PRESENCE OF HUMAN CELL RECEPTOR ACE2 AND TMPRSS2 IN HUMAN LUNG CELL PRODUCED BY BAT CORONAVIRUSES FROM EAST COAST MALAYSIA

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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

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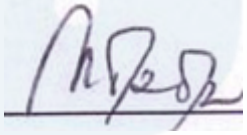
CERTIFICATION

This is to certify that we have read this research paper entitled Presence of host cell factor ACE2 and TMPRSS2 in Human lung cell produced by bats' coronaviruses from East Coast Malaysia by Hazael Sharmila A/P Albert Charles, 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.



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Family

DVM 5 Class of 2018/2023

Thank you.

DEDICATIONS

I dedicate my dissertation to my wonderful parents, Albert Charles and Paramesuary, for always loving and supporting me. Also, to my beloved siblings, Bethany and Daniel who always encouraged me to do my best.

I dedicate this dissertation to my helpful and highly motivated Supervisor Associate Professor Dr. Maizan Mohamed and Dr. Brenda Sabrina Gilbert. Without them, it will not be possible for me to complete this dissertation successfully.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

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

A recent human pandemic was brought on by the novel severe acute respiratory syndrome coronavirus 2, or SARS-CoV-2. The genome of SARS-CoV-2 has revealed a tight link with bat SARS-like coronavirus strains from Asia, and these bats are thought to constitute the virus's reservoir. Human SARS-CoV-2 has identified human receptor angiotensin-converting enzyme 2 (ACE2) and human cellular serine protease TMPRSS2 for the viral S protein to bind and enter the host cells. Early this year, our group has isolated five SARS-CoV-2 like viruses (98-99% RdRp sequence identity with human SARS-CoV-2) from bats in East Coast Malaysia (Four from Sekayu, Terengganu and one from Gunung Reng, Kelantan) and the zoonotic potential of these viruses to human is still unknown. The goal of this study is to examine the zoonotic potential of these five SARS-CoV- 2 like coronaviruses that were isolated from bats in Terengganu and Kelantan, Malaysia. Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to determine whether the viruses could cause pathogenicity in humans. The viruses have shown able to use the ACE2 human receptors but did not use the serine protease TMPRSS2 for the binding and enter the host cell. This indicates that the SARS- CoV- 2 like viruses isolated from bats in East Coast, Malaysia might possess some degree of

zoonotic potential, as it can enter the human lung cell A549 through the binding to ACE2 receptor but without the help of TMPRSS2 as by human SARS-CoV-2. So, these viruses may have a zoonotic potential to human.

Keywords: SARS-CoV-2-like coronaviruses, ACE2 receptors, TMPRSS2 serine protease, Human Lung Cell, Zoonotic

ABSTRAK

Abstrak kertas penyelidikan yang disampaikan kepada Fakulti Perubatan Veterinar dalam syarat sebahagian kursus DVT55202 - Projek Penyelidikan.

Pandemik manusia baru-baru ini disebabkan oleh novel sindrom pernafasan akut teruk coronavirus 2, atau SARS-CoV-2. Genom SARS-CoV-2 telah mendedahkan kaitan rapat dengan strain coronavirus seperti SARS kelawar dari Asia, dan kelawar ini dianggap sebagai takungan virus.SARS-CoV-2 manusia telah mengenal pasti enzim penukar angiotensin manusia 2 (ACE2) dan protease serin selular manusia TMPRSS2 untuk protein S virus untuk mengikat dan memasuki sel perumah. Pada awal tahun ini, kumpulan kami telah mengasingkan lima virus seperti SARS-CoV-2 (98-99% RdRp jujukan identiti dengan SARS-CoV-2 manusia) daripada kelawar di Pantai Timur Malaysia (Empat dari Sekayu, Terengganu dan satu dari Gunung Reng, Kelantan) dan potensi zoonosis virus ini kepada manusia masih belum diketahui.Matlamat kajian ini adalah untuk mengkaji potensi zoonosis lima jenis coronavirus SARS-CoV-2 ini yang diasingkan daripada kelawar di Terengganu dan Kelantan, Malaysia. Reverse transcriptase-polymerase chain reaction (RT-PCR) digunakan untuk menentukan sama ada virus boleh menyebabkan patogenik pada manusia. Virus tersebut telah menunjukkan kebolehan menggunakan reseptor manusia ACE2 tetapi tidak menggunakan serine protease TMPRSS2 untuk mengikat dan memasuki sel hos.Ini menunjukkan bahawa virus seperti SARS- CoV- 2 yang diasingkan daripada kelawar di Pantai Timur, Malaysia mungkin mempunyai beberapa tahap potensi zoonosis, kerana ia boleh memasuki sel paru-paru manusia A549 melalui pengikatan kepada reseptor ACE2

tetapi tanpa bantuan TMPRSS2 seperti oleh manusia SARS-CoV-2. Jadi, virus ini mungkin mempunyai potensi zoonosis kepada manusia.

Kata kunci: Koronavirus seperti SARS-CoV-2, reseptor ACE2, protease serin TMPRSS2, Sel Paru-paru Manusia, Zoonotik

1.0 INTRODUCTION

Research background

SARS-coronavirus 2 is the causative agent of the 2019 Coronavirus illness outbreak (SARS-CoV-2). After SARS-CoV-1 and MERS-CoV, SARS-CoV-2 is the third beta-coronavirus to produce a pandemic. As far as we know, this is the sixth coronavirus to infect humans (Grudlewska-Buda *et al.,* 2021). This virus has a spherical shape with a positive, single-stranded RNA genome, and it has an encapsulation diameter of 120 nm. Initial symptoms of this virus infection included fever, weakness, a dry cough, and trouble breathing (Liu Y-C *et al.,* 2020). It is currently unknown where this virus originated or how it spread. Numerous investigations on animals, particularly bats of the genus Rhinolophus, have been done since the appearance of this virus to discover the potential reservoir and intermediate hosts. *R.affinis*, RaTG13 bats in China carry SARS-CoV-2 more closely than bats in Thailand, Cambodia, or Japan, with a whole-genome level similarity of 96.1%. (Temmam *et al.,* 2022). It is likely that bats serve as reservoir hosts for SARS-CoV-2, however it is unknown whether the virus infected humans directly or was spread from animals to humans via intermediate hosts. The bat-isolated virus may pose a zoonotic threat to people, according to this evidence. Entry of coronaviruses into cells depends on the priming of the viral spike (S) protein by a protease from the infected cell and the binding of the S protein to cellular receptors. For entry, SARS-CoV-2 uses the SARS-CoV receptor ACE2, and for S protein priming, the virus employs the serine protease TMPRSS2 (Hooffmann *et al.,* 2020)

Bats in Malaysia should be tested for the SARS-CoV-2 related virus after similar viruses were discovered in bats in nearby countries like Thailand and Laos (Temmam *et al.,* 2022). Five viruses similar to SARS-Coronavirus-2 have been isolated and identified by our team from bats in Terengganu and Kelantan, Malaysia (unpublished manuscript). However, the zoonotic potential of these viruses has not been studied. Human lung cells were infected with them to test their ability to adhere to and enter the host cell via the ACE2 receptor and the serine protease TMPRSS2. Like human SARS-CoV-2, it may be able to infect humans if it can enter cells through connecting with the ACE2 receptor and the serine protease TMPRSS2 for S protein binding.

1.1 Research problem

The entire country has been affected by COVID-19, which is caused by SARS-CoV-2. It's highly contagious and has rapidly travelled over the globe. Humans and animals equally can contract respiratory diseases from them. The viruses that spread from bats to humans are likewise connected to these. Therefore, the purpose of this study is to determine whether the SARS-CoV-2-like virus recently identified from Malaysian bats is zoonotic and whether or not it is pathogenic by their ability to express ACE2 receptor and the cellular serine protease TMPRSS2 as done by SARS-CoV-2.

1.2 Research questions

1.2.1 Can SARS-CoV-2 like virus isolated from bats in East Coast, Malaysia able to infect human lung cells by expressing the ACE2 receptor and cellular serine protease TMPRSS2 similar to human SARS-CoV-2?

1.2.2 Do SARS-CoV-2-like viruses isolated from bats in East Coast, Malaysia have a zoonotic potential?

1.3 Research hypothesis

1.3.1 SARS-CoV-2 like virus isolated from bats in East Coast, Malaysia able to express ACE2 receptor and cellular serine protease TMPRSS2 in human lung cells.

1.3.2 SARS-CoV-2 like virus isolated from bats in East Coast, Malaysia may have a potential to become a zoonotic virus.

1.4 Objectives

1.4.1 To determine the zoonotic potential of the SARS-CoV-2 like virus isolated from bats in East Coast, Malaysia.

1.4.2 To determine the ability of the SARS-CoV-2 like virus to express ACE2 receptor and the cellular serine protease TMPRSS2 in human lung cells.

2.0 LITERATURE REVIEW

2.1 Morphology and characteristics of SARS-CoV-2

The biggest RNA viral genomes are coronaviruses' (26-32kb). It is categorised as a member of the Nidovirales order, which includes the subfamily Corovirinae of the Coronaviridae family (Liu Y-C *et al.,* 2020). The average size of a virions is between 50 and 200 nm. SARS-CoV-2 virion can take on spherical, pleomorphic, or oval forms. Surface viral protein spike, membrane, and envelope of coronavirus enclosing the helical nucleocapsid containing viral RNA are embedded in the host membrane-derived lipid bilayer (Finlay *et al.,* 2004). Figure 1 shows that the S-spike protein, M-membrane protein, and E-envelope protein make up the three protein envelopes of this virus. While the M-membrane and E-envelope proteins create the ring structure, the S-spike protein generates peplomers and provides the unique crown shape. The N- nucleocapsid protein is a phosphoprotein and the fourth protein found in the nucleocapsid (Grudlewska-Buda *et al.,* 2021).

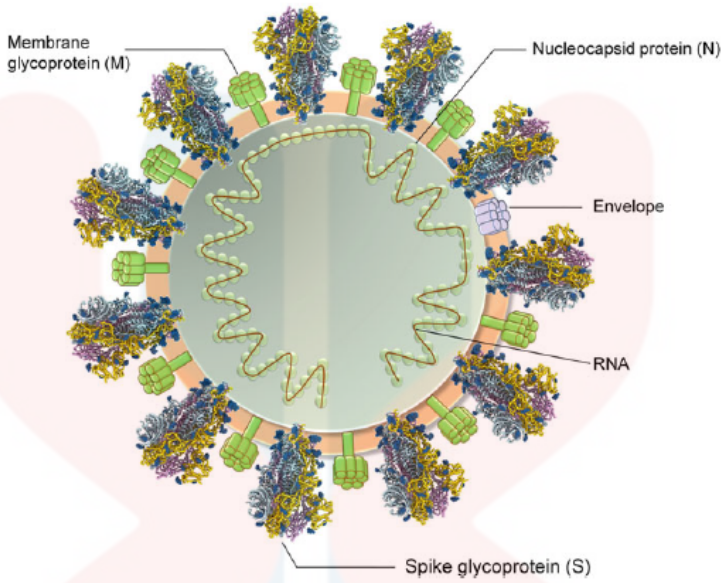

Figure 1: Structure of SARS-CoV-2 virus.

2.2 Entry and Replication of SARS-CoV-2 in Host Cells.

The spike glycoproteins of a coronavirus attach to cellular receptors, and the S protein is primed by a protease from the host cell, allowing the virus to enter the cell. Both the ACE2 receptor and the serine protease TMPRSS2 are involved in S-protein priming by SARS-CoV-2 (Hoffman *et al.,* 2020). According to studies, SARS-CoV-2 has 10-20 times higher affinity for the ACE2 receptor (Wrapp *et al.,* 2020). Afterward, the viral RNA is released into the host cytoplasm, where it is translated to produce the replicase polyproteins pp1a and pp1b, which are subsequently broken into smaller proteins by virus-encoded proteases. A virion is assembled when RNA from a virus interacts with a protein in the endoplasmic reticulum or Golgi complex. Afterward, the vesicles transport the virus particles outside of the cells (Hoffmann *et al.,* 2020)

2.3 Bats and coronaviruses

Every human coronavirus has animal origins which are also natural hosts (Liu Y-C *et al.,* 2020). Bats are a unique group of mammals of the order *Chiroptera* and an important source of zoonotic viruses particularly Coronaviruses (Frutos *et al.,* 2021). They also play a crucial role as natural reservoirs for both alpha and beta coronaviruses. The ability to fly is one of the most distinguishing features of bats. Throughout their evolution, they achieved a unique set of antiviral immune response genes that controls virus propagation while limiting self-damaging inflammatory responses. Strong antiviral immune response against RNA viruses has been made possible by a number of modifications (Subudhi *et al.,* 2019). Migratory bats can lead to the dissemination of viruses over a large area. Some are very close in contact with each other providing opportunities for viral exchange. The *Rhinolophus affinis* BatCov RaTG13 in China shares 96% identity with SARS-CoV-2. (Zhou *et al.,* 2020). Bats are likely reservoir hosts for SARS-CoV-2 but whether they are directly transmitted to humans or transmitted to intermediate hosts to cause the animal-to-human transmission is still unknown (Liu Y-C *et al.,* 2020). There is also a study stating that Malayan pangolins (*Manis javanica*) in China might be the intermediate host from bats to humans because of the similarity of the pangolin coronavirus to SARS-CoV-2 (Temmam *et al.,* 2022).

3.0 MATERIAL AND METHODS

3.1 Sample collection

Five samples collected from bats which were positive of coronavirus were obtained from achieved samples in the Virology Laboratory, FPV, UMK. Oropharyngeal swabs were taken from the bats from Gunung Reng, Kelantan and Sekayu, Terengganu. Four samples were from Sekayu, Terengganu and one was from Gunung Reng, Kelantan. These samples were similar to SARS-CoV-2 in humans with 98-99% sequences similarity (unpublished findings).

3.2 Sample preparation

3.2.1 A549 Cells Growth and Maintenance

In this study we used A549 (CCL-185) cells, a lung carcinoma of human cells obtained from American type culture collection (ATCC). A growth media containing 10% (v/v) Fetal Bovine Serum (FBS), 1% (w/v) penicillin-streptomycin, and DMEM (Dulbecco's Modified Eagle Medium) 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. In a biosafety cabinet, 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

The cell suspension of A549 cells (105 cells/ml) were seeded into T25 flasks and incubated for 24 hours at 37°C with 95% humidity and 5% CO2. After 24 hours and the cell reached desired confluency (>70%), the virus suspension from the third-passage in A549 cells was inoculated and incubated for 7 days at 37°C with 5% CO2 with 95% of humidity. Daily changes were observed until cytopathic effect (CPE) was observed and virus was harvested when CPE was observed. Once CPE was observed, the supernatant from the flask was collected and centrifuged at 12,320g for 10 minutes. The cleared supernatant was collected and kept inside a 1.5ml vial tube and stored in -80°C freezer for the next use.

3.2.3 Viral Inoculation

The A549 cells were seeded inside a 6-well plate with negative control for 24 hours prior to inoculation as shown in Figure 2. Post-seeding after 24 hours, the media was removed and washed with 1 X PBS and each of the wells was filled with 2 ml of 2% DMEM (Gibco, USA). One well served as negative control well without inoculation of SARS-CoV-2 like of Bats virus. Each plate was inoculated with 200µL of fourth-passaged SARS-CoV-2 like Bats virus suspension and incubated for 24 hours at 37°C with 5% CO2 and 95% humidity. After 24 hours, the supernatant from each well was centrifuged at 12,000g for 10 minutes and 0.5ml of the cleared supernatant was collected into a sterile cryovial tube until the next use for RNA extraction.

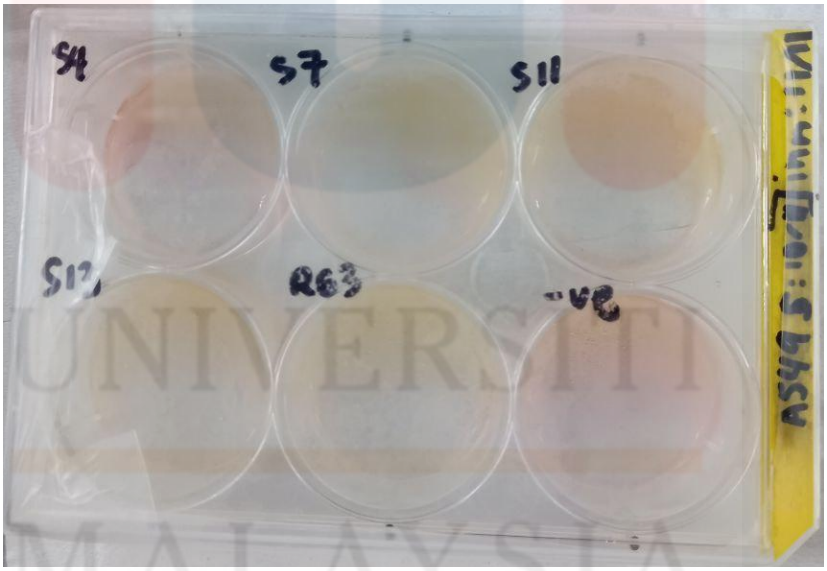

Figure 2. Arrangement of the cell lines inside the 6-well plate.

3.2.4 RNA extraction

A549 cells suspension was inoculated with SARS-CoV-2 like of bats and the negative control (A549 without inoculation) were extracted using GENEzol reagent (Geneaid Biotech Ltd, Taiwan) which contain phenol, chloroform and guanidine isothiocyanate. RNA extraction protocol was done following the manufacturer's protocol. RNA extraction started by adding 750 µl GENEzol reagent into 250µl of the tissue suspensions and was incubated at room temperature for 5 minutes. A 200 µl chloroform was added and shake vigorously for 15 seconds using vortex (Corning, Japan). Then, the mixture was centrifuged at 16,800g for 15 minutes at 4°C and all the tubes were inserted into the centrifuge.

The RNA precipitate can be observed after centrifuging at the bottom of the tube as white pellet. The supernatant from each tube was removed using micropipette and 1m of isopropanol solution was added into the pellet inside the tube using micropipette and was shaken vigorously for 15 seconds using a vortex (Corning, Japan). It was then continued to be centrifuged at 4,800g for 5 minutes at 4°C. The supernatant was removed and the pellet was-air dried for 15 minutes. 30 µl of nuclease free water was added into the pellet in each of the tubes and gentle mixing was done by pipetting up and down. The tubes were then incubated at 55°C for 10-15 minutes and were stored in -80°C until further used for RT-PCR.

3.2.5 One-Step RT-PCR reaction

The reaction was carried out in 25µl reaction mixture using AccessQuick RT-PCR reagent kit (Promega, USA) from the extracted RNA products from A549 cells infected with SARS-CoV-2 like of Bats. A total of 5 µl of RNA extracted product from each sample was preheated at 92°C for 5 minutes and was immediately put on ice soon after. Each of the extracted RNA from 4 samples were added into 20µl of reaction mixture containing 12.5µl of 2 x AcessQuick buffer, 0.5µl of AMV reverse-transcriptase enzyme, 1µl of forward primer (0.5µl (8U) Taq polymerase, 4.3µl of nuclease free water following the protocol by the manufacturer (Promega USA) with slight modification. RT-PCR reaction was performed (Biorad T100, USA). The primers used are as listed in Table 1.

Table 1. List of designed primers use for RT-PCR

Primer	Sequence 5'-3'	Gene	Product size (bp)	Reference
ACE2_F ACE2_R	5'- AGAGTTTCTGGGGATACAG - 3' 5'- GGTCTGAACATCATCAGTG - 3'	ACE2	244	This study
SARS-CoV-2_F SARS-CoV-2_R	5' TCTACTCTTRYRCAGAATG – 3' 5' GTGGYTCTTTMAMDTMCTC - 3'	RdRp	120	This study
TMPRSS2_F TMPRSS2_R	5'- GAACTCAGGGTCACCACCAG - 3' 5'- GGTCAAGGTGATGCACAGTG- 3'	TM-PRSS2	262	This study

GAPDH_F GAPDH_R	5’-GAGTCAACGGATTTGGTCGT-3’ 5’-GACAAGCTTCCCGTTCTCAG-3’	GAP-DH	195	This study

Table 2. RT-PCR reaction mixture for designed primers

Solution	Volume per sample (µl)
Nucleus Free Water	4.3
2X RT-PCR buffer (AccessQuick)	12.5
AMV Reverse Transcriptase (10 unit/ul)	0.5
RNAse inhibitor (30 unit/µl)	0.2
Primer 1 ● Forward primer(20 ρmol)- Designed primer	1.0
Primer 2 ● Reverse primer(20 ρmol)- Designed primer	1.0
Taq polymerase (8 unit/µl)	0.5
Extracted RNA	5
Total	20

After that, an RT-PCR reaction was performed in a single-tube reaction system using a thermocycler (Biorad cT100 touch). The RT-PCR protocol used is as shown in Table 3.

Table 3. Reaction cycle of One- Step RT-PCR protocol used.

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

3.3 Gel Electrophoresis analysis

The PCR product was analyzed in 2 % (w/v) agarose gel. The agarose gel was prepared by weighing 0.8 g of agarose powder (Vivantis) and dissolved with 40 ml of 1X Tris-Borate EDTA (TBE) buffer in a conical flask and heated with a microwave to dissolve the agarose. 1.0 µl of Midori Green dye (Nippon genetics, Germany) was added and mixed well before pouring to the pre-casted tray. After 20 minutes, the gel solidified and the comb was removed gently. 10 µl of each PCR product containing the 1 µl of loading dye (Promega, USA) and 100 bp DNA marker was loaded into each well of the gel. The electrophoresis was carried out at 100 volts for 40 minutes in a submarine gel electrophoresis system. The PCR band was analyzed and photographed using a gel documentation system (GEL doc TM EZ Imager, BIORAD, USA)

4.0 RESULTS

Gel electrophoresis of housekeeping gene GAPDH was done to show that all the RNA viruses were prepared in the same concentration and that virus bands confirms that the treatments contain the virus (Figure 3). Based on Figure 5, bands were observed in all cells except in the control uninfected cells for the presence of ACE2 receptor with the expected band size, 244bp. Based on Figure 6, there was no bands, showing negative for TMPRSS2 including positive SARS-CoV-2 because the product should be 262 bp.

4.1 RT-PCR of housekeeping gene GAPDH in A549 cells.

Expected band of 195 bp product was observed in all infected and uninfected cells, indicating all the RNAs (Figure 3) were extracted in the same manners with same concentration of RNAs for further proceed to RT-PCR.

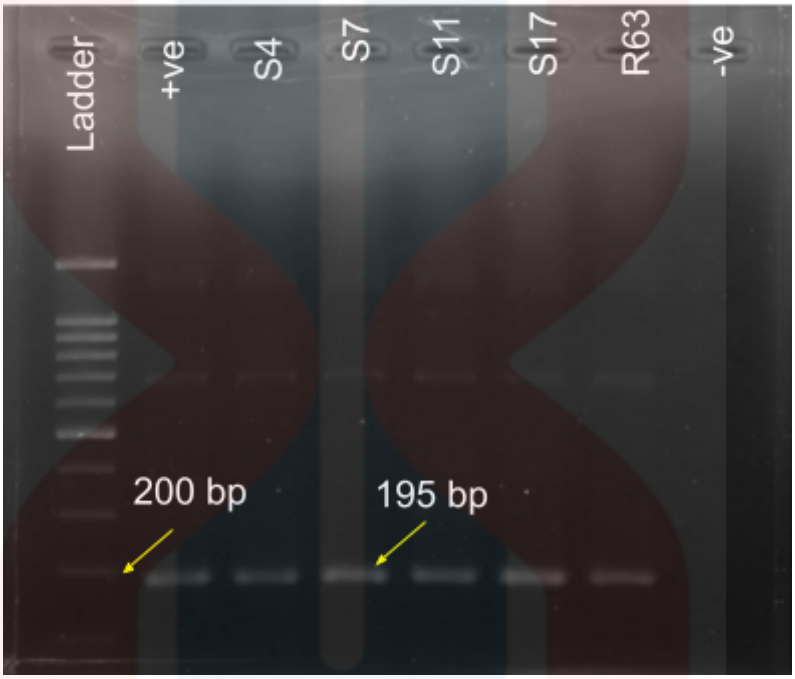

Figure 3: Gel electrophoresis results of housekeeping gene GAPDH

+ve: Human SARS-CoV-2 positive sample.

S4: Positive sample of SARS-CoV-2 like virus of bats inoculated in A549 cell from Sekayu, Terengganu.

S7: Positive sample of SARS-CoV-2 like virus of bats inoculated in A549 cell from Sekayu, Terengganu.

S11: Positive sample of SARS-CoV-2 like virus of bats inoculated in A549 cell from Sekayu, Terengganu.

S13: Positive sample of SARS-CoV-2 like virus of bats inoculated in A549 cells from Sekayu, Terengganu.

R63: Positive sample of SARS-CoV-2 like virus of bats inoculated in A549 cells from Gunung Reng, Kelantan.

-ve: Negative control of A549 cell.

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

Band of an expected product of 120 bp (Figure 4) was observed in all infected and uninfected cells, indicating all the RNAs of SARS- CoV-2 was present in all cells.

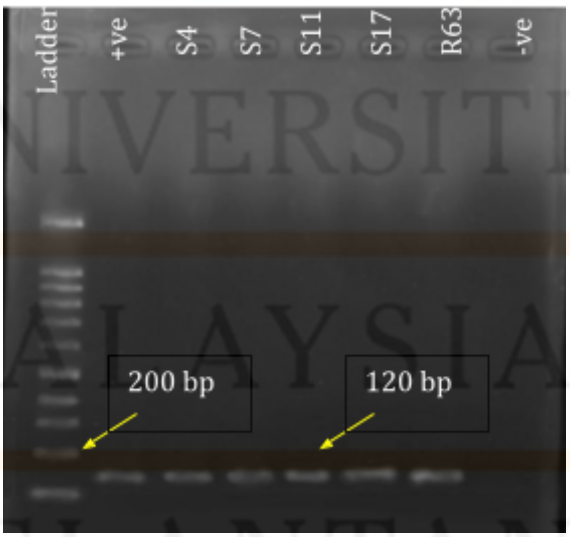

Figure 4. Gel electrophoresis results of SARS-CoV-2 in A549 cells.

+ve: Human SARS-CoV-2 positive sample.

S4: Positive sample of SARS-CoV-2 like virus of bats inoculated in A549 cell from Sekayu, Terengganu.

S7: Positive sample of SARS-CoV-2 like virus of bats inoculated in A549 cell from Sekayu, Terengganu.

S11: Positive sample of SARS-CoV-2 like virus of bats inoculated in A549 cell from Sekayu, Terengganu.

S13: Positive sample of SARS-CoV-2 like virus of bats inoculated in A549 cells from Sekayu, Terengganu.

R63: Positive sample of SARS-CoV-2 like virus of bats inoculated in A549 cells from Gunung Reng, Kelantan.

-ve: Uninfected Human Lung Cells

4.3 RT-PCR of ACE2 receptor from treated Human Lung Cell icoulated with SARS- CoV-2 like virus isolated from bats in East Coast Malaysia.

Band of expected size 244 bp for ACE2 receptor was observed in all SARS- CoV-2 like virus infected cells and human SARS-CoV-2 positive control but not in uninfected cells as negative control (Figure 5).

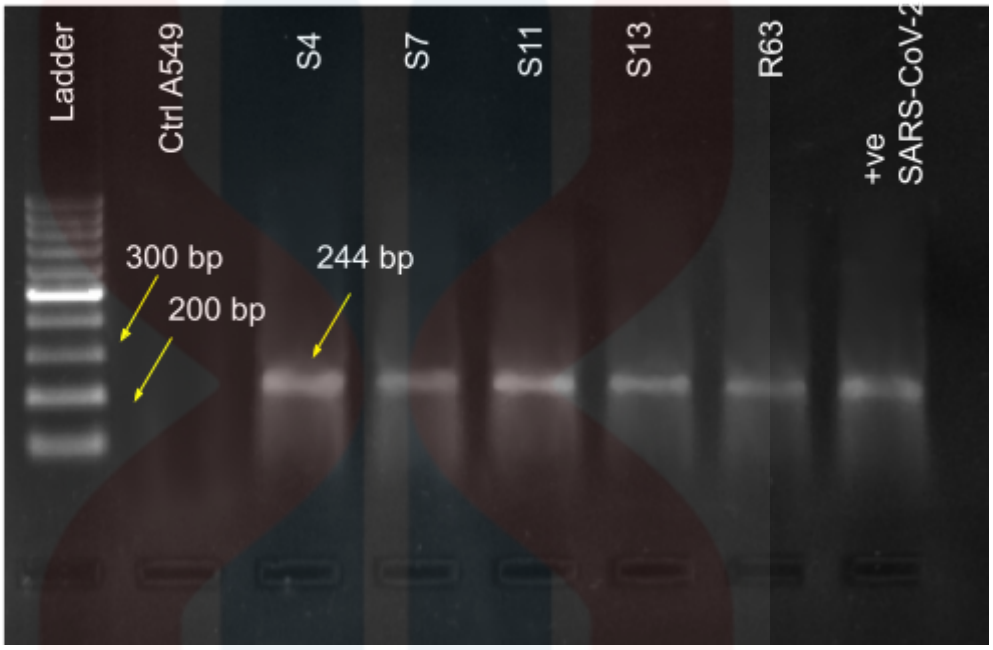

Figure 5. Results of gel electrophoresis of ACE2 receptor on infected Human Lung Cell.

Ctrl A549: Uninfected Human Lung Cells

SARS-CoV-2: Human SARS-CoV-2 positive sample.

S4: Positive sample of SARS-CoV-2 like virus of bats inoculated in A549 cell from Sekayu, Terengganu.

S7: Positive sample of SARS-CoV-2 like virus of bats inoculated in A549 cell from Sekayu, Terengganu.

S11: Positive sample of SARS-CoV-2 like virus of bats inoculated in A549 cell from Sekayu, Terengganu.

S13: Positive sample of SARS-CoV-2 like virus of bats inoculated in A549 cell from Sekayu, Terengganu.

R63: Positive sample of SARS-CoV-2 like virus of bats inoculated in A549 cell from Gunung Reng, Kelantan.

4.4 RT-PCR of cellular serine protease TMPRSS2 from treated Human Lung Cell inoculated with SARS- CoV-2 like virus isolated from bats in East Coast Malaysia.

No expected band of 262 bp of TMPRSS2 was presence in all infected cells, positive SARS-CoV-2 control and uninfected negative control (Figure 6).

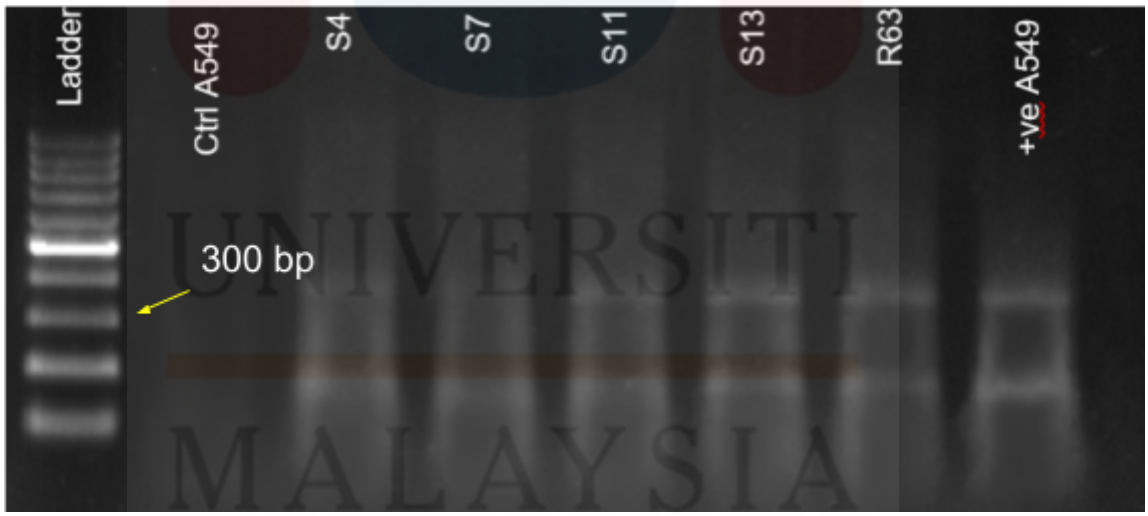

Figure 6. Results of gel electrophoresis of TMPRSS2 serine protease of the infected A549 cells.

Ctrl A549: Uninfected Human Lung Cells

SARS-CoV-2: Human SARS-CoV-2 positive sample

S4: Positive sample of SARS-CoV-2 like virus of bats inoculated in A549 cells from Sekayu, Terengganu.

S7: Positive sample of SARS-CoV-2 like virus of bats inoculated in A549 cells from Sekayu, Terengganu.

S11: Positive sample of SARS-CoV-2 like virus of bats inoculated in A549 cells from Sekayu, Terengganu.

S13: Positive sample of SARS-CoV-2 like virus of bats inoculated in A549 cells from Sekayu, Terengganu.

R63: Positive sample of SARS-CoV-2 like virus of bats inoculated in A549 cells from Gunung Reng, Kelantan.

5.0 DISCUSSION

The Coronavirus disease 2019 (COVID-19) is a prime example of viruses that sometimes leave their sylvatic environment and accidentally infect humans (Yiu *et al.,* 2020). Compatibility between the virus and the host is a key to the emergence of an infectious disease such as COVID-19. The presence of adequate receptors for the virus on animals and human cells shows the compatibility for the virus to enter the host cells through the recognition of the virus to the receptor binding on the host cells (Frutos *et al.*, 2021). The structural basis of SARS-CoV-2 with ACE2 interaction for host entry has been shown by (Yan *et al.,* 2020). Therefore, this study was conducted to check if the SARS-CoV-2-like viruses isolated from bats in East Coast, Malaysian have a similar potential and receptor binding mechanism as human SARS-CoV-2 to infect human cells and cause the disease by binding onto the ACE2 receptor and using the cellular serine protease TMPRSS2.

This study creating awareness towards the public about the possibility of future infections that could also lead to another pandemic. Based on Figure 5, expected bands were observed in all cells except in the control uninfected cells, this confirmed that the SARS-CoV-2 like virus of bats isolated from East Coast, Malaysian bats can use human ACE2 receptors to enter the human A549 lung cells which is like human SARS-CoV-2 virus. However, no band was observed in Figure 5 for TMPRSS2 including for positive human SARS-CoV-2, this indicates that the SARS-CoV-2 like viruses from bats does not use the cellular serine protease TMPRSS2 to prime the S protein to enter the host cells. It may be possible that these viruses do not require TMPRSS2 for their virus entry.

Figure 5 also showed that no band was observed with positive control SARS-CoV-2, suggesting that it also does not require TMPRSS2 for their virus entry into human host cells. It is likely that the SARS-CoV-2 positive control we used belongs to Omicron variant strain, as this strain has been shown does not require TMPRSS2 for their cell entry compared to the wild type of SARS-CoV-2 (Bo Meng *et al.,* 2022). Hence, this SARS-CoV-2 like viruses in bats from East Coast, Malaysia may have a potential to become a zoonotic virus since they can recognise ACE2 human receptor. This is confirmed by the ability of the viruses to produce cytopathic effect in these cells (Data not shown). However, further studies such as sequence analysis of the receptor binding on the viruses need to be conducted to confirm this statement.

In this research, we report evidence on the presence of SARS-CoV-2 like virus from bats found in Malaysia and has the potential of zoonosis. However, the presence of the viruses in bats is not sufficient to trigger a pandemic in the human population. Human activity is required to amplify the frequency of viruses which then leads to amplification loops to trigger an epidemic. Human population density and wet markets were key factors in the emergence of COVID-19. The ability to act before the human population amplifies by spreading the virus will be the main challenge of the future pandemic.

6.0 CONCLUSION AND RECOMMENDATIONS.

In conclusion, these SARS-CoV-2 like viruses of bats isolated from East Coast Malaysia uses the ACE2 receptors but does not require TMPRSS2 to enter the human lung cells, indicating that it might possess a zoonotic-potential to humans. However, further research such as sequence analysis of receptor binding on the virus and inhibition of interferon type I (α and β) that specific to virus infection need to be done to conclude this statement.

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