SEROLOGICAL DETECTION OF SARS-CoV-2 (COVID-19) IN CATS IN KOTA BHARU AND BACHOK, KELANTAN

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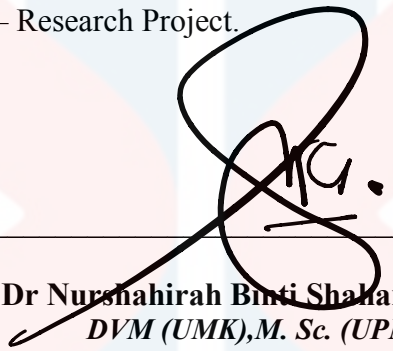
CERTIFICATION

This is to certify that we have read this research paper entitled **'Serological Detection of SARS-CoV-2 (COVID-19) In Cats in Kota Bharu and Bachok, Kelantan'** by Husna Athirah Binti Hasaruddin, and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course DVT 5436 – Research Project.



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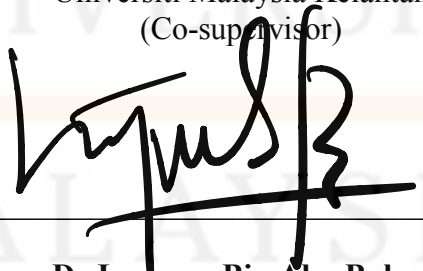

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DEDICATIONS

I dedicate my dissertation work to my parents, Zuraidah and Hasaruddin for their endless love and financial support throughout these 24 years of raising me. My sister Husna Izzati for always being there to lend me a shoulder.

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ABSTRACT

An abstract of the research paper presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement on the course DVT 5436 – Research Project

COVID-19 is a disease caused by SARS-CoV-2 where it can be transmitted from humans to humans via contaminated respiratory droplets. However there are limited studies regarding the possibility of human to animal transmission of COVID-19. The aim of this research is to detect the presence of SARS-CoV-2 antibodies in cats who had previous exposure of COVID-19 from their owners who were tested positive within the region Kota Bharu and Bachok, Kelantan using serological detection via ELISA. Posters and consent form were blasted through social medias and a total of 18 blood samples were collected from the respondents' cats. Serum samples were used for enzyme-linked immunosorbent assay (ELISA) test kit by IDvet in detecting any presence of antibodies against SARS-CoV-2 virus' nucleocapsid protein. The negative ELISA test kit indicated the absence of any SARS-CoV-2 specific antibodies from the serum sample obtained. To sum up this research, it was concluded that all cats involved were tested negative for SARS-CoV-2 even with prior exposure to COVID-19 from their owners as no antibodies were detected.

Keywords: *COVID-19, SARS-CoV-2, ELISA, Nucleocapsid protein*

ABSTRAK

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

Tujuan kajian ini dijalankan adalah untuk mengkaji kejadian jangkitan penyakit Corona 2019 (COVID-19) dalam kalangan kucing yang pernah terdedah kepada COVID-19 melalui tuan mereka yang pernah disahkan positif dalam kawasan Kota Bharu dan Bachok, Kelantan dengan mengesan antibody melalui pengesanan serologi ELISA. Poster serta borang kebenaran telah diagihkan melalui media social dan sejumlah 18 sampel darah berjaya dikumpulkan daripada kucing milik responden, di mana serum darah telah digunakan untuk enzyme-linked immunosorbent assay (ELISA) kit ujian dari IDvet untuk mengesan antibody terhadap protein nucleocapsid virus SARS-CoV-2 di dalam sampel serum. Untuk merumuskan kajian ini, kesimpulannya kesemua kucing yang terlibat tidak dijangkiti SARS-CoV-2 walaupun dengan pendedahan terhadap COVID-19 daripada tuan mereka.

Kata kunci: *COVID-19, SARS-CoV-2, ELISA, Nucleocapsid protein*

1.0 Introduction

The infectious disease, COVID-19, is caused by the SARS-CoV-2 virus. Most infected individuals will only have mild to moderate respiratory symptoms and will recover without the need for medical care. On the other side, some people might get really ill and need medical care. Elderly people and people with underlying medical illnesses including cancer, diabetes, pulomonary disease, or cardiovascular disease are more prone to have serious illness. (WHO, 2020).

The first cases of COVID-19 can be traced back during the 31st December 2019 where the Wuhan Municipal Health Commission in China had filed a report of numerous cases of pneumonia occurring in Wuhan which was soon identified as a novel coronavirus. Only 12 days later on the 12th January 2020 when China had finally publicize the genetic sequencing of this virus known as COVID-19 (WHO, 2020). SARS-CoV-2 quickly expanded to additional countries such as South Korea, Thailand, Singapore, Japan, Italy, Spain, the United States, and the United Kingdom, and was declared a pandemic by the World Health Organization on March 12, 2020.

On January 25, 2020, the first case of COVID-19 was found in Malaysia. It was later determined that three Chinese nationals who had previously been in close contact with an infected person in Singapore were to blame. (Kiros et al., 2020). As for 28th December 2021, there is a total of 42,357 active cases reported in Malaysia with a total accumulation of 2,739,480 local and 7,353 imported cases reported (MOH,2021).

The genomic sequence of SARS-CoV-2 was found to be 96.2 percent similar to the coronavirus RaTG13, which was discovered in horseshoe bats (Rhinolophus sp.) in Yunnan province in 2013. Farm animals, such as pigs, chickens, and ducks, were shown to be immune to the virus in a study of animals that had direct contact with humans. SARS -CoV-2 was not contagious in dogs, but it was highly contagious in ferrets and, notably, young cats (Kiros et al., 2020).

2.0 Research problem

From the start of the pandemic of COVID-19, many lives were severely affected which includes the citizen of Kelantan, Malaysia. Regardless of the thousands of cases of human infection, there are still no demonstration of cats infected with this disease from this state to prove the transmission of this disease from humans to animals. This study aims to use serological detection method in distinguishing the incidence of COVID-19 in cats around Kota Bharu and Bachok.

3.0 Research questions

3.1 What is the incidence of COVID-19 in cats around Kota Bharu and Bachok that had previous exposure from their owner?

4.0 Research hypothesis

4.1 Using ELISA, SARS-CoV-2 antibodies can be detected in cats with previous exposure from their owners in Kota Bharu and Bachok, Kelantan

5.0 Objectives

5.1 To detect the presence of SARS-CoV-2 antibodies in cats serologically using an ELISA antibody test kit.

5.2 To determine the prevalence of COVID-19 in cats with previous exposure from their owners in Kota Bharu and Bachok, Kelantan.

6.0 Literature review

6.1 Characteristic of SARS-CoV-2

Coronaviruses belong to the Orthocoronavirinae subfamily of the Coronaviridae family. The Orthocoronovirinaea subfamily is divided into four genera (Alpha-, Beta-, Gamma-, and Delta-coronaviruses). Coronaviruses are single-chained enclosed ribonucleic acid (RNA) viruses that lack RNA-dependent RNA polymerase enzymes yet encode for them in their genomes. On their surfaces, they feature rod-like extensions (Gönültaş et al., 2020).

They have a broad host range due to receptor protrusions, a high frequency of mutations, and RNA instabilities, and may be found in people, bats, pigs, cats, dogs, rodents, and poultry (Gönültaş et al., 2020).

6.2 Clinical Manifestation

COVID-19 individuals might present with a variety of symptoms. At least 26 of these symptoms were identified. This includes symptoms such as dyspnea, fever, headache, cough and loss sense of taste. COVID-19 can be asymptomatic to some people and causes severe pneumonia that may cause fatality. This means that the severity of the infection might vary. In COVID-19 infection, fever was the most prevalent symptom among the patients. The second most frequent symptom was coughing, which is related to the virus being spread by respiratory droplets. Fatigue has also been frequently observed in various research, making it one of the most common symptoms of the

condition. This symptom might be linked to a rise in viral load as well as the immune system's reaction to the infection (Rosa et al., 2020).

6.3 Transmission of SARS-CoV-2

SARS-CoV-2 is transmitted primarily by contaminated respiratory droplets, with viral infection occurring through direct or indirect contact with nasal, conjunctival, or oral mucosa when respiratory particles are inhaled or deposited on these mucous membranes. The majority of target host receptors are found in the epithelium of the human respiratory system, which includes the oropharynx. Conjunctiva and the digestive system, which can serve as transmission gateways, are additional routes by which infection may spread. (Cevik et al., 2020).

The risk of transmission is influenced by variables such as contact pattern, environment, host infectiousness, and socioeconomic factors. Close range contact, such as 15 minutes face to face and within 2 metres, is the most common mode of transmission. Within houses and during meetings, spread is extremely effective (Cevik et al., 2020).

6.4 Studies of COVID-19 in Pets

A Pomeranian dog originating from China had become the first pet tested positive for SARS-CoV-2 in February 2021 while the second positive case was a cat, also originating from China. The genetic sequences of SARS-CoV-2 between the owner and their pets were similar, suggesting the possibility of human to animal transmission (Kiros et al., 2020).

6.5 Detection of SARS-CoV-2 in Cats Through ELISA

An experimental study of SARS-CoV-2 infection in cats was done in Italy using both molecular and serological detection. A serum sample was collected from a 10-year old European shorthair male cat who was previously exposed by the owner who was diagnosed positive with COVID-19. The cat showed respiratory distress and vomiting for 7 days. At days 14 and 31, the cat's specific serum antibodies to SARS-CoV-2 was examined and detected using ELISA (Natale et al., 2021).

7.0 Materials and methods

7.1 Ethics and Statement Permit Approval

The sampling procedures were done by Veterinarians from Faculty of Veterinary Medicine, Universisi Malaysia Kelantan after duely obtaining approval from the Instituitional Animal Care and Use Committee (IACUC). The ethics number was listed as UMK/FPV/ACUE/FYP/014/2022.

7.2 Sample Collection

Posters and google form were blasted in social media including Facebook, Instagram and Whatsapp to reach the targeted audience. A list of names and info were obtained and interested volunteers were contacted and appointments for blood sample collection were set. (Appendix A.1 & A.2). Personnel involved in sample collection wore equipments including face masks and gloves. Prior to sampling, personnels involved also used COVID-19 self test kits to test themselves to reduce any risk of virus transmission to volunteers and vice versa. A total of 18 blood samples were collected from different household via venepuncture of the jugular vein and stored in plain blood tubes. The plain blood tubes were stored in icebox while transported to the laboratory. The tubes were then centrifuged and the serum were collected and stored at -30°C using an Eppendorf tube storage box.

7.3 Double Antigen Enzyme-Linked Immunosorben Assay (ELISA) Test Kit

IDvet's ELISA test kit ID Screen® SARS-CoV-2 Double Antigen was used to detect SARS-CoV-2 antibodies directed to the nucleocapsid of SARS-CoV-2.

All reagents were allowed to thaw until reaching room temperature before there were homogenized thoroughly. All samples were replicated in order to ensure the accurancy of the data. Then 25 µl of dilution buffer 13 was added to each of the wells. Then the 25 µl of negative control were added to both the wells A1 and B1 while 25 µl positive control were added to the wells C1 and D1. Next, 25µl each of the sample collected were added to the remaining wells. The whole plate was covered and incubated for 45 minutes at 37°C. Next the wells were then emptied thoroughly and each wells were washed 5 times using a minimum of 300 µl of the wash solutions. No drying were done between washes but there were emptied thoroughly.

The next step done was adding 100 µl of conjugate ix into each of the well and the plate was covered again and incubated for another 30 minutes at 21°C. After 30 minutes, the wells were emptied again and washed 5 times with at least 300 µl of wash solution. Wells were not dried between the washes.

The next step involved adding 100 µl of the substrate solution to each of the well. Then the plate was covered again and this time it was incubated for 20 minutes in a dark cupboard. Post 20 minutes, 100 µl of the stop solution were added to each well in the same order how the substrate solution was added. The O.D was then observed, read and recorded at 450 nm.

7.4 Data Interpretation

The sample-to-positive proportion (S/P percent) of each samples were determined and calculated as shown below :

$$\text{S/P \%} = \frac{\text{OD(Sample)} - \text{OD(NC)}}{OD(PC) - OD(NC)} x\ 100$$

Validation of the test was done by calculating the mean of the Optical Density Positive Control O.D. (ODPC) where it was greater than 0.350 and the ratio of the mean of the Positive and Negative Controls O.D. (ODNC) was greater than 3.

If the S/P % of a sample shows less than or equal to 50%, then it is deemed as negative while S/P% between the range 50-60% is deemed doubtful. Only 60% or more are considered as a positive result.

8.0 Results

Table 8.1 shows the result of the optical density reading of the serum samples at 450nm. (Appendix B.2)

Table 8.1: Optical Density Reading Value

	1	**2**	**3**	**4**	**5**
A	0.0072	0.084	0.091	0.078	0.078
B	0.078	0.073	0.081	0.072	0.075
C	1.087	0.072	0.082	0.068	0.074
D	1.016	0.061	0.081	0.071	0.076
E	0.08	0.065	0.107	0.086	0.176
F	0.074	0.08	0.084	0.072	0.178
G	0.128	0.066	0.12	0.096	0.109
H	0.251	0.101	0.125	0.129	0.081

Table 8.2 demonstrate the calculation of Sample to Positive (S/P%) of the serum sample as shown by the formula above

Table 8.2: S/P% of Each Serum Sample

Sample	S/P%	Result
1	0.204	Negative
2	11.725	Negative
3	0.358	Negative
4	-0.870	Negative
5	-0.256	Negative

6	0.870	Negative
7	1.126	Negative
8	0.665	Negative
9	2.099	Negative
10	4.864	Negative
11	0	Negative
12	-56.323	Negative
13	40.962	Negative
14	3.840	Negative
15	18.360	Negative
16	0	Negative
17	10.445	Negative
18	2.048	Negative

9.0 Discussion

The test carried out was valid as the mean value of OD_{PC} was 1.051 while the ratio of the mean values of OD_{PC} and OD_{NC} was 24.671. In this study there were no detection of the SARS-CoV-2 specific antibodies via serological detection using IDvet's ELISA test kit ID Screen® SARS-CoV-2 Double Antigen MultiSpecies ELISA. Based on the result of this research, all the total sample collected from 18 cats were tested negative as the S/P% calculated were all less than 50%. These findings shows no proof of evidence that these cats were infected with COVID-19 even after previous exposure to their COVID-19 positive owners.

The negative results obtained may be due to the small sample size. Serological investigations on cats in Wuhan revealed that SARS-CoV-2 infected cat populations there during the outbreak where 15 of 102 cats tested were positive in ELISA (Sailleau.,et al 2020). Comparing to the sample size of this study to the study held in Wuhan, the ratio is 1:5. Chances of obtaining a positive result might arise with a bigger sample size.

Another plausible cause to the negative findings may be due to the low antibodies titer present in the serum sample hence producing a false negative results. Based on the internal validation report from ID.vet, the ID Screen SARS-CoV-2 Double Antigen Multi Species ELISA test kit has limited sensitivity data as the documented positive sera in animal species, includings cats were not readily available.

Despite the negative results obtained from this study, there are still published research conducted in different countries such as Hong Kong, Belgium, France and the United States of America that proved the ability of transmission of SARS-CoV-2 from humans to both cats and dogs, hence establishing an infection. (Kiros M., et al 2020).

In spite of that, in a similar investigation for SARS-CoV-2 screening where oropharyngeal swabs were collected concurrently, one of the RT-PCR results shows positive for one of the 25 samples. Unfortunately due to the nervous and fierce behaviour shown by the cat, the blood sampling was not done and comparison via serological method isn't able to be conducted.

In addition, during the active infection of COVID-19, the exposure of the owners to their cats were not enlisted hence we are unsure regarding the contact pattern between them which could also contribute to the probability of an infection to occur. Close range contact, such as 15 minutes face to face and within 2 metres, is the most common mode of transmission (Cevik et al., 2020).

Based on the interview with the owners, no clinical symptoms relating to the COVID-19 infections were seen in their pets as well. This differs to a research done in Italy where after being exposed to a positive COVID-19 owner, for the next seven days, the cat had respiratory discomfort and vomiting. The cat's specific blood antibodies to SARS-CoV-2 were investigated and found using ELISA at days 14 and 31(Natale et al., 2021). This gives another stronger proof of evidence that the COVID-19 infection in cats within the district of Kota Bharu and Bachok, Kelantan didn't occur as expected.

10.0 Conclusion

No evidence of COVID-19 infection were found in cats, indicating that none of the cats involved in this study had been or were infected with SARS-CoV-2. SARS-CoV-2 as antibodies were not detected in cats in Kota Bharu and Bachok, Kelantan, according to ELISA data. It must, however, be confirmed by looking into the SARS-CoV-2 cases that occurred during this epidemic and utilising more samples. SARS-CoV-2 transmission from humans to cats requires more research.

11.0 Recommendations and future work

Several recommendation can be done to improve the study. One of it is by creating a more systemic method of data collection for easier access during conducting the experiment. This can be done using data tabulation in Microsoft Excel.

My next recommendation would be increasing the sample size involved and extending the area of interest to collect the sample. Hence the target would be increasing the sample size to at least 50 sample and instead of just around Kota Bharu and Bachok, the area of interest could involved the whole Kelantan state.

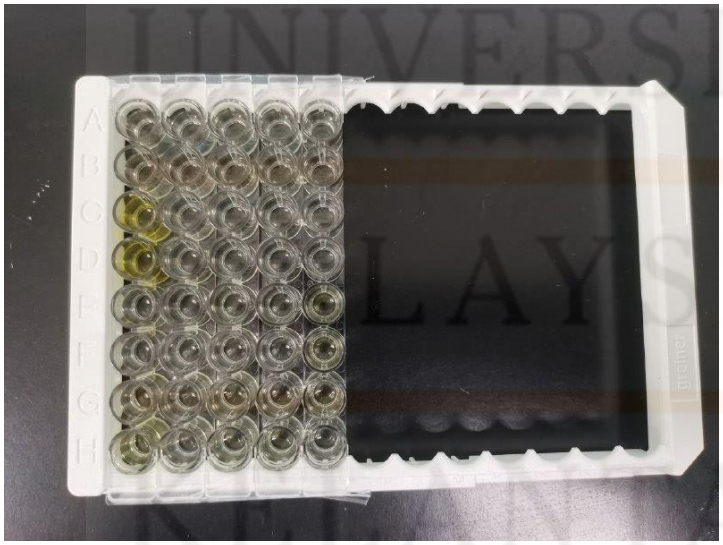
Appendix A

Appendix A.1: Poster used in the study to recruit volunteers

Appendix A.2: Google form in the study to collect data from volunteers

Appendix B

Appendix B.1: Performing the ELISA using serum sample



Appendix B.2: ELISA plate after stop solution was added

References

Cevik, M., Kuppalli, K., Kindrachuk, J., & Peiris, M. (2020). Virology, transmission, and pathogenesis of SARS-Cov-2. *BMJ*, m3862. https://doi.org/10.1136/bmj.m3862

Coronavirus. (2020, January 10). WHO | World Health Organization. https://www.who.int/health-topics/coronavirus#tab=tab_1

Gönültaş, S., Karabağlı, M., Baştuğ, Y., Çilesiz, N. C., & Kadıoğlu, A. (2020). COVID-19 and animals: What do we know?. *Turkish journal of urology*, *46*(4), 249–252. Advance online publication. https://doi.org/10.5152/tud.2020.140520

Kiros, M., Andualem, H., Kiros, T., Hailemichael, W., Getu, S., Geteneh, A., Alemu, D., & Abegaz, W. E. (2020). COVID-19 pandemic: Current knowledge about the role of pets and other animals in disease transmission. *Virology Journal*, *17*(1). https://doi.org/10.1186/s12985-020-01416-9

Sailleau C;Dumarest M;Vanhomwegen J;Delaplace M;Caro V;Kwasiborski A;Hourdel V;Chevaillier P;Barbarino A;Comtet L;Pourquier P;Klonjkowski B;Manuguerra JC;Zientara S;Le Poder S;, C. (n.d.). *First detection and genome sequencing of SARS-COV-2 in an infected cat in France*. Transboundary and emerging diseases. Retrieved May 27, 2022, from https://pubmed.ncbi.nlm.nih.gov/32500944/

Natale, A., Mazzotta, E., Mason, N., Ceglie, L., Mion, M., Stefani, A., Fincato, A., Bonfante, F., Bortolami, A., Monne, I., Bellinati, L., Guadagno, C., Quaranta, E., Pastori, A., & Terregino, C. (2021). SARS-Cov-2 Natural Infection in a Symptomatic Cat: Diagnostic, Clinical and Medical Management in a One

Health Vision. *Animals : an open access journal from MDPI*, *11*(6), 1640. https://doi.org/10.3390/ani11061640

da Rosa Mesquita, R., Francelino Silva Junior, L. C., Santos Santana, F. M., Farias de Oliveira, T., Campos Alcântara, R., Monteiro Arnozo, G., Rodrigues da Silva Filho, E., Galdino Dos Santos, A. G., Oliveira da Cunha, E. J., Salgueiro de Aquino, S. H., & Freire de Souza, C. D. (2021). Clinical manifestations of COVID-19 in the general population: systematic review. *Wiener klinische Wochenschrift*, *133*(7-8), 377–382. https://doi.org/10.1007/s00508-020-01760-4