INHIBITION OF ALPHA AND BETA INTERFERON BY CORONAVIRUSES ISOLATED FROM BATS IN EAST-COAST MALAYSIA

REXZANA ROSE HENRY

(D18A0024)

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

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR

THE DEGREE OF

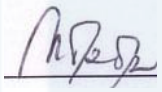
DOCTOR OF VETERINARY MEDICINE

DECEMBER 2022

UNIVERSITI MALAYSIA KELANTAN

CERTIFICATION

This is to certify that we have read this research paper entitled **Inhibition of alpha and beta Interferon by Coronavirus Isolated from bats in East-Coast Malaysia** by Rexzana Rose Henry, and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course DVT 55204 – Research Project.



Prof. Madya. Dr. Maizan Binti Mohamed
BSC (UKM), MSc (UPM), PhD (University of St. Andrew, UK),
Senior Lecturer
Faculty of Veterinary Medicine
Universiti Malaysia Kelantan
(Supervisor)

Dr. Siti Nor Azizah Binti Mahamud
DVM (UPM), MSc (UPM)
Lecturer,
Faculty of Veterinary Medicine
Universiti Malaysia Kelantan

(Co-supervisor)

ACKNOWLEDGEMENT

Special recognition and appreciation for the names that have been very helpful and supportive throughout the completion of this thesis paper:

God Almighty

Prof. Madya. Dr. Maizan

Dr. Siti Nor Azizah Binti Mahamud

Dr. Brenda Sabrina Gilbert

Lab Assistant of PFV UMK Virology Laboratory

Family and friends

Rotation Group 2 DVM5

DVM 18/23 Classmates

Thank You

DEDICATIONS

I dedicate this work to my very loving and supportive family who has been there throughout my journey in the program of Doctor Veterinary Medicine. Everything I have accomplished throughout this degree has been made possible by their physical, emotional, financial and spiritual support. My parents, Henry and Ruslin, My brothers, Hezron, Hitborn, and Harvey and lastly, my pet dogs had inspired me throughout this program and none of this would be possible without them.

I would also like to express my gratitude to my supervisor, Prof. Madya. Dr. Maizan and co-supervisor, Dr. Siti Nor Azizah binti Mahamud for giving me ideas and guidance throughout the preparation of this project. Same goes to Dr. Brenda Sabrina Gilbert for guiding me throughout the completion of the laboratory works related to this project.

I would also like to take this opportunity to give a special thanks to my DVM18/23 classmates for being helpful and supportive throughout the completion of this project. Especially to Taufiq, Hazael, Kosheila, Kirthekka, Yamunasri, thank you for being the best cheerleaders throughout my journey in this program.

TABLE OF CONTENTS

Table of Contents

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

An abstract of the research paper presented to the Faculty of Veterinary Medicine. Universiti Malaysia Kelantan, in partial requirement on the course DVT 55204 – Research Project.

INHIBITION OF ALPHA AND BETA INTERFERON BY CORONAVIRUSES ISOLATED FROM BATS IN EAST-COAST MALAYSIA

By:

REXZANA ROSE HENRY

2022

The novel coronavirus SARS-CoV-2 or also known as the COVID-19 virus were identified in December 2019 in Wuhan, China which had caused a pandemic affecting millions of people in the world. Bats were suggested to be the natural reservoir of this virus where the presence of zoonotic transmission helped in causing the infection in humans. Interferons are part of the human immune system which can induce antiviral activity when produced upon the recognition of viruses present in the body. In order to infect humans, the viral agents need to inhibit the production of interferons. This study was conducted with the aim of evaluating the zoonotic potential of SARS-CoV-like coronavirus isolated from bats in East-Coast Malaysia. Ability of SARS-CoV-like coronavirus isolates to inhibit interferon-α and interferon-β was determined by inoculating the viruses into human lung cells A549 and inhibition of the interferons by RT-PCR using specific primers of the interferons. The viruses able to inhibit the

production of interferons indicates that it can infect and cause infection to humans. In this study, the expression of both interferon-α and interferon-β were not inhibited by the isolated SARS-CoV-like coronaviruses. This indicates that these SARS-CoV- like coronaviruses from bats in East-Coast Malaysia do not have a zoonotic potential to humans. Further study involving the presence of human receptor binding domain (RBD) of the virus needs to be conducted for better understanding of this zoonotic-potential of the virus.

Keywords: COVID-19, SARS-CoV-2, interferon, coronavirus, zoonosis

ABSTRAK

Abstrak kertas penyelidikan yang disampaikan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan dalam syarat sebahagian kursus DVT 55204 ─ Projek Penyelidikan.

PENGHALANGAN PENGHASILAN INTERFERON ALPHA DAN BETA OLEH ISOLAT CORONAVIRUS DARI KELAWAR DI PANTAI TIMUR, MALAYSIA

Oleh:

REXZANA ROSE HENRY

2022

Coronavirus SARS-CoV-2 atau juga dikenali sebagai virus COVID-19 telah dikenal pasti pada Disember 2019 di Wuhan, China yang telah menyebabkan pandemik yang menjejaskan berjuta-juta orang di dunia. Kelawar telah dicadangkan sebagai takungan semula jadi virus ini di mana kehadiran jangkitan zoonotik membantu menyebabkan jangkitan pada manusia. Interferon adalah sebahagian daripada sistem pertahanan badan yang akan melindungi badan dari jangkitan virus. Untuk menjangkiti manusia, virus-virus perlu menghalang penghasilan interferon dari badan manusia. Tujuan kajian ini dijalankan adalah untuk menilai potensi zoonotik coronavirus SARS-CoV-like yang diasingkan daripada kelawar di Pantai Timur Malaysia. RT-PCR telah

dijalankan keatas RNA yang telah diekstrak dari sel paru-paru manusia yang telah diinokulasi dengan virus SARS-CoV-like isolat. Keupayaan virus untuk menghalang penghasilan interferon menunjukkan virus mempunyai potensi zoonotik. Dalam eksperimen ini, ekspresi kedua-dua interferon-α dan interferon-β tidak dihalang oleh coronavirus SARS-CoV-like. Ini menunjukkan bahawa coronavirus SARS-CoV-like yang diisolasi daripada kelawar di Pantai Timur Malaysia tidak mempunyai potensi zoonotik. Walaubagaimanapun, kajian yang lebih lanjut seperti kehadiran domain perlekatan reseptor sel manusia perlu dijalankan untuk lebih memahami keupayaan zoonotik virus ini.

Kata kunci: COVID-19, SARS-CoV-2, interferons, coronavirus kelawar, penghalangan interferon

1.0 INTRODUCTION

1.1 Research background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a β-coronavirus from the *Coronaviridae* family (Xia *et al*., 2020) that causes the coronavirus disease 2019 (COVID-19). According to Callaway *et al.*, 2020, most researchers have agreed that this virus originated from bats, especially horseshoe bats. Two studies conducted by Zhou *et al*. in 2020 found two bat coronaviruses that have been reported to have 96.2% and 93.3% of overall genome similarity to SARS-CoV-2.

The human interferons (IFNs) response is part of the immune system against viral infections (Acharya *et al.,* 2020). There are two types of IFNs. Type I IFNs include IFNs-α, β, and ω, while Type II IFNs only include IFN-γ (Bekisz *et al.*, 2004). This study only focuses on IFNs-α and IFNs-β. IFN-α can induce an antiviral state, inhibiting cell growth, stimulating the activity of natural killer cells and the cytotoxic activities of lymphocytes and macrophages, and finally inducing cell differentiation in normal cells and some neoplastic cells (Pestka *et al.*, 1987) while antiviral and antiproliferative effects are produced by IFN-β (Markowitz, 2007). The inhibition of IFNs productions will lead to the infection from pathogens. This research was done to see the zoonotic potential of coronavirus isolated from bats in East-Coast Malaysia by acting as an interferon antagonist that can prevent immune response and then cause infections in humans.

1.2 Research problem

The emergence of the SARS-CoV-2 pandemic has caused the loss of millions of lives in less than a year and it's been suggested that bats from South East Asia were the source of this disease. SARS-CoV-2-like coronaviruses have been isolated from Thailand. Considering that Kelantan is neighboring Thailand and to date, no study has been conducted on the presence of SARS-CoV-2-like coronaviruses in bats from East Coast, Malaysia.

1.3 Research question

Do the SARS-CoV-2 viruses isolated from East Coast Malaysia have a zoonotic potential to humans?

1.4 Research hypothesis

The SARS-CoV-2 virus does have a zoonotic potential to humans by combating the production of IFN-α and IFN-β.

1.5 Objectives

To investigate the zoonotic potential of SARS-CoV-like virus isolated from bats in East-Coast Malaysia by determining the ability of the virus to inhibit the production of IFN-α and IFN-β.

2.0 LITERATURE REVIEW

2.1 Characteristics and Functions of Human Interferon Alpha and Beta

Interferon (IFN) was initially discovered by Alick Isaacs and Jean Lindermann through a study that they conducted in 1957. The two major categories of IFNs are Type I and Type II IFNs in which Type I IFNs include IFNs-α, β, and ω while Type II IFNs only include IFN-γ. IFNs-α and IFNs-β possess the ability to induce antiviral activities in the immune system (Pestka *et al*,. 1987;Markowitz, 2007). The presence of dsRNAs, viruses, other microorganisms, cytokines and growth factors will lead to the production of IFNs which is mediated by the interferon regulatory factors (IRFs) such as IRF-3 and IRF-7 (Bekisz *et al.*, 2004). Type I IFNs act through the Type I IFN Receptor Complex (IFNAR) which consists of IFNAR-1 and IFNAR-2. IFNAR has an ability to distinguish various types and subtypes of IFN and to elicit different types of responses depending on bound ligands (ref?).

2.2 Coronaviruses

The SARS-CoV-2 virus is one of the SARS-related coronaviruses which belongs in the genus β-coronavirus of the *Coronaviridae* family (Wacharapluesadee *et al*., 2021). Coronavirus is a type of enveloped RNA virus that is spherical in shape and has a diameter of approximately 125 nm. They have club-shape spikes projecting from their surface and giving the appearance of a solar corona, hence the name, coronavirus. These spikes play a role in receptor binding and membrane fusion

(MALIK, 2020).

Coronavirus has four structural proteins that play primary roles in the structure of the viral particles which are the S protein, the M protein, the E protein and finally the N protein as shown in Figure 1. The S protein binds cellular receptors and mediates membrane fusion (Kirchodoerfer, 2016) which will subsequently result in viral entry (MALIK, 2020). The M protein defines the shape of the envelope and is also the most dominant structural protein of the coronavirus (Neuman *et al*., 2011). The E protein is the smallest structural protein of the coronavirus. This structural protein plays an important role in virus production and maturation (Nieto-Torres *et a*l., 2014). Lastly, the N protein is the one that binds to the RNA genome (MALIK, 2020).

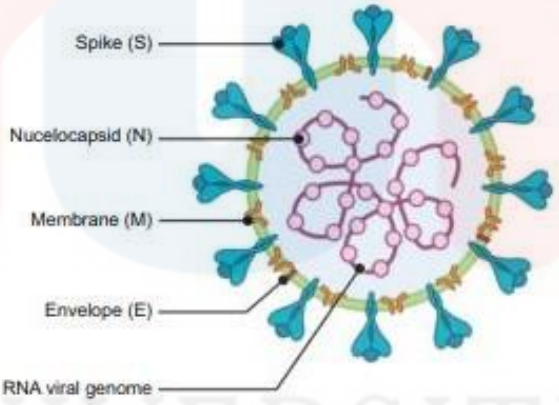


Figure 1 Schematic drawing of the coronavirus structure (Amawi et al., 2020)

2.3 Bats Origin of SARS and MERS

According to Hernandez-Aguilar *et al*., 2021, it has been suggested that the severe acute respiratory coronavirus (SARS-CoV) infection in humans and coronaviruses that are hosted in bats are related. Not only that, the Middle East Respiratory Syndrome (MERS) was also found to be associated with bats according to Hu *et al.*, 2015. Severe Avute Respiratory Syndrome (SARS) first started in the Guangdong Province, southern China around late 2002 and MERS emerged from the Kingdom of Saudi Arabia in June 2021 which both were presentedwith respiratory symptoms such as coughing, dyspnea and pneumonia (Hu *et al.*, 2015). Johara *et al.*, has shown that two other zoonotic viruses such as the Nipah virusand Hendra virus were both known to have come from bats. Previous study reported a bat coronavirus (BatCoV RaTG13) was detected in intermediate horseshoe bat, *Rhinolophus affinis* in Yunnan province (Zhou *et al.*, 2020). Another study wasalso managed to isolate a bat coronavirus which was also collected from bats in Yunnan Province (Zhou *et al.*, 2020). These two viruseshave 96.2% and 93.3% of overall genome similarity to SARS-CoV-2 respectively. This shows that bats, especially horseshoe bats, are natural reservoirs forcoronaviruses. There are also studies that suggest pangolins to be part of the emergenceof SARS-CoV-2 pandemic. A study conducted by Liu *et al.*, managed to detectβ-coronaviruses, pangolin-CoV-2020 in three Malayan pangolins, *Manis javanica* (Liu *et al.*, 2020). Genomic analyses had suggested that the pangolin-CoV-2020 has nucleotide sequence similarity of 90.32% and 90.24% to SARS-CoV-2 and

Bat-CoV-RaTG13 respectively (Liu *et al*., 2020).

2.3 SARS-CoV, SARS-CoV-2, and MERS-CoV IFN Inhibition

Coronaviruses possess a multifunctional protein, the papain-like protease (PLP). This protein has the ability to antagonize the productions of type I IFNs (Clementz *et al*., 2010) by suppressing the host gene expression. This can be supported by a study by Lokugamage *et al.,* 2020 that has reported the low expression of IFN type I in cells infected with SARS-CoV-2 which suggest that there are possibilities that the SARS-CoV-2. These PLPs play a role in the formation of double-membrane vesicles for replication-transcription which restrict the response of IFN-I (Xia *et al*., 2020). Lastly, a study also reported that the most potent IFN-I inhibitor of the coronavirus is through the accessory proteins of the coronavirus in which they would cause the silencing of the IFN-I gene expression (Lei *et al*., 2020).

3.0 MATERIALS AND METHOD

3.1 Sample Collection

Altogether, five positive coronavirus samples used in this project were archived RNA samples retrieved from the Virology Laboratory of Faculty of Veterinary Medicine, Universiti Malaysia Kelantan. The samples were taken from the oropharyngeal swabs of bats. Four of the samples were from Sekayu, Terengganu and one was from Gunung Reng, Kelantan (Table 1). These samples were already confirmed positive as coronavirus with 98-99%sequences similarity to SARS-CoV-2 in humans.

Table 1. List of coronavirus samples used in this study

Samples from Sekayu, Terengganu ● S4 ● S7 ● S11 ● S13	Samples from Gunung Reng, Kelantan ● R63

3.2 Cell Culture

3.2.1 A549 Cells Growth and Maintenance

The A549 (CCL-185) cells, a lung carcinoma of human cells obtained from American type culture collection (ATCC) was used in this study. A growth media containing 10% Fetal Bovine Serum (FBS), 1% penicillin-streptomycin, and Dulbecco's Modified Eagle Medium (DMEM) was used to grow the cells and to ensure they

have sufficient nutrients and energy before cell cycle regulation. For maintenance of the cells, the cells were grown in a T25 flask using a maintenance media containing DMEM with 2% FBS and 1% penicillin-streptomycin. To remove cell debris and dead cells, the culture media was changed every day. First, the T25 flask containing the cells was taken out from the incubator and viewed under the microscope. The old medium was discarded and the flask was rinsed with 1 X phosphate buffered saline (PBS) twice and the PBS was discarded. 6 mL of new growth medium was added into the T25 flask and incubated at 37˚C.

3.2.2 Viral Propagation and Inoculation

The cell suspension of A549 cells were seeded into a T25 flask and incubated for 24 hours at 37˚C with 95% humidity and 5% CO_2. When the cells had reached the desired confluency which was more than 70%, the cells were trypsinised and 10^5 cells/ mL cells were seeded inside a 6-well plate for 24 hours before the inoculation. After the cells reached 70% confluency, the media was removed and washed with PBS twice. Two hundred (200) µL of the supernatant containing confirmed coronavirus were then inoculated into the cells and incubated for one hour in a 37°C incubator. The infected cells were washed twice with PBS and the wells were then filled with 2mL of 2% DMEM. One well of uninoculated was used as a negative control. Figure 3 shows the arrangement of the wells. The plate was incubated for 1 week at 37˚C with 95% humidity and 5% CO_2. Then, the supernatant from the flask was collected and kept inside a 1.5 mL vial tube and stored at -80˚C.

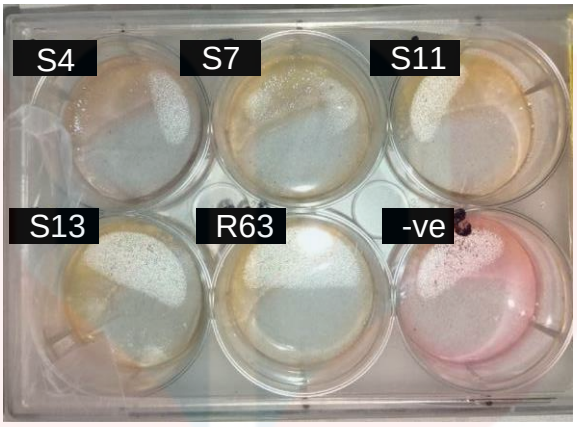


Figure 2 Arrangement of the cell lines inside of the 6-well plate. (Note: S4, S7, S11, S13, and R63 are the infected cells and -ve is the uninfected cells.)

3.3 RNA Extraction

The RNA from the A549 cells suspension was extracted by mixing 250 μL of the cells suspension with 750μL of GeneZOL reagent in a microcentrifuge tube and stored at room temperature for five minutes to permit the complete dissociation of nucleoprotein complexes. Then, 200μL of chloroform was added and the microcentrifuge tube was shaken vigorously for 15 seconds by using a vortex. Then, the mixture was centrifuged at 1400 rpm for 15 minutes at 14 ˚C. The aqueous phase was transferred into a new Eppendorf tube and 600μL of isopropanol was added. The mixture was kept at room temperature for 10 minutes. The mixture was then centrifuged at 14000 rpm for 10 minutes at 4˚C. The RNA formed a white pellet on the bottom of the tube. The supernatant was removed. 1 mL of 75% ethanol was added to the pellet and centrifuged at 140000 rpm for 5 minutes at 4˚C. The supernatant was removed, leaving the pellet on the bottom of the tube. The pellet was air-dried for 10 minutes and then dissolved in 30μL of nuclease free water after

it had dried completely. The pellet was mixed with the nuclease free water by pipetting the mixture up and down for a few times. Lastly, the tube was incubated for 10 minutes at 60℃. Then, the tube was stored at -80℃. Table 2 shows the list of reagents used in this procedure.

Table 2. Reagents used in RNA extraction

Reagents	Volume
GeneZOL reagent	750 µL
Chloroform	200 µL
Isopropanol	600 µL
75% Ethanol	1 mL
Nuclease Free Water	30 µL

3.4 One-Step RT-PCR

The cDNA from the RNA samples was retrieved from the laboratory by taking 5µL of the RNA to a microcentrifuge tube. Then, 1µL of forward primer and 1µL of reverse primer was added into the PCR tube containing the RNA. This mixture was then heated for 5 minutes at 95℃. Once the heating process was complete, the PCR tube was put on ice immediately to cool down.

Then, a Master Mix for One-Step RT-PCR was added into the same PCR tube. This Buffer Mix contains 12.5µL of AccessQuick (Promega, USA), 0.5µL of AMV-RT,

0.2µL of Rnase Inhibitor, 0.3µL of DNA Taq Polymerase (8U) and 4.5µL of nuclease free water, 1 µL of forward primer and 1µL of reverse primer. This BufferMix was mixed for five RNA samples, one negative cell sample, one negative control and one positive control. The steps were the same for virus detection, IFN-α, IFN-β, and GAPDH using different sets of primers. The volume for each reagent is shown in Table 3 and the list of primers that were used in this sudy is as shown in Table 4.

Table 3. Reagents used in RT-PCR

Reagents	Volume (µL)
RNA	5
Forward Primer (20 ρMol)	1
Reverse Primer (20 ρMol)	1
AccessQuick	12.5
AMVRT	0.5
RNase Inhibitor	0.2
DNA Taq Polymerase (8U)	0.3
Nuclease Free Water	4.5

Table 4. Primers for RT-PCR.

Primers	Sequence	Gene	Expected band size	Reference
IF_F IFN_AR	5'-GACTCCATCTTGGCTGGCTGTGA-3' 5'-TGATTTCTGCTCTGACAACCT-3'	IFN-α	140 bp	Designed for this study
IFN_F IFN_BR	5'-CAACTTGCTTGGATTCCTACAAAG-3' 5'-TATTCAAGCCTCCCATTCAATTG-3'	IFN-β	101 bp	Designed for this study
SARS-CoV-2_F SARS-CoV-2_R	5'-TCTACTCTTRYRCAGAATG-3' 5'-GTGGYTCTTTMAMDTMCTC-3'	RdRp	120 bp	Designed for this study
GAPDH_F GAPDH_R	5'-GAGTCAACGGATTTGGTCGT-3' 5'-GACAAGCTTCCCGTTCTCAG-3'	GAPDH	195 bp	Xie *et al.*

The mixture was then loaded in a thermocycler with the RT-PCR protocol setting for

35 cycles. Amplification was optimized and performed by using the Biorad thermocycler where cDNA synthesis was conducted at 42°C for 60 minutes for 1 cycle. 32 cycles of RNA inactivation was conducted at 65°C for 5 minutes, denaturation at 92°C for 1 minute, annealing at 50°C for 1 minute, extension at 72°C for 1 minute were done followed by post-extension at 72°C for 5 minutes. The reaction cycle of One-Step RT-PCR is shown in Table 5.

Table 5. Reaction cycle of One-Step RT-PCR Protocol

Steps	Reactions	Temperature (˚C)	Time (min)
1	cDNA synthesis	42	60
2	RT inactivation	65	5
3	Denaturation	92	1
4	Annealing	50	1
5	Extension	72	1
6	Post-extension	72	5

3.5 Gel Electrophoresis

A 2% agarose gel was prepared by mixing 40 ml of Tris-Borate (TBE) buffer solution with 0.8 g of agarose powder inside a glass media bottle. The mixture was heated in a microwave for 4 minutes on medium high heat then cooled down at room temperature for approximately 3-5 minutes or until the bottle was cool enough to be held with gloved hands. One µL Midori green dye was mixed in and the mixture was swirled

gently and then poured into a casting tray with 10 combs and was let set for 20 minutes. After the gel had set, 10µL of amplified samples was mixed with 2µL of loading dye and loaded into the wells of the agarose gel. The gel electrophoresis was run for 45 minutes on 100 volts (V). The results of the gel electrophoresis were then visualized with a UV transilluminator imager. The same concentration of agarose powder was used for both IFN-α and IFN-β reactions.

4.0 RESULTS

4.1 RT-PCR of housekeeping gene GAPDH in A549 cells

Expected band of 195 bp GAPDH housekeeping gene was observed in all the infected and untreated cells with almost the same intensity, indicating that all the cells have the same concentration of RNA before proceeding to RT-PCR (Figure 3).

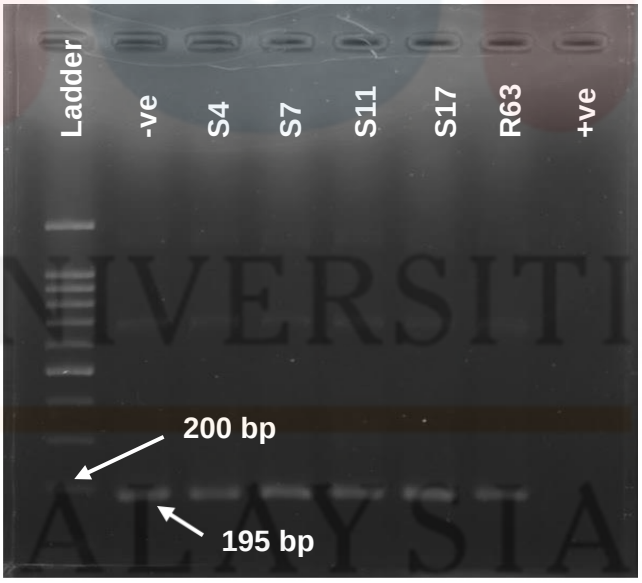


Figure 3 Gel electrophoresis results of housekeeping gene GAPDH

-ve = Negative control of A549 cell.

S4 +ve = A549 cell inoculated with S4 virus sample.

S7 +ve = A549 cell inoculated with S7 virus sample.

S11 +ve = A549 cell inoculated with S11 virus sample.

S13 +ve = A549 cell inoculated with S13 virus sample.

R63 +ve = A549 cell inoculated with R63 virus sample.

+ve = Positive control of A549 cell.

4.2 RT-PCR of SARS-CoV-2 in A549 cells

An expected band of 120 bp was observed in all infected cells and positive control, but no band was observed in the negative control (Figure 4). This shows that the virus was present in the infected samples and in the positive control.

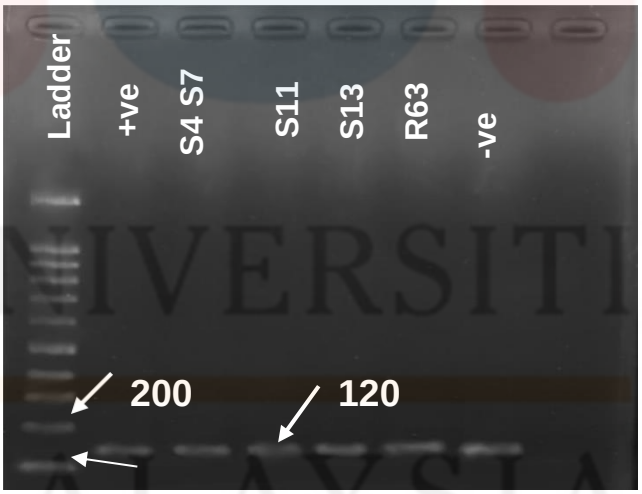


Figure 4 Gel electrophoresis results of SARS-CoV-2 in A549 cells

+ve = Positive control of A549 cell.

S4 +ve = A549 cell inoculated with S4 virus sample.

S7 +ve = A549 cell inoculated with S7 virus sample.

S11 +ve = A549 cell inoculated with S11 virus sample.

S13 +ve = A549 cell inoculated with S13 virus sample.

R63 +ve = A549 cell inoculated with R63 virus sample.

-ve = Negative control of A549 cell.

4.3 RT-PCR for IFN-α IFN-β and from treated A549 cells.

An expected band of 140 bp was expressed in all of the infected cells and the negative control (Figure 6). This indicates that the virus did not inhibit the production of IFN-α. An expected band of 101 bp was expressed in all the infected cells and uninfected cells, but not in the positive control and negative control (Figure 7). Hence, IFN-β production was not inhibited in the infected and uninfected cells.

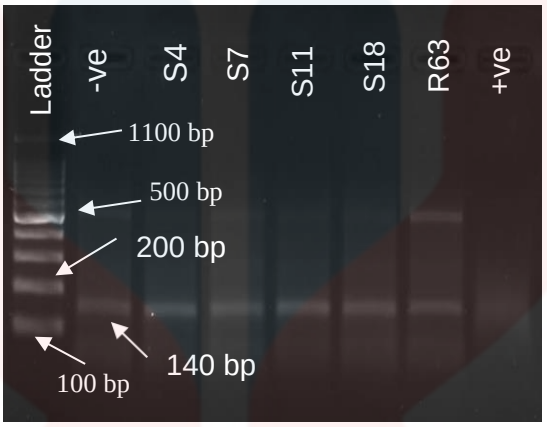


Figure 5 Gel electrophoresis results for IFN- α

-ve = Negative control (Uninfected cells)

S4 +ve = A549 cell inoculated with S4 virus sample.

S7 +ve = A549 cell inoculated with S7 virus sample.

S11 +ve = A549 cell inoculated with S11 virus sample.

S13 +ve = A549 cell inoculated with S13 virus sample.

R63 +ve = A549 cell inoculated with R63 virus sample.

+ve = Negative control of A549 cell.

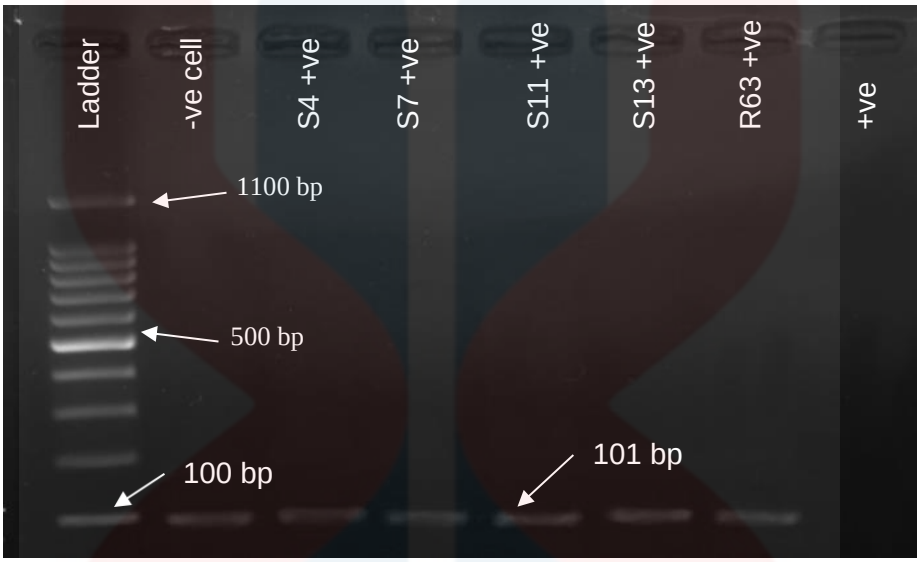


Figure 6 Gel electrophoresis results for IFN-β

-ve cell = Negative control (Uninfected cells).

S4 +ve = A549 cell inoculated with S4 virus sample.

S7 +ve = A549 cell inoculated with S7 virus sample.

S11 +ve = A549 cell inoculated with S11 virus sample.

S13 +ve = A549 cell inoculated with S13 virus sample.

R63 +ve = A549 cell inoculated with R63 virus sample.

+ve = Positive control SARS-CoV-2.

5.0 DISCUSSION

As previously mentioned in this study, a study conducted by Zhou *et al*. in 2020 and another study conducted by Zhou *et al*. in 2020 managed to detect bat coronaviruses with 96.2% and 93.3% of overall genome similarity to SARS-CoV- 2 respectively. This suggested there was presence of interspecies transmission of the virus either from bat to human or involved the intermediate species such as pangolin (Liu *et al*., 2020).

The IFN system is an important system that plays a role in the immune response in humans which will be induced in the presence of viral antigens (Kumar *et al.*, 2021). This system works in a way where viral infections will trigger the production of IFNs, especially IFN Type I that will induce IFN-stimulated genes (ISGs) transcription that will lead to the immune response against the viral infections (Mesev *et al.*, 2019). Therefore, viruses need to evolve or find ways to escape the IFN system in order to evade the human host cells. This study was conducted to see the zoonotic potential of the ability of the SARS-CoV-like viruses isolated from bats from East Coast, Malaysia

through their ability to inhibit the production of IFN Type I, particularly with the virus infection that involves the IFN-α and IFN-β in human immune system.

SARS-CoV proteins have inhibitory effects on type I IFN-mediated antiviral immune responses and since it has 82% nucleotide similarity with the SARS-CoV-2 genome, the SARS-CoV-2 proteins were expected to have the same inhibitory effect on type I IFNs (Kim *et al.*, 2021). This study revealed that IFN-α and IFN-β bands were present in both infected and non-infected A549 cells. This indicated that coronaviruses isolated from bats in East Coast, Malaysia cannot inhibit the production of IFN-α and IFN-β. Hence, they may not cause the disease in humans and not the virus of public health concern although these coronaviruses are closely related to human SARS-CoV-2 (98-99% sequences identities).

6.0 CONCLUSION AND RECOMMENDATION

From this study, it can be concluded that the SARS-CoV-like coronaviruses isolated from bats in East-Coast Malaysia cannot inhibit the production of IFN in human host cells. Hence, these viruses may not have a zoonotic potential. However, further studies should be done continuously to evaluate the zoonotic potential of these coronaviruses since these coronaviruses may evolve to have the ability to infect humans in the future. The studies may be conducted by using more samples from different areas in East-Coast Malaysia to have more accurate results.

REFERENCES

Bekisz, J., Schmeisser, H., Hernandez, J., Goldman, N. D., & Zoon, K. C. (2004). Human interferons alpha, beta, omega. *Growth Factors, 22*, 243-251. 10.1080/08977190400000833

Callaway, E., Ledford, H., & Mallapaty, S. (2020). Six months of coronavirus: the mysteries scientists are still racing to solve. *Nature, 583*, 178-179. 10.1038/d41586-020-01989-z

Cheetham, B. F., McInnes, B., Matamadiotis, T., Murray, P. J., Alin, P., Bourke, P., Linnane, A. W., & Tymms, M. J. (1991). Structure-function studies of human interferons-α: enhanced activity on human and murine cells. *Antiviral Research, 15*, 27-40.

Guan, Y., Zheng, B. J., He, Y. Q., Liu, X. L., Zhuang, Z. X., Cheung, C. L., Luo, S. W., Li, P. H., Zhang, L. J., Guan, Y. J., Butt, K. M., Wong, K. L., Chan, K. W., Lim, W., Shortridge, K. F., Yuen, K. Y., Peiris, J. S. M., & Poon, L. L. L. (2003) Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. *Science (New York, N.Y.), 302*(5643), 276-278. 10.1126/science.1087139

Halpin, K., Young, P. L., Field, H. E., & Mackenzie, J. S. (2000). Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra

virus. *The Journal of General Virology*, *81*, 1927-1932. 10.1099/0022-1317-81-8-1927

Hernández-Aguilar, I., Lorenzo, C., Santos-Moreno, A., Naranjo, E. J., & Navarrete-Gutiérrez, D. (2021). Coronaviruses in Bats: A Review for the Americas. *Viruses.*, *13*(7), 1226. 10.3390/v13071226

Johara, M. Y., Field, H., Rashdi, A. M., Morissy, C., Heide, B., Rota, P., Adzhar, A., White, J., Daniels, P., Jamaluddin, A., & Ksiazek, T. (2001). Nipah Virus Infection in Bats (Order Chiroptera) in Peninsular Malaysia. *Emerging Infectious Diseases*, *7*(3), 439-441. 10.3201/eid0703.017312

Kumar, A., Ishida, R., Strilets, T., Cole, J., Lopez-Orozco, J., Fayad, N., Felix-Lopez, A., Elaish, M., Evseev, D., Magor, K. E., Mahal, L. K., Nagata, L. P., Evans, D. H., & Hobman, T. C. (2021). SARS-CoV-2 Nonstructural Protein 1 Inhibits the Interferon Response by Causing Depletion of Key Host Signaling Factors. *Journal of Virology*, *95*(13). 10.1128/JVI.00266-21

Liu, P., Jiang, J.-Z., Wan, X.-F., Hua, Y., Li, L., Zhou, J., Wang, X., Hou, F., Chen, J., Zou, J., & Chen, J. (2020). Are pangolins the intermediate host of the 2019 novel coronavirus (SARS-CoV-2)? *PLoS Pathogens*, *16*(5). 10.1371/journal.ppat.1008421

Malik, Y, A. (2020) Properties of coronavirus and SARS-CoV-2. *The Malaysian Journal of Pathology*, 42(1), 3-11 PMID: 32342926

Markowitz, C. E. (2007). Interferon-beta: mechanism of action and doing issues. *Neurology*, 68. 10.1212/01.wnl.0000277703.74115.d2

Mesev, E. V., LeDesma, R. A., & Ploss, A. (2019). Decoding type I and III interferon signalling during viral infection. *Nature Microbiology*, *4*(6), 914-924. 10.1038/s41564-019-0421-x

Neuman, B. W., Kiss, G., Kunding, A. H., Bhella, D., Baksh, M. F., Connelly, S., Droese, B., Klaus, J. P., Makino, S., Sawicki, S. G., Siddell, S. G., Stamou, D. G., Wilson, I. A., Kuhn, P., & Buchmeier, M. J. (2011). A structural analysis of M protein in coronavirus assembly and morphology. *Journal of structural Biology*, *174*(1), 11-22. 10.1016/j.jsb.2010.11.021

Nieto-Torres, J. L., DeDiego, M. L., Verdiá-Báguena, C., Jimenez-Guardeño, J. M., Jose A Regla-Nava, J. A., Fernandez-Delgado, R., Castaño-Rodriguez, C., Alcaraz, A., Torres, J., Aguilella, V. M., & Enjuanes, L. (2014). Severe acute respiratory syndrome coronavirus envelope protein ion channel activity promotes virus fitness and pathogenesis. *PLoS Pathogens*, *10*(5), e1004077. 10.1371/journal.ppat.1004077

Park, A., & Iwasaki, A. (2020). Type I and Type III Interferons – Induction, Signaling, Evasion, and Application to Combat COVID-19. *Cell Host*

Microbe, *27*(6), 870-878. 10.1016/j.chom.2020.05.008

Pestka, S., Langer, J. A., Zoon, K. C., & Samuel, C. E. (1987). Interferons and their actions. *Ann. Rev. Biochem*, 727-777.

Shi, C.-S., Qi, H.-Y., Boularan, C., Huang, N.-N., Abu-Asab, M., Shelhamer, J. H., & Kehrl, J. H. (2014, Sep 15). SARS-coronavirus open reading frame-9b suppresses innate immunity by targeting mitochondria and the MAVS/TRAF3/TRAF6 signalosome. *Journal of Immunology (Baltimore, Md.: 1950)*, *193*(6), 3080-3089. 10.4049/jimmunol.1303196

Wacharapluesadee, S., Tan, C. W., Maneeorn, P., Duangkae, P., Zhu, F., Joyjinda, Y., Kaewpom, T., Chia, W. N., Ampoot, W., Lim, B. L., Rodpan, A., Noradechanon, K., Phaichana, T., Jantarat, N., Thongnumchaima, B., Tu, C., Crameri, G., Stokes, M. M., Hemachudha, T., & Wang, L.-F. (2021, February 09). Evidence for SARS-CoV-2 related coronaviruses circulating in bats and pangolins in Southeast Asia. *Nature Communications*, *12*, 1-9. 10.1038/s41467-021-21240-1

Wong, A. C. P., Lau, S. K. P., & Woo, P. C. Y. (2021, Oct 29). Interspecies Jumping of Bat Coronaviruses. *Viruses*, *13*(11), 2188. 10.3390/v13112188

Xiao, K., Zhai, J., Feng, Y., Zhou, N., Zhang, X., Zou, J.-J., Li, N., Guo, Y., Li, X., Shen, X., Zhang, Z., Shu, F., Huang, W., Li, Y., Zhang, Z., Chen, R.-A., Wu, Y.-J., Peng, S.-M., Huang, M., … Shen, Y. (2020, July). Isolation of

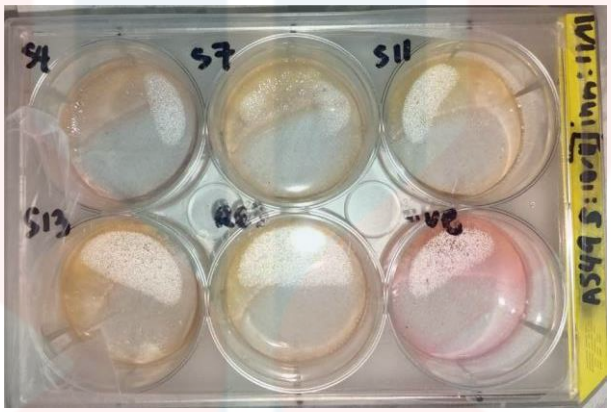
SARS-CoV-2-related coronavirus from Malayan pangolins. *Nature*, *583*(7815), 286-289. 10.1038/s41586-020-2313-x

Yu, D., Li, H., Xu, R., He, J., Lin, J., Li, L., Li, W., Xu, H., Huang, S., & Huang, J. (2003, October 17). *Prevalence of IgG Antibody to SARS-Associated Coronavirus in Animal Traders --- Guangdong Province, China, 2003*. CDC. Retrieved December 16, 2022, from https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5241a2.htm

Zhou, H., Chen, X., Hu, T., Li, J., Song, H., Liu, Y., Wang, P., Liu, D., Yang, J., Holmes, E. C., Hughes, A. C., Bi, Y., & Shi, W. (2020, June 8). A Novel Bat Coronavirus Closely Related to SARS-CoV-2 Contains Natural Insertions at the S1/S2 Cleavage Site of the Spike Protein. *Curr Biol*, *30*(11), 2196-2203. 10.1016/j.cub.2020.05.023

Zhu, H., Wei, L., & Niu, P. (2020, March 2). The novel coronavirus outbreak in Wuhan, China. *Global Health Research and Policy*, *5*. 10.1186/s41256-020-00135-6

APPENDIX A



Cells arrangement in a 6-well plate after the virus inoculation.

Samples used in this study.