish the majority of FIPVs from FECVs. These mutations, located in the putative fusion peptide of the S protein, are hypothesized to affect the cellular tropism of the virus, enhancing monocyte/macrophage tropism. Tekes *et al., (*2016).

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2.8 Risk Factors related to FIP development

The findings support previous research indicating that age, breed, and sex predispositions exist for FIP in Australia. Pedigree cats were significantly over-represented in FIP cases, while domestic crossbreeds were under-represented, consistent with earlier studies. Notably, certain pedigree breeds such as the Devon Rex, British Shorthair, and Abyssinians were significantly over-represented in the FIP group, whereas domestic crossbreeds, Persians, and Himalayan cats were under-represented. This pattern of breed susceptibility aligns with previous smaller case series reported in North America and Australia, highlighting the over-representation of British Shorthair, Devon Rex, and Abyssinian cats, and the under-representation of domestic crossbreeds, Himalayan, and Persian cats. The observation that not all pedigree breeds are overrepresented complicates the idea that the primary risk factor for pedigree cats is living in or being born in a multi-cat household. While this factor likely contributes to the risk for these cats, if it were the most critical risk factor for FIP, it is expected that all pedigree cats would be overrepresented. Since this is not the case, the overrepresentation of specific breeds might suggest that certain breed lines within these breeds have a higher risk of FIP. It has been proposed that there may be a genetic component influencing the effectiveness of a cat's immune response and its susceptibility to FIP. For instance, cats from specific breed lines might be more likely to inherit susceptibility to FIP, especially if they come from a small population with limited genetic diversity. Genetic monomorphism at the major histocompatibility complex was

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implicated in an outbreak of FIP among a group of closely related captive cheetahs, which resulted in the death of 60% of the population. Cats that are direct relatives of those who have died from FIP are significantly more likely to develop the disease than unrelated cats, indicating that susceptibility to FIP is at least partially hereditary. The existence of susceptible lines within breeds might explain why not all pedigree cats are overrepresented in FIP cases and why the pattern of breed susceptibility varies between countries, where different breed lines likely exist.Certain breeds might have a higher risk of developing FIP due to the possibility that the catteries they come from carry more virulent strains of FIPV compared to other catteries. This study does not aim to investigate whether the prevalence of specific breeds is due to susceptible bloodlines that raise the chances of in vivo mutation. Worthing et al., (2012).

Based on these observed sequence differences, a diagnostic assay for FIP has been developed. Short fragments of the S gene from fecal and tissue samples of both FECVs and FIPVs revealed that methionine at position 1058 was predominantly found in fecal samples, while leucine at the same position was found in tissue samples, regardless of the infection type. This M1058L substitution is considered a marker for systemic FCoV infection rather than for FIP. Further studies identified another substitution, I1108T in the HR1 region, which is exclusively found in FIPVs and may alter the fusogenic activity of the S protein, affecting the virus's cellular tropism.

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Additionally, another study investigated the furin cleavage site between the S1 and S2 domains of the S protein in FECV and FIPV samples. All the FECVs had an intact and functional furin cleavage, while 10 out of 11 FIPVs had amino acid changes at or near the site. This will affect the efficiency of furin-mediated S protein cleavage because the fusion activity of the coronaviral S protein requires activation by cellular proteases. These substitutions might indirectly support viral spread and FIP development. Even with the correlation which the author identified between genetic changes in the S gene and the occurrence of FIPV, it is important to note that these FECV-FIPV substitutions were identified only through comparative sequence analyses only. The functional relevance to cellular tropism and the biotype switch in FIP pathogenesis remains to be experimentally proven. Tekes *et al*.,(2016).

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2.9 The Spike protein in Type I and Type II FCov

A study by Xia et al., was done at various pet hospitals in Liaoning Province, China, from October 2017 to May 2019. The researchers had examined 39 samples. From those samples are Ascites samples from 31 cats with suspected FIP and feces from 8 healthy cats were collected. The adaptive evolution of FCoV, focusing on selective pressure, was analyzed using PAML and Datamonkey for key proteins involved in viral entry, replication, and virulence. This study aimed to enhance understanding of FCoV's adaptive evolution, potentially shedding light on their pathogenic mechanisms. The finding was 3 positive for FCoV from those samples taken. Other coronaviruses such as SARS and MERS are almost similar to FIPV infections, to which they are globally distributed and can lead to the fatal disease that poses a significant health risk to every animal. The more prevalent FECV typically causes mild or asymptomatic enteric infections in cats. Xia et al., discovered that FIPV and FECV are classified into types I and II based on differences in the S gene's nucleotide sequence. These codes for the spike protein. While most natural feline coronavirus infections are type I FCoV, it is difficult to be cultured and unlike type II FCoV that can proliferate in various cell lines. The pathogenesis of FIP remains unclear even until this day. However it is believed that the viral quasispecies resulting from RNA replication errors are the factors that overwhelm the weak immune system of a cat leading to FIP. The spike (S) glycoprotein is crucial for receptor binding and viral entry. Like the

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CHAPTER 3

3.0 METHODS

SAMPLE COLLECTION

Samples are collected from random cats which are owned. They are either healthy cats or cats exhibiting infectious clinical signs reminiscent of Feline Infectious Peritonitis with or without treatment and definitive FIP diagnoses. A total of 28 samples have been collected over the course of two months. 25 of the samples are taken from healthy cats which had never been diagnosed with Feline infectious peritonitis nor suspected. Three of the samples being from cats which exhibit clinical signs from respiratory tract infections, gastrointestinal infections, ascites, and neurological abnormalities. The sample collection was conducted using a 21 G needle for venipuncture, some from preplaced or during indwelling catheter placements, and rectal swab. The blood is stored into EDTA tubes whilst the rectal swabs are frozen in Viral Transport Media.

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GENE EXTRACTION

Gene extraction of the blood and rectal samples were done using manual extraction without a kit. The first samples are separated at 300 µl into 1.5 ml centrifuge tubes and reagent GeneZOL© is added 3 times the volume of sample as well as vortexed for 10 seconds. It is then left to incubate at room temperature for five minutes. After wards 200 µl of chloroform is added into the tube then is vortexed for 10-15 seconds. Fourthly, the mixture was vortexed at 14 000 rpm under 4 °C for 15 minutes. Next the supernatant which is a clear aqueous solution will be collected and transferred into a new centrifuge tube of 550 µl of volume. The fifth step is to add the same volume of supernatant used one to one ration and spin at 14 000 rpm in 10 mins at 4°C. Then the supernatant will be discarded, in this step there could be an RNA pellet which could be appreciated, whether it is cloudy or transparent. Right after, the pellet will be washed with 70% cold ethanol stored at – 20 °C, and centrifuged at 14 000 rpm for 5 mins in 4°C. Finally, the ethanol will be discarded using pipette, left to dry for 10-15 minutes and finally be rehydrated with 30µl Nuclease free water in an incubator at 60 °C for 10-15 minutes. Now the RNA has been extracted and ready to be used instantly or stored at -80°C freezer.

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RT PCR

From the stored samples 5 µl of the sample will be inserted into a microcentrifuge tube. The samples will be preheated at 95 °C for 5 minutes. After wards 20 µl of the master mix will be inserted into the sample tube (s). The master mix consists of 12.5 µl of 2x Access Quick buffer, 0.5 µL of AMV-RT, 1.0 µl of forward and reverse primers (RTPCR P205 5' GGCAACCCGATGTTTAAAACTGG 3'P211 5' CACTAGATCCAGACGTTAGCTC 3') , RNAsin 0.2 µl as well as 4.8 µl of Nuclease free water which are timed to the number of samples respectively. After that, the samples will be centrifuged at 5000rpm for 10 seconds and is inserted into thermal cycler for the reaction process. The product can be stored in a chiller at 4°C. All the transfer of samples and master mix preparation must take place under or at 4 °C.

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NESTED PCR

The Nested PCR will use the products of the RT PCR. 2 µl of the RT PCR product and mixed with the Nested PCR master mix. The Nested PCR master mix are, Taq Polymerase 12.5µL, 1.0µl of forward and reverse primers (NESTED PCR P204 5' GCTCTTCCATTGTTGGCTCGTC 3' P276 5' CCGAGGAATTACTGGTCATCGCG 3') and nuclease free water. Then the supernatant is centrifuged at 5000 rpm for 10 s and placed into the Thermal Cycler.

GEL ELECTROPHORESIS

The agarose powder is first weighed at 1.2 g using a weighing device. Then the powder would be mixed with 80 ml of Tris-acetic-acid-EDTA (TAE) to then be heated via microwave in a media bottle for 3 minutes. Next Midori green stain of 1 µl will be mixed into the cooled agarose-TAE mixture. Afterwards, the mixture is cooled down and set into the casting tray and well is created. Then the 10 µl of DNA ladder is placed in the first well followed by 10 µl of samples and controls. Then Gel Electrophoresis was run at 100 mV for 40 minutes for each gel plate.

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CHAPTER 4

4.0 RESULTS

4.1 NESTED PCR & GEL ELECTROPHORESIS

There were 20 out of 50 samples that were found positive. Those account for 3 out of 25 (12.5%) blood samples and 17 being from 25 (70.8%), but 14 of 25 true positives (58%) rectal swabs. The far end of the well which is number 19 of each gel plate is a positive control using FCoV sample that was extracted from patients which are highly suspected of Feline Infectious Peritonitis. The well 20 of each gel plate is the negative control and the first well of each plate being the DNA ladder of 100 bp. The bp of the product is 177 bp. Some of the results appear misaligned mainly due to placement of gel on Ultraviolet gel imaging. It is important to note that well with sample number 43,44 and 46 are suspected of cross contamination from. Hence the true positives are 17 out of 50 samples. Patient sample numbers 49 and 50 are from blood and rectal samples of patients which are recovering from Feline Infectious Peritonitis respectively.

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Specimen	Positive	True Positive	Negative	Remarks
Blood	3	3	22	-
Rectal fluids	17	14	11	Suspected cross contamination
Positive control	1	1	0	No error in preparing the positive control
Negative control	0	0	1	No error in master mix preparation for both types of PCR

Table 1: Displaying results of the samples collected

CHAPTER 5

5.0 DISCUSSION

The aim of this study was initially to compare the gene sequence of FCoV of patients with and without symptoms reminiscent of Feline Infectious Peritonitis. However, due to further literature review like ones by Doenges et al. (2016) it was proven that should there be mutations in the gene then the virus would not have been detectable and hence why RT-PCR with the spike protein gene remain viable in detection of the virus. When looking for viral presence specificity and sensitivity are important. This also proves difficulty in diagnosing patients. Which is why samples collected

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about patient clinical sign and specific organ failure and history are the only true way to diagnose Feline Infectious Peritonitis (Felten et al., 2017). The results that were detected showed an overall 34% positive of total samples tested using S gene primer. The control is Coronavirus which was collected in a previous study done in Kelantan whereby the cats were highly suspected of Feline Infectious Peritonitis. The base pairing of 177 for the positive results proves that the S gene remain a reliable gene to detect Feline Coronavirus as stated by Doenges et al. (2016). This proves the null hypothesis proposed which there would be no differences of genetic variation as if there were mutations of S gene of Feline infectious Peritonitis suspected patients, the results would have been negative for the sample collection.

Next the 12% positive results of blood samples are values that suite the prediction in studies like that was done where similar collection via blood and body fluid only showed positive of 3/25 patients. (Emmler et al., 2019). Other than the rectal results which show 58% true positive of total sample indicates that the prevalence of FCoV is higher in the gastrointestinal tract than in blood which can be explained by the Feline Enteric Coronavirus presence and how it is easily more transmissible via fecal to oral route and that viremia is usually secondary due to mutations which can cause the virus to develop into early stage of FIP. However, this is more of a suggestion as pointed out by Sharif *et al.,* (2011) in their study, and can be compared to the fact where there is positive samples for blood of 12.5% but without any visible clinical signs. The more plausible evidence that many studies such as by Tekes *et al.,* (2016), Porter et al. (2014) and Doenges et al. (2016) have agreed on is the 3c gene mutation of FCoV which is high suggestive of l formation of Feline Infectious Peritonitis by invading through the peripheral Mononuclear cells. But the formation of the disease is more complicated and requires more details like lesion formation and various other clinical signs to piece the puzzles together. Other than that, the question of Feline Coronavirus being a commensal was explored by Van Brussel et al. (2022) where it was mentioned how it was the most abundant virome in gastrointestinal tract of healthy cats among other viruses. Which can be why there was a 58% prevalence of the samples from the rectum. But no conclusive evidence stating it is normal to find circulating Feline Coronavirus as mentioned before where it could be an early development of FIP.

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Furthermore, the limitations of this study would be discussed. The main challenge of this study was to get samples from one region as not many owners would agree to allow the blood collection of their pet. Then the manpower which was lacking only allowed 50 total cats of the target to be reached of 60 sample frame size. Another was time and transport constraint where vital samples like blood can only be kept for a maximum of 14 days in the EDTA tubes. Finally, the study took place around flooding season which made processing samples also be delayed hence the need to send the samples for gene sequencing had to be revised. But as proposed earlier, there might not be any difference in the sequencing as using the same primer RTPCR (P205 5' GGCAACCCGATGTTTAAAACTGG 3' ,P211 5' CACTAGATCCAGACGTTAGCTC 3') and Nested PCR (P204 5' GCTCTTCCATTGTTGGCTCGTC 3' P276 5' CCGAGGAATTACTGGTCATCGCG 3') would have theoretically produce the same sequence which could be a hypothesis or further researched in the future.

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CHAPTER 6

6.0 CONCLUSION & RECOMMENDATIONS

6.1 Conclusion

The null hypothesis of no genetic variation is accepted as the results of PCR were positive when using positive control from FCoV of highly suspected FIP and the same primers from the previous study. However, gene sequencing is still required to fully prove this hypothesis.

6.2 Recommendation

The recommendations of this study are to perform gene sequencing in the near future or future research to better support the hypothesis. Moreover, having more manpower and time is essential to perform sample processing without errors like cross contamination which occurred in three samples of this current study. Additionally having more options of primers like for gene 3c and M gene can better help explore more about the variations of FCoV strains.

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7.0 APPENDICES

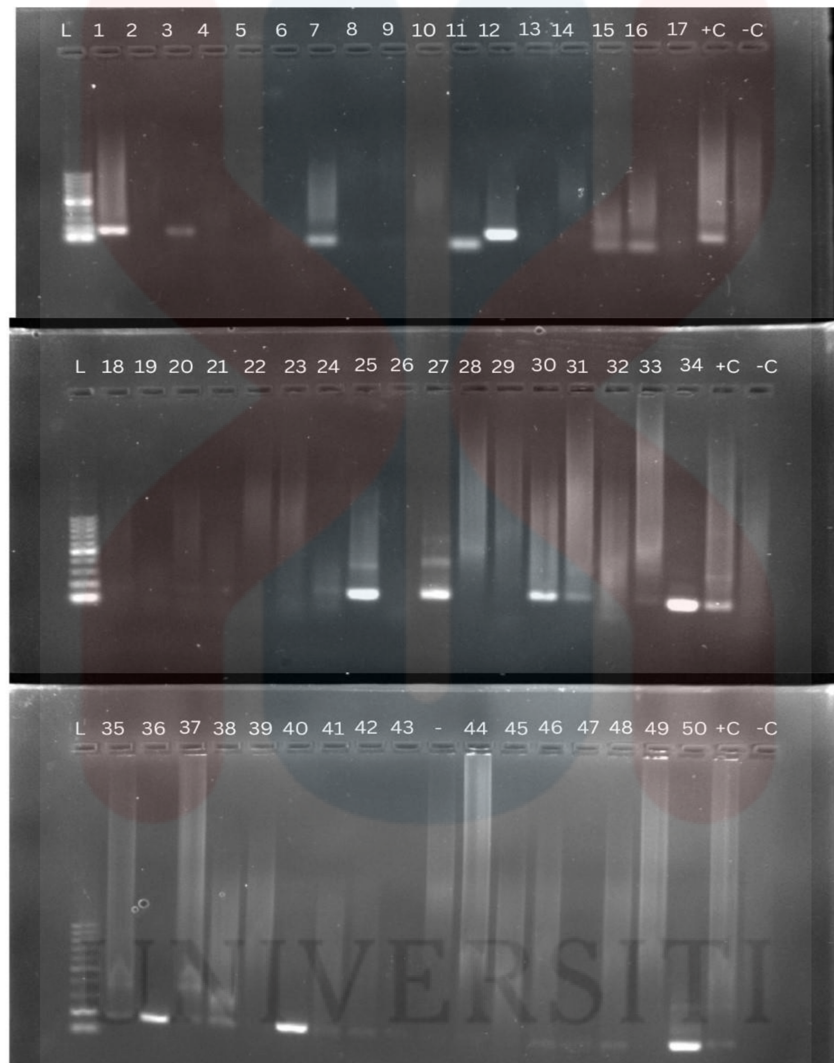

Figure 1: GEL ELECTROPHORESIS RESULTS AFTER NESTED PCR

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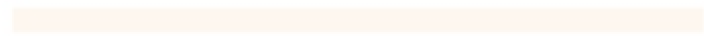

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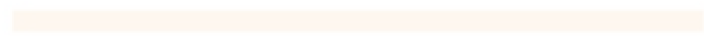

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