# MOLECULAR DETECTION OF SARS-COV-2 (COVID-19) IN CATS IN KOTA BHARU AND BACHOK, KELANTAN

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(D17B0008)

A RESEARCH PAPER SUBMITTED TO THE FACULTY OF VETERINARY MEDICINE, UNIVERSITI MALAYSIA KELANTAN
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THE DEGREE OF
DOCTOR OF VETERINARY MEDICINE

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UNIVERSITI MALAYSIA KELANTAN

It is as a result of this certification that we have read this research paper entitled "**Molecular Detection of SARS-CoV-2 (COVID-19) in Cats in Kota Bharu and Bachok, Kelantan**" by Hafizuddin Bin Gos Hambali, and in our opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course DVT 5436 – Research Project.

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Family and Friends

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**Thank You**

## DEDICATIONS

This thesis is dedicated to:

For the sake of Allah, my Creator and my Master,

The Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, my second home,

My supervisor, guides me through the darkest parts of the valley while shining a ray of hope and assisting me,

My dad, never stops giving of himself in an infinite number of different ways,

My dear brothers are always there for me even when things do not appear promising.

To every member of my family, the embodiment of kindness and generosity,

My friends and classmates who are always there to cheer me on and support me,

I would want to dedicate this study to everyone who had an impact on my life.

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**ABBREVIATIONS**

| | |
|---|---|
| COVID-19 | Coronavirus Disease 2019 |
| WHO | World Health Organization |
| SARS-CoV-2 | Severe Acute Respiratory Syndrome Coronavirus 2 |
| MERS | Middle East Respiratory Syndrome |
| RNA | Ribonucleic Acid |
| RBD | Receptor Binding Domain |
| ml | Millilitre |
| µl | Microlitre |
| rpm | Revolution per minute |
| RT-PCR | Reverse Transcriptase Polymerase Chain Reaction |
| ACE2 | Angiotensin-Converting Enzyme 2 |

**ABSTRACT**

An abstract of the research paper was presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement on the course DVT 5436-Research project.

This research aimed to explore the COVID-19 occurrence in domestic cats in Kota Bharu and Bachok, Kelantan, and subsequently demonstrate the natural transmission of SARS-CoV-2 between people and domestic cats. Samples were collected from 26 SARS-CoV-2 positive household cats after obtaining consent from the Faculty of Veterinary Medicine at Universiti Malaysia Kelantan (UMK) and the Final Year Project (FYP) Committee (UMK/FPV/ACUE/FYP/014/2022). Oropharyngeal swabs were used to prepare samples and then processed for reverse transcriptase-polymerase chain reaction assay (RT-PCR). The results show that only 1 of the cats tested positive for the SARS-CoV-2 while the other 25 tested negative. In conclusion, there is a possibility that cats in Kota Bharu and Bachok, Kelantan, were infected with SARS-CoV-2 from the owners. Positive control showed a bright band of predicted size, confirming this finding.

Keywords: COVID-19, SARS-CoV-2, cats, oropharyngeal, RT-PCR

## ABSTRAK

Abstrak daripada kertas penyelidikan dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan untuk memenuhi sebahagian daripada keperluan kursus DVT 5436 – Projek Penyelidikan.

Kajian ini bertujuan untuk meneroka kejadian jangkitan COVID-19 pada kucing di Kota Bharu dan Bachok, Kelantan, dan seterusnya untuk menunjukkan penularan semula jadi SARS-CoV-2 di antara manusia dan kucing. Sampel telah diambil daripada 26 ekor kucing dimana isi rumah merupakan positif SARS-CoV-2 setelah mendapat kebenaran daripada Fakulti Perubatan Veterinar Universiti Malaysia Kelantan (UMK) dan Jawatankuasa Projek Tahun Akhir (UMK/FPV/ACUE/FYP/014/2022). Sampel disediakan daripada calitan orofarinks kemudian diproses untuk ujian tindak balas rantaian transkriptase-polimerase terbalik (RT-PCR). Hasil menunjukkan bahawa hanya 1 kucing yang diuji positif SARS-CoV-2 manakala 25 yang lain diuji negatif. Kesimpulannya, terdapat kemungkinan kucing di Kota Bharu dan Bachok, Kelantan, dijangkiti SARS-CoV-2 daripada pemiliknya. Kawalan positif menunjukkan jalur terang saiz yang diramalkan, mengesahkan penemuan ini.

Kata kunci: COVID-19, SARS-CoV-2, kucing, orofarinks, RT-PCR

## 1.0 INTRODUCTION

Coronaviruses are part of the Nidovirales order, the Coronaviridae family, and the Orthocoronavirinae subfamily (Malik., 2020). They are spherical (125nm diameter) and have club-shaped spikes on the surface, making them look like solar corona. (Malik., 2020). The new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the seventh coronavirus to infect humans and cause respiratory disorders. It is the causal agent of the current coronavirus disease 2019 (COVID-19) pandemic (Kim *et al*., 2021). It belongs to the genus Betacoronavirus, which contains the other two zoonotic coronaviruses (SARS-1 and MERS) which have recently caused epidemics in humans and animals (Ruiz *et al*., 2020). As a result, the WHO proclaimed the COVID-19 a public health emergency of worldwide concern on the 30th of January 2020 due to serious illness consequences for many nations (Dansana *et al*., 2021).

According to the World Health Organization (WHO), there is no indication that pets may function as a source of human infection by spreading SARS-CoV-2 or that they can spread the virus to other humans (Ruiz *et al*., 2020). However, as of December 2019, when the SARS-CoV-2 epidemic was first recognised, many studies proved that SARS-CoV-2 might infect various animal species, both naturally and experimentally (Gaudreault *et al*., 2020). Due to the close relationship between humans and animals, there are concerns about SARS-CoV-2 transmission from COVID-19 patients to animals, also known as "reverse zoonosis". This theory is crucial because the association between humans and animals, including companion animals, livestock, and wildlife species, are so close. (Gaudreault *et al*., 2020).

The present research was prompted by the fact that many cases have been documented in the Kelantan area, which raises the likelihood that their pets may be afflicted. In

addition, identifying the SARS-CoV-2 virus in cats in various countries raises concerns about the virus's zoonotic potential. Therefore, this research intends to determine whether cats in the Kelantan area are at risk of contracting SARS-CoV-2.

## 2.0 RESEARCH PROBLEM

Various studies suggest that there is a possibility that SARS-CoV-2 can be transmitted from animals to humans; even though it is very unlikely, thus it is of the utmost importance to determine the occurrence of SARS-CoV-2 in animals since animals often contact people. Furthermore, on the 8th of May in 2022, the most recent data revealed that there are currently 4.46 million cumulative cases across Malaysia, with 30,120 instances occurring in the Kelantan area. Thus, the SARS-CoV-2 possibly infected the cat, especially during the infected period of close contact with humans. Hence, this research aims to employ polymerase chain reaction to assess COVID-19 in cats in Kota Bharu and Bachok, Kelantan.

## 3.0 RESEARCH QUESTIONS

1. Is there any SARS-CoV-2 infection evidence in cats in Kota Bharu and Bachok, Kelantan, Malaysia?

## 4.0 RESEARCH HYPOTHESIS

1. Cats in Kota Bharu and Bachok, Kelantan were detected with SARS-CoV-2 using Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR).

## 5.0 RESEARCH OBJECTIVES

1. To detect the presence of SARS-CoV-2 infection in cats in Kota Bharu and Bachok, Kelantan, Malaysia, using Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR).

## 6.0 LITERATURE REVIEW

### 6.1 SARS-CoV-2 characteristics

SARS-CoV-2 is a single-stranded, positive-sense RNA virus with a 30-kb non-segmented genome (Uddin *et al*., 2020). The genome had genomic and phylogenetic similarities with SARS-CoV, especially in the S gene and RBD, suggesting direct human-to-human transmission (Uddin *et al*., 2020).

The SARS-CoV-2 S glycoprotein attaches to the ACE2 receptor on the host's target cell (Kiros *et al*., 2020). Moreover, the M glycoprotein is the most abundant structural protein in the virus, where it helps viral assembly with other structural proteins (Kiros *et al*., 2020). Finally, the primary function of N glycoprotein is to bind and package viral RNA genomes into nucleocapsids (Kiros *et al*., 2020).

### 6.2 SARS-CoV-2 transmission

The earliest hypothesis shows that the virus originated in animals, was then transmitted to people, and then continued transmission among humans since there is evidence that multiple infected individuals were exposed to seafood at Wuhan City's wet animal market (Kiros *et al*., 2020).

In light of the available data, SARS-CoV-2 is likely to have a natural origin. It is transmitted predominantly by inhaling droplets released by an infected individual through coughs (Uddin *et al*., 2020). In addition to the transmission of the disease via

droplets, one of the most important routes of infection is through hands that have touched contaminated surfaces and come into contact with the face, eyes, or nose (Uddin *et al*., 2020).

Changing environmental elements and changes in human behaviour are two important variables that may impact seasonality and should be considered (Uddin *et al*., 2020). For example, outdoor environmental factors, such as temperature and humidity, and indoor such as temperature and moisture, sunlight, and vitamin D status (Uddin *et al*., 2020). Although the seasonality of SARS-CoV-2 transmission has not been confirmed, there is mounting evidence that climate may have a role in the transmission and spread of the illness. (Uddin *et al*., 2020).

**6.3 Clinical manifestation of SARS-CoV-2 infection**

The reported symptoms have ranged from moderate to severe, with some even leading to death accidence in extreme circumstances (Adhikari *et al*., 2020).

In addition, in recent case series from China and the United States, individuals with COVID-19 have experienced other neurological symptoms without showing evidence of direct viral invasion into the brain.

**6.4 SARS-CoV-2 in animals**

Coronaviruses may be transferred from animals to people, albeit this is uncommon (Singla *et al*., 2020). Previous human outbreaks of the primary coronaviruses indicated that bats could act as reservoirs for these viruses, which can transcend the species barrier and infect people and other domestic and wild animals (Singla *et al*., 2020).

The Animal and Plant Health Inspection Service (APHIS) in Ames, Iowa, reported that the National Veterinary Services Laboratories found Malayan Tigress at the Bronx

Zoo infected with SARS-CoV2 (Leroy *et al*., 2020). Additionally, the Bronx Zoo veterinarian verified that the Malayan tiger, her sibling, two Amur tigers, and three African Lions had a non-productive cough. (Leroy *et al*., 2020). Two household dogs and cats also were detected with SARS-CoV-2 (Leroy *et al*., 2020). However, the animals were mostly asymptomatic, and there was no evidence of productive or infectious infection (Leroy *et al*., 2020). Nevertheless, the findings confirm that tramsssion between humans and animals may occur in specific circumstances (Newman *et al*., 2020). Therefore, animals that are SARS-CoV-2 positive test must be monitored and quarantined from humans and other animals until they fully recover (Vetter *et al*., 2020).

**6.5 SARS-CoV-2 detection using Polymerase Chain Reaction**

The clinical diagnosis has conventionally been validated using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) examination of nasopharyngeal swabs. (Wang *et al*., 2020). In tests conducted on the cat, RNA of SARS-CoV-2 was found in consecutive faeces samples and stomach contents using conventional, qualitative PCR, which was later verified by next-generation sequencing (Leroy *et al*., 2020). Quantitative PCR testing and serological examinations are necessary to get a deeper understanding of this infection and the range of outcomes, even though these results demonstrate the existence of viral RNA (Leroy *et al*., 2020).

## 7.0 MATERIALS AND METHOD

### 7.1 Ethics statement and permit approval

Veterinarians supervised the students to ensure the procedures were acceptable and ethical. Sample procedures were carried out with consent from the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan (UMK), and the Final Year Project (FYP) Committee, with the ethics number UMK/FPV/ACUE/FYP/014/2022.

### 7.2 Study area and cat swab sampling

Twenty-six nasal swab samples were obtained from the SARS-CoV-2 positive household's cats around Kota Bharu and Bachok, Kelantan area. When the cat was presented, information such as vaccination status, indoor or outdoor cat and any clinical signs during the owner's COVID-19 infection were documented on an online google form that the owner filled before the sample collection.

Because molecular detection of SARS-CoV-2 is compared to SARS-CoV-2 antibody detection, oropharyngeal swab and blood collection were conducted concurrently, and blood was drawn from the jugular vein. During the swab and blood collection, complete personal protective equipment (PPE) was worn, including a face shield, a face mask, eye goggles, double gloves, and a raincoat shield, to minimise any viral transmission to the handler. All samples were transported in an icebox to the faculty of veterinary medicine's molecular laboratory. After viral extraction and molecular testing, the swabs were refrigerated at -80°C.

### 7.3 Sample Collection

A consent letter was presented to the owner while filling out the online google form before collecting the oropharyngeal swab. The approach and the potential complications that may arise from the sampling process were outlined in advance. The procedures performed on all cats were overseen by a senior veterinarian who ensured acceptable and ethical. Personal protection equipment, which included a raincoat, a face shield, a facemask, double-layer gloves, and a laboratory coat, was worn by the handler to keep them safe and protected. A towel wrap restraining approach was used where the animal was held with its mouth wide open, allowing the oropharyngeal swab to be put directly into the oropharyngeal region. While collecting the swab, care was taken to avoid contamination. After sampling, the swab tip was placed in a 1.5ml screw cap tube prefilled with virus shield solution, permitting nucleic acid stability during sample collection and inactivating nucleases and infectious agents. The swabs are then preserved in the icebox and returned to the laboratory, promptly stored at -80°C.

### 7.4 RNA Extraction

The SARS-CoV-2 is an RNA virus. Thus, the virus must be isolated before it can be amplified using reverse transcriptase-polymerase chain reaction techniques. A Qiagen RNA Viral RNA Mini Kit (50), which contains 50 QIAamp Mini Spin Columns, carrier RNA, collection tubes (2ml), and RNase-free buffer, is used to purify and extract viral RNA from 26 samples in the Faculty of Veterinary Medicine at the Universiti Malaysia Kelantan's biosafety level 3 containment laboratory. The positive control employed in this experiment was obtained from the government hospital.

560 µl Buffer AVL is pipetted into a 1.5 ml microcentrifuge tube containing carrier RNA. In the microcentrifuge tube, 140 µl sample solution was added to the Buffer

AVL–carrier RNA. The tube was incubated at room temperature for 10 minutes and then centrifuged to remove any remaining drops inside the lid. Next, 560 µl ethanol (96–100%) was added to the sample and stirred for 15 seconds using pulse vortexing. Without wetting the rim, 630 µl of the solution was applied from step 4 to the QIAamp Mini column (in a 2 ml collecting tube). Close the cover and centrifuge for 1 minute at 6000 x g (8000 rpm). The QIAamp Mini column was inserted into a clean 2 ml collection tube, with the filtrate tube being discarded. This process was repeated. In the QIAamp Mini column, 500 µl Buffer AW1 was inserted. The cover was closed and centrifuged for 1 minute at 6000 x g (8000 rpm). The QIAamp Mini column was put in a clean 2 ml collection tube (supplied), and the filtrate tube was discarded. In the QIAamp Mini column, 500 µl Buffer AW2 was inserted. The cap was closed and centrifuged at maximum speed for 3 minutes (20,000 x g; 14,000 rpm). The previous collection tube holding the filtrate was discarded, and a new 2 ml collection tube was inserted into the QIAamp Mini column. It was centrifuged at full speed for one minute. A QIAamp Mini column was placed in a clean 1.5 ml microcentrifuge tube, and 60 µl of AVE buffer was equilibrated. The cap was sealed and incubated for one minute at room temperature. Next, it is centrifuged for 1 minute at 6000 x g (8000 rpm). A 60 µl Buffer AVE was used to elute the viral RNA from the QIAamp Mini column into a PCR tube. The collected material is then stored at -80°C.

### 7.5 Detection of SARS-COV-2 by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Covid F3 (5' GAAATGGTCATGTGTGGCGG 3') and Covid R3 (5'GAGACACTCATAAAGTCTGTG 3') primers were employed to detect SARS-CoV-2 specifically. The master mix was made in a sterile 1.5 ml micro centrifuged tube for all samples for a one-step polymerase chain reaction, and the mixture was

prepared according to table 1. Each run includes a positive sample from positive human SARS-C0V-2 and nuclease-free water as a negative control.

RT-PCR reactions were carried out under the following conditions: Initial denaturation at 95 ° C for 5 minutes, 35 cycles of 30 seconds of denaturation at 70 ° C, 30 seconds of annealing at 50 ° C, 30 seconds of extension at 72 °C, and a final extension step at 72 ° C for 5 minutes.

***Table 1 Reverse transcriptase-polymerase chain reaction (RT-PCR) components***

| Reagent | Volume per reaction (µL) | Volume for 20 reactions (µL) |
|---|---|---|
| **2X access quick buffer** | 4.3 | 86 |
| **Nuclease free water** | 12.5 | 250 |
| **AMV-RT** | 0.5 | 10 |
| **Taq polymerase** | 0.5 | 10 |
| **RNAsin** | 0.2 | 4 |
| **Forward primer** | 1 | 20 |
| **Reverse primer** | 1 | 20 |

**7.6 Agarose Gel Electrophoresis**

With 80 ml of TBE water, 2% of 1.6g of agarose gel was added. The gel was microwaved until it became translucent, indicating dissolved. Before pouring the heated agarose gel into the gel-casting tray, 1 ml of Midori green was added. Then, a comb was placed on the tray. After allowing the gel to be set at room temperature for 40 minutes, the gel was used to harden. Then, the comb is removed from the tray it sits on. The tray was placed in the electrophoresis chamber with the wells towards the

cathode side of the instrument. By pipetting up and down multiple times, 1 ml of DNA markers and 4 ml of PCR products were mixed. The mixture was used many times. The electrophoresis lid was closed, and the electrical lead was attached. For 50 minutes, the gel was run at 100 V and 400 mA. The gel was collected once the electricity was turned off. The predicted product size was visualised using a Gel Doc TM EZ Imager (Bio-rad, USA). The expected product size for SARS-CoV-2 is 220bp.

**8.0 RESULTS**

Between April to May 2022, 16 indexes (2 men, 14 women) participants from 14 households with 26 cats were enrolled in this research. The subject consists mainly of women (n = 14, 87.5%), with most aged 18-30-year-old (n = 11, 68.6%). Most respondents stated university education (n = 15, 93.8%). Moreover, all participants were quarantined, and most took care of their cats during the COVID-19 infection period (n = 10, 62.5%). Most respondents were infected with COVID-19 in March 2022 (n= 9, 56.3%) while most did not seem aware of reverse zoonosis (n = 13, 81.3%).

***Table 2: Baseline characteristics of 16 human index participants between April 2022 and May 2022 in Kota Bharu and Bachok, Kelantan (***Calvet *et al*., 2021)

| Characteristics | n (%) |
|---|---|
| **Sex, n (%)** | |
| Men | 2 (12.5) |
| Women | 14 (87.5) |
| **Age, n (%)** | |
| 18-30 | 11 (68.6) |
| 31-40 | 3 (18.8) |
| 41-50 | 2 (12.5) |
| **Highest formal educational attendance, n (%)** | |
| University/post-graduation | 15 (93.8) |
| High school/technical college | 1 (6.3) |
| **Quarantine status, n (%)** | |
| Quarantine at home | 15 (93.8) |
| Quarantine at the quarantine centre | 1 (6.3) |
| **Took care of the cat during the quarantine period, n (%)** | |
| Ownself | 10 (62.5) |
| Someone else | 6 (37.5) |
| **Month infected with COVID-19, n (%)** | |
| March 2022 | 9 (56.3) |
| February 2022 | 2 (12.5) |
| December 2021 | 1 (6.25) |
| October 2021 | 2 (12.5) |
| March 2021 | 1 (6.25) |
| November 2020 | 1 (6.25) |
| **Reverse zoonosis awareness, n (%)** | |
| Yes | 13 (81.3) |
| No | 3 (18.9) |

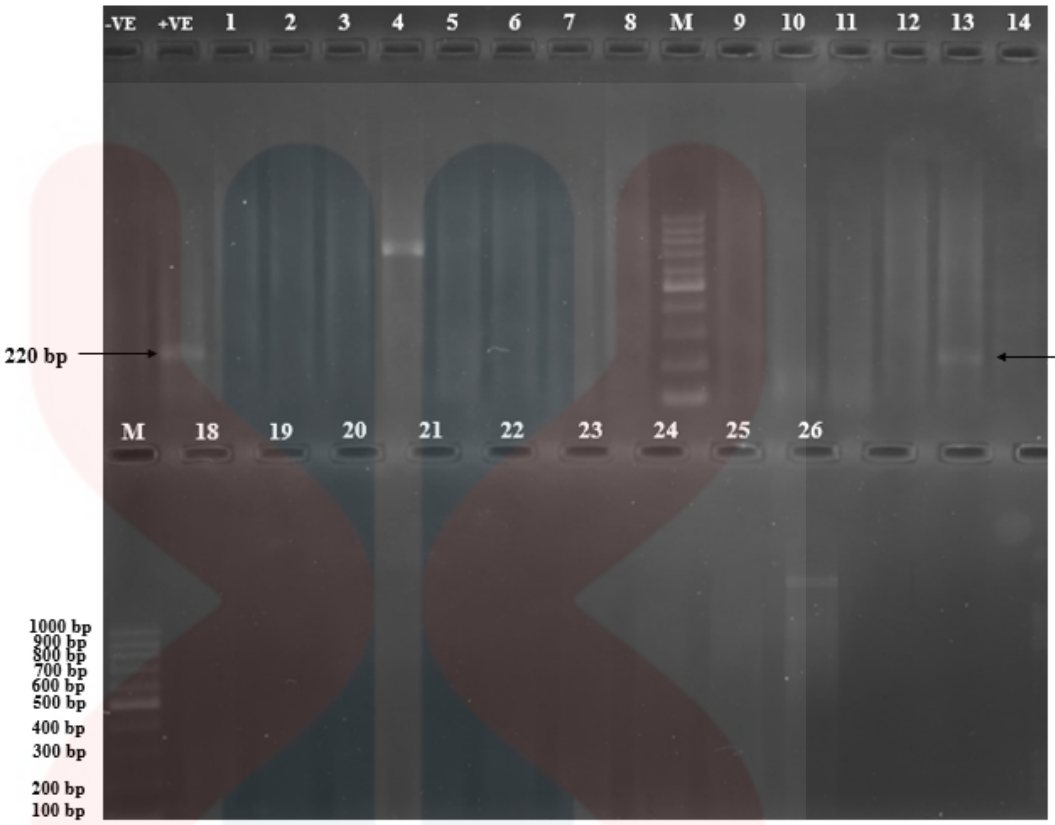



***Figure 1: SARS-C0V-2 detection in cats by agarose gel electrophoresis where M represents ladder, -ve negative control, +ve positive control. Well 1 to 26 represent samples from the oropharyngeal swab.***

Twenty-six (26) oropharyngeal samples were acquired from positive household cats and examined for a reverse transcriptase-polymerase chain reaction. The findings are displayed in Figure 1. Figure 1 reveals a positive outcome for sample number 13. However, Figure 1 also shows samples number 4 and 26; the band is over the predicted size, 220 bp, and thus did not send for sequencing as the bands were not laid on the expected band size. Furthermore, for sample no 13, sequencing should be submitted to confirm the polymerase chain reaction findings. However, due to time constraints, the sample cannot be sent for confirmation.

*Table 3: SARS-CoV-2 (COVID-19) occurrence in positive household cats in Kota Bharu and Bachok, Kelantan*

| Location | Positive | Negative |
|---|---|---|
| Kota Bharu | 1 | 18 |
| Bachok | 0 | 7 |

## 9.0 DISCUSSION

Samples were obtained from 26 cats, and 1 was positive while the other 25 were tested negative, which suggests that there is a chance that cats in Kota Bharu and Bachok, Kelantan were affected by SARS-CoV-2.

Furthermore, the band that indicated a positive result lay within the 220bp, predicted band size and was comparable to the positive control; a human sample tested positive. Based on this finding, the SARS-CoV-2 virus that may infect the cat is the same virus that infected people. Therefore, there is a potential that the human owner may have infected their cat during the quarantine period in which they tested positive for COVID-19. In addition, several studies have shown that cats and dogs in Italy have been exposed to the SARS-CoV-2 virus, where dogs from positive COVID-19 homes are more likely than dogs from negative COVID-19 homes to test positive for SARS-CoV-2 (Patterson *et al*., 2020).

Moreover, there was less than one month gap between when the owner became infected with SARS-CoV-2 in March 2022 and when we obtained the sample in April 2022. SARS-CoV-2 RNA persistence was identified in cats in investigations done in China and the United States for 8 to 17 days, corresponding to 22 to 34 days following the beginning of symptoms in the human COVID-19 case (Calvet *et al*., 2021). This

study is one of the probable explanations for the probability of receiving positive findings even if the owner had SARS-CoV-2 infection long before the sample was taken. Moreover, the cat may be immunocompromised due to its Sporotrichosis infection, which boosts the risk of getting infected with SARS-CoV-2 in this investigation. According to the research findings, the simultaneous presence of SARS-CoV-2 and other secondary infections would raise the viral loads, ultimately resulting in the direct transmission of the coronavirus (Carneiro *et al*., 2022).

Besides, we could not get blood samples from the RT-PCR positive cat in this investigation due to the cat's unwillingness to cooperate throughout the process. Because of this, we cannot compare the detection of SARS-CoV-2 by serological methods in this cat. Moreover, in an experimental investigation, cats infected with the virus ceased shedding the virus 10 days after infection (Patterson *et al*., 2020). Even though various studies may have varied time frames, the infected cat produces antibody responses between 7-13 days after the infection (Patterson *et al*., 2020). Thus, if we collect the cat serum sample and test it for antibodies, we can get a positive result.

However, the negative findings in 25 cats may be broken down into various aspects, such as sample technique. For example, low viral load and extensive degradation in the sample may cause false-negative findings as the swab sample has been kept for 2 weeks under - 80°C before continuing with RNA extraction, which may influence the results. Furthermore, the time interval for the oropharyngeal swab sample to be transferred back to the laboratory also may affect the viral survival rate, although it was transported on the ice. Nevertheless, as the data reveal, it is a real negative for this relatively small sample population, which is confirmed by the positive control.

Moreover, while or after the owner had been infected with COVID-19, most of the cats did not display clinical signs such as coughing, runny nose, or fever, which were also absent throughout the sampling period; this may explain why the majority of cats were found to be negative for the virus. However, one of the cats was not in good condition as it displayed clinical indications such as respiratory distress due to Sporotrichosis, which came out as positive in this investigation.

In addition, several publications describe taking nasal swabs, and rectal swab samples from cats in Hong Kong that is at the centre of a major SARS cluster and viral RNA was found in the rectal swab specimens (Barrs *et al*., 2020). Thus, it is possible that collecting samples from various multiple sites of the same animals will boost the likelihood of detecting SARS-Cov-2 infection.

## 10.0 CONCLUSION

In conclusion, the reverse transcriptase-polymerase chain reaction gave a positive result for 1 sample and a negative result for the remaining 25 samples, indicating a possibility that cats in Kota Bharu and Bachok, Kelantan were infected with SARS-CoV-2 from the owners. Positive control showed a bright band of predicted size, confirming this finding.

## 11.0 RECOMMENDATION AND FUTURE WORK

Detection of SARS-CoV-2 in cats can be enhanced by expanding the sample size to at least 100 cats and incorporating particular sample testing criteria, such as a cat exhibiting respiratory symptoms.

In addition, the scope of research can be increased to learn more about SARS-CoV-2 occurrence in cats.

Furthermore, sampling methods and collection can be improved to preserve the viability of the virus, hence increasing the likelihood of future positive outcomes.

**APPENDIX A**


*Appendix A.1: The poster used in this study*

**APPENDIX B**


***Appendix B.1: The online Google form used in this study***

**APPENDIX C**

Consent form/ Borang persetujuan: *

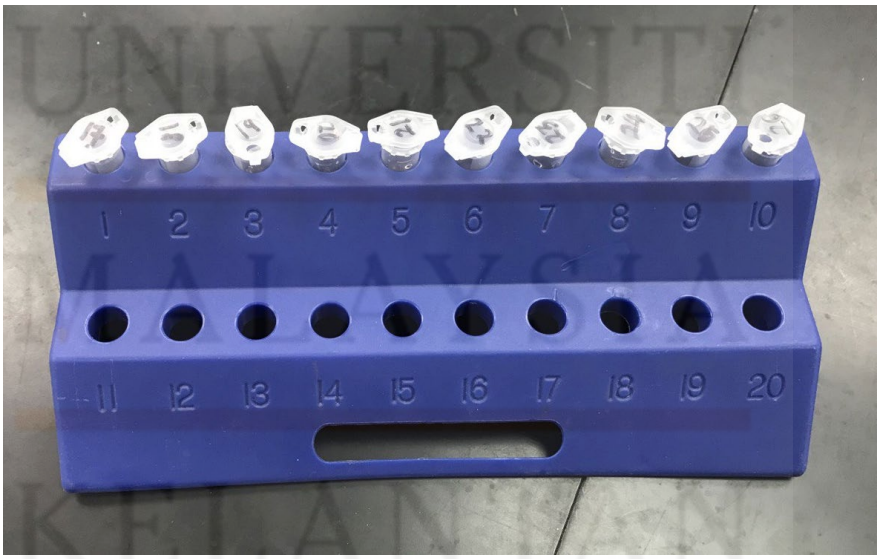
1. I confirm that **I have read** the information above.
2. I understand that **my participation is voluntary** and that I am free to withdraw at any time without giving any reason, without my legal rights being affected.
3. I understand that individuals from the partner universities may look at research data collected during the study, to ensure the study is conducted appropriately.
   I permit for these individuals to have access to my records and there will be **no personal or confidential data will be collected.**

☐ I agree to take part in this study and would like to participate (Saya bersetuju untuk mengambil bahagian)

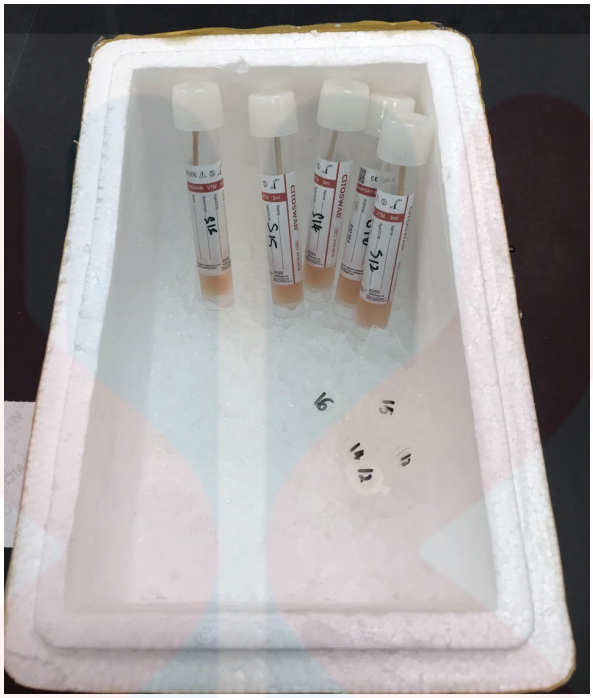
***Appendix C.1: The consent form used in this study***

**APPENDIX D**

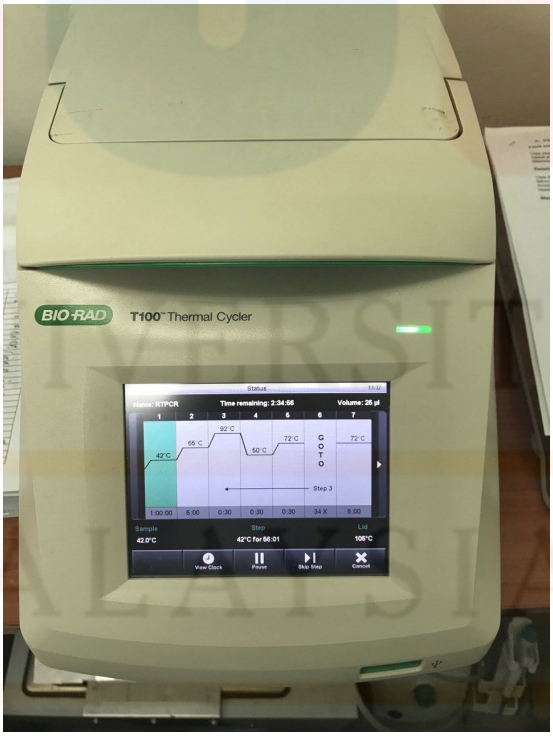

*Appendix D.1: A Qiagen RNA Viral RNA Mini Kit (50), which contains 50 QIAamp Mini Spin Columns, carrier RNA, collection tubes (2ml), and RNase-free buffer.*




*Appendix D.2: The sample during the RNA extraction procedure*



*Appendix D.3: Oropharyngeal sample stored in the icebox*




*Appendix D.4: Thermocycler used for the RT-PCR*

## 12.0 REFERENCES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