MOLECULAR DETECTION OF BUNYAVIRUS IN BAT FLIES IN KELANTAN AND TERENGGANU, MALAYSIA

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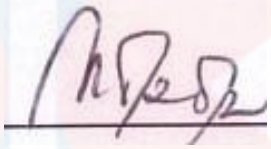
CERTIFICATION

This is to certify that we have read this research paper entitled **'Molecular Detection of Bunyavirus in Bat flies in Kelantan and Terengganu, Malaysia'** by Nur Nerina Aerisya binti Zameri, 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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Thank You

DEDICATIONS

I dedicate my dissertation work to my family and friends. This work is wholeheartedly dedicated to my beloved parents, who have been my strongest support system and source of inspiration. My best friend, Maryam Salsabeela who has never left my side.

I also dedicate this work to many of my lecturers and laboratory staff who have guided me well in completing my research project and have been there throughout the process. I will always appreciate all of the things they have done, especially Prof Maizan, Dr. Tan Li Peng and Ms Nani Izreen.

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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

Bunyavirus is an RNA virus that belongs to the *Bunyaviridae* family. The *Bunyaviridae* family is one of the largest virus families, with five genera, four of which contain animal-infecting viruses and one carrying plant-infecting viruses. Arthropods are required as vectors for Orthobunyavirus, Phlebovirus, and Nairovirus (arboviruses), whereas rodents are required for Hantavirus (roboviruses). Bats are known to be the natural reservoirs for a wide range of zoonotic diseases that can spread and cross species barriers and are home to a wide range of ectoparasitic species. The most frequent ectoparasites on bats are bat flies (*Nycteribiidae* and *Streblidae*). The purpose of this study is to detect the presence of Bunyavirus from ectoparasites of bats for the possibility of bats as the reservoir host of Bunyavirus. A total of 98 ectoparasite samples were collected from bats from 2 different places in East Coast, Malaysia which are in Sekayu, Terengganu and Gunung Reng, Kelantan. The ectoparasites were further classified based on their family morphology. In this study, the ectoparasites were mainly bat flies. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) using universal primers of the Small (S) RNA segment of Bunyavirus was used to detect Bunyavirus. Agarose gel electrophoresis results showed none of the samples appear at the expected amplicon band size. This result will contribute to the new knowledge on the diversity of the virus, their vectors and possible public health importance in Malaysia.

Keywords: *Bunyavirus, bat flies, RT-PCR, Kelantan, Terengganu.*

ABSTRAK

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

Bunyavirus adalah virus RNA yang tergolong dalam keluarga *Bunyaviridae.* Keluarga *Bunyaviridae* adalah salah satu keluarga virus terbesar, dengan lima genera, empat daripadanya mengandungi virus yang menjangkiti haiwan dan satu membawa virus yang menjangkiti tumbuhan. Arthropod diperlukan sebagai vektor untuk Orthobunyavirus, Phlebovirus, dan Nairovirus (arbovirus), manakala tikus diperlukan untuk Hantavirus (robovirus). Kelawar dikenali sebagai takungan semula jadi untuk pelbagai jenis penyakit zoonosis yang boleh merebak dan merentas halangan spesies dan menjadi rumah kepada pelbagai spesies ektoparasit. Ektoparasit yang paling kerap pada kelawar ialah lalat kelawar (Nycteribiidae dan Streblidae). Tujuan kajian ini adalah untuk mengesan kehadiran Bunyavirus daripada ektoparasit kelawar dan kemungkinan kelawar sebagai perumah takungan Bunyavirus. Sampel ektoparasit dikumpul daripada kelawar dari dua tempat berbeza di Pantai Timur, Malaysia iaitu di Sekayu, Terengganu dan Gunung Reng, Kelantan. Ektoparasit selanjutnya diklasifikasikan berdasarkan morfologi keluarga mereka. Dalam kajian ini, ektoparasit kebanyakannya adalah lalat kelawar. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) menggunakan primer universal segment RNA kecil (S) Bunyavirus digunakan untuk tiada satu sampel pun termasuk kawalan mempunyai saiz jalur yang dijangkakan dan beberapa sampel menghasilkan saiz jalur yang berbeza-beza. Keputusan ini akan menyumbang pengetahuan baru berkenaan diversiti virus,

vector dan berkemungkinan menjadi virus berkepentingan kesihatan awam di Malaysia.

Kata kunci: *Bunyavirus, lalar kelawar, RT-PCR, Kelantan, Terengganu.*

1.0 Introduction

Bats are one of the most diverse, abundant and widely distributed groups of mammals and the only mammals having capacity of powered flight, which can fly using their wings (Li *et al*., 2010) as far as 1000 km (Fleming, 2019). Except in the northern and southern polar region, nearly 1,000 species of bats can be found all over the planet (Omatsu *et al.*, 2007). Their existence provides critical ecosystem services ranging from pest control (Reiskind and Wund 2009, 2010; Reydell *et al*., 2002), reseeding cut forests, and pollination of plants, all of which ensures humans and animals are supplied with enough food. On the other hand, their existence brings out harms as well. They are known to be the natural reservoirs for a wide range of zoonotic diseases that can spread and cross species barriers (Li *et al*., 2010). More than 80 virus species from diverse families and numerous parasites have been isolated or found in bats to date, with each of them having the ability to cause a variety of illness. Their large population and gregarious roosting behaviour, in particular incline the risk of disease transmission both within (inter-) and between (intra-) species (Calisher *et al*., 2006). Plus, bats with migratory habits provide a good opportunity for long distance spread of the pathogens (Messenger *et al.*, 2003). Pathogens are transmitted from bats to people and other animals and vice versa by direct contact with diseased animals, their blood and tissue or via vector species. Insects and ectoparasites are one of the most important vector groups with over a million described species, responsible for disease transmission. Bats are home to a variety of ectoparasitic species, including bat flies, bugs, fleas and various bat-specific arachnids, including mites and ticks. Bat flies (*Nycteribiidae* and *Streblidae*) are the most common bat ectoparasites found on bats (Patterson *et al*., 2009)

Bunyavirus is an RNA virus under the family *Bunyaviridae*. The *Bunyaviridae* family is one of the largest virus families, divided into five genera, four of which contain viruses that infect animals and one containing plant virus. The genera under the *Bunyaviridae* family are *Orthobunyavirus, Phlebovirus, Nairovirus, Hantavirus* and *Tospovirus* (Machlalan & Dubovi, 2016). *Orthobunyavirus, Phlebovirus* and *Nairovirus* need arthropods as vectors (arboviruses) while *Hantavirus* require rodents to be the vector (roboviruses) (Machlalan & Dubovi, 2016). Bunyaviruses may infect bats via arthropods especially the ticks, however it is still unclear whether bats are important reservoir hosts of the virus (Calisher *et al*., 2006) and which ectoparasites of bats does Bunyavirus opt as its vector.

Bunyaviruses are responsible for causing several illnesses in human and domestic animals characterized by clinical manifestation of fever, haemorrhagic fever, renal failure, encephalitis, meningitis, blindness and birth abnormalities in domestic animals. Majority of the diseases are self-limiting fevers that last for 1-4 days, accompanied by headaches, muscle aches, generalised weakness and nausea. Although most of these viruses infect a variety of vertebrate hosts, only a few are responsible for zoonoses. Some of important viruses under this family are Rift Valley fever virus under the genus *Phlebovirus* that is transmitted by the mosquitoes, causing abortion and hepatitis in animals and haemorrhagic fever in human, La Crosse virus under the *Orthobunyavirus* genus, transmitted by mosquitoes causing congenital malformation in sheep and La Crosse encephalitis in human and Crimean-Congo haemorrhagic fever virus under the genus *Nairovirus* that is transmitted by ticks causing haemorrhagic fever in human (Zeller and Bouloy, 2000).

Bunyaviruses have the ability to infect a wide range of hosts and some even possess zoonotic risk. Most of the viruses under this family are transmitted from one animal to another and to humans by the aid of arthropod vectors. Therefore, the aim of the study is to detect the presence of Bunyavirus from the flies of bats to have better visualization of the possibility of bats as the reservoir host and involved in the transmission of Bunyavirus.

2.0 Research problem

Bunyavirus is known to be transmitted inter- and intra-species by the means of vector, either by arthropod or rodent vector. Bats are also known to be the natural reservoirs for a wide range of zoonotic diseases that can spread and cross species barriers. However, there is no information on the transmission of Bunyavirus by the arthropod, particularly by ectoparasite in Malaysia. Hence, this is a pilot study investigating the presence of Bunyavirus and its prevalence in Bat flies in East Coast (Kelantan and Terengganu), Malaysia.

3.0 Research questions

3.1 Do bat flies in Kelantan and Terengganu, Malaysia carry the Bunyavirus?

4.0 Research hypothesis

4.1 Bats flies in Kelantan and Terengganu, Malaysia are the potential vector for Bunyavirus transmission to and from bats to other species.

5.0 Objectives

5.1 To detect the presence of Bunyavirus in the bat flies in Kelantan and Terengganu, Malaysia via RT-PCR method

6.0 Literature review

6.1 *Bunyaviridae*

The *Bunyaviridae* family is one of the largest virus families, with over 350 viruses. It was named *Bunyaviridae* after Bunyamwera in Uganda, where the virus was first discovered in 1943. The members of this virus family were divided into five genera, four of which contain viruses that infect animals and one (the genus *Tospovirus*) that only infect plants. A large number of bunyaviruses have yet to be classified into a genus or a serogroup (Machlalan & Dubovi, 2016). The genera under the *Bunyaviridae* family are *Orthobunyavirus* (from the Greek orthos meaning "true or proper"), *Phlebovirus* (from phlebotomus or sandfly fever), *Nairovirus (*from Nairobi sheep disease), *Hantavirus* (named after the location it was first isolated which was Hantaan River, Korea) and *Tospovirus* (from tomato-spotted wilt virus, a pathogen spread by sap-sucking insects). The viruses under this family are mainly arthropod-borne.

6.2 Morphology, structural characteristics, and properties of Bunyavirus virion.

Virions are spherical or pleomorphic in shape, 80-120nm in diameter, and have two surface glycoproteins (Gc and Gn) projections of 5-10nm enclosed in a lipid bilayer envelope which is approximately 5nm thick. Virion envelopes are usually made of cellular Golgi membranes, or on occasion can also be made of cell surface membranes. The envelope surrounds the tripartite single-stranded negative sense RNA genome, designated according to their relative size as large (L), medium (M) and small (S) (Bishop *et al.*, 1980). These three segments are presented in the virions as

ribonucleoproteins (RNPs) in which every each of them containing a single genomic RNA encapsidated by the viral nucleocapsid (N) and (L) proteins (Obijeski *et al*., 1976) with a diameter of 2-2.5nm, a length of 200-3000nm, and usually but not always, display helical symmetry (Figure 1). The morphological features differ across viruses of each of the five genera, especially the length of the S, M and L segments. However, the sizes and coding strategies of proteins encoded by viruses in the same genus are somehow similar but different between the genera (Elliott, 1990)

6.3 Life cycle and transmission of Bunyavirus

Viruses from three genera (Orthobunyavirus, Phlebovirus, and Nairovirus) coexist in arthropod-vertebrate-arthropod cycles, each with its own arthropod vectors and vertebrate reservoir hosts. Bunyaviruses are transmitted to rodents, birds, and bigger animals by arthropods such as mosquitoes, ticks, midges, and biting flies (Phlebotomus flies). When humans come into contact with the insect vector's surroundings, they become infected with the virus. Hantaviruses, on the other hand, do not have an arthropod vector and are kept in rats (rodents) that are distinct to each virus (Machlalan & Dubovi, 2016). When humans come into direct touch with infected rats or inhale aerosolized mouse urine, they become infected with the virus. Bunyaviruses cause transient infection in mammals and birds, as well as life-long chronic infection in their arthropod vectors, whereas viruses in the genus Hantaviruses can infect rodent reservoir hosts and cause persistent illness. The majority of Bunyaviruses are incapable of infecting humans or domestic animals, but those that do can result in catastrophic illnesses ranging from congenital abnormalities to systemic "haemorrhagic fever" disease syndromes (CDC Works 24/7, 2022).

6.4 Vectors

Bat flies (Diptera: Nycteribiidae and Streblidae), bugs (Hemiptera: Cimicidae and Polyctenidae), fleas (Siphonaptera: Ischnopsyllidae), and other bat-specific arachnids, such as mites (Mesostigmata: Spinturnidae and Macronyssidae) (Argas spp, Ixodes spp, Ornithodoros spp., and Carios spp). The most frequent bat ectoparasites observed on bats are bat flies (Nycteribiidae and Streblidae). There are documented cases of bat-specific ectoparasites biting humans (Piksa *et al*.,2013) increasing the potential of bat-borne pathogen transmission. Bat flies can spread viruses to bats and humans, according to research (Patterson et al., 2009). The first Orthobunyavirus was discovered in bat flies called Eucampsipoda africana (Diptera: Nycteribiidae) that were collected from Egyptian fruit bats (Rousettus aegyptiacus) in the wild in South Africa. Wolkberg virus (WMV) was named after the cave where Egyptian fruit bats and ectoparasites were kept. The virus was given the name Wolkberg virus (WMV) after the cave where the Egyptian fruit bats were kept and the ectoparasites were gathered (Jansen Van Vuren *et al.*, 2017).

6.5 Bunyavirus infection cases in Malaysia

To date, there is no infection of Bunyavirus in humans in Malaysia. The common infection of Bunyavirus found in Malaysia is from the genus *Hantavirus*, spread by rodents. In animals, a novel *Orthobunyavirus,* Kedah Fatal Kidney Syndrome Virus (KFKSV) which causes severe kidney lesions was isolated from broilers in Kedah, Malaysia. This virus is closely related to the Umbre virus which causes fatal encephalitis in immunocompromised human patients (Perot *et al.*, 2021). Oya virus, under the simbu serogroup of *Orthobunyavirus,* was isolated in pigs with clinical signs similar to Nipah virus was isolated in Sibu, Sarawak in 2000 (Kono et

al., 2002). However, there is still a lack of study done on the possibility of bats ectoparasites as the vector for this virus.

7.0 Materials and methods

7.1: Sample collection

The samples used in this study were collected from 2 different states, Kelantan and Terengganu in the East Coast of Malaysia. The bat flies were collected from bats in Sekayu, Terengganu and Gunung Reng, Kelantan. We were unable to collect mosquitoes and ticks due to the unavailability of mosquito's trap and none of the ticks was present on the sampled bats. The method used was approved by the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, University Malaysia of Kelantan (UMK/FPV/ACUE/PG/6/2021)

7.2: Sample sorting and identification

All the samples were stored in the freezer at -80ºC. The sorting procedure was conducted to classify the ectoparasites into their family. The procedure was done by placing each ectoparasite into a petri dish or sauce plate filled with 80% diluted ethanol and was observed under light microscope. The thumb forceps were used to extend the legs, and to gently manipulate the position of the ectoparasite for better view. Photos were taken on two views, dorsal and lateral view and each ectoparasite was further identified by its family and genus. The findings were recorded in a table. After the identification procedure, the ectoparasites were separated into new micro centrifuges and were labelled with new identification (ID) based on the criteria recorded.

7.3: RNA extraction

The samples were pooled based on its family, (Nycteribiidae or Streblidae). Then, sample homogenization was done where 1 ml GENEzol ™ (Geneaid, New Taipei City,

Taiwan) was added into a 30mg tissue sample. Then the sample was homogenized with the pipette tips and ground several times until it is homogenized in the Eppendorf tube. The homogenized sample was placed in an incubator for 5 minutes at room temperature to allow complete dissociation of the nucleoprotein complex. Chloroform was added per 1 ml of GENEzol ™ reagent used in the homogenization. The tube was shaken vigorously for 10 seconds. Then, the sample was centrifuged at 14,000 x g for 15 minutes at 4°C to separate the phases. Only 500 µL of the aqueous (clear) phase containing the RNA was transferred to a new micro centrifuge tube by slowly pipetting out the solution and not disturbing the interphase layer (white) or the organic phase layer (red).

Next, the RNA was precipitated using the following steps. Isopropanol (500 µL) was added into the aqueous phase and was mixed together by inverting the tube several times so that it can combine together completely. Then, the sample mixture was left for incubation for 10 minutes at room temperature. The sample mixture was centrifuged at 14,000 x g for 10 minutes at 4°C to allow the formation of a dense RNA pellet. The supernatant was removed carefully by pouring it into a special waste tube specifically for isopropanol waste. The pellet was resuspended with the addition of 1 ml of 70% cold ethanol in order to wash the RNA pellet and briefly vortex for 5 seconds. The sample mixture was centrifuged at 14,000 x g for 5 minutes at 4°C. The supernatant was discarded with a micropipette. The RNA pellet was allowed to air dry for 5 minutes at room temperature. 30 µL of nuclease-free water was added into the pellet for RNA resuspension. Then, the RNA pellet was incubated for 10 minutes at 60°C so that it can completely dissolved and then was stored at -80°C

7.4: Master Mix preparation

Five µL of the extracted RNA was preheated at 95ºC for 5 minutes. Then, a master mix was added and proceeded with Reverse transcription-polymerase chain reaction (RT-PCR) using the component in Table 1.

Table 1: Component used in RT-PCR

Solution	Volume (uL)
2X buffer	12.5
AMV-RT	0.5
Primer Forward	1
Primer Reverse	1
RNase inhibitor (RNAsin)	0.2
Taq Polymerase (8 Unit)	0.5
dH_2O	4.3
Total volume	25

7.5: RT-PCR

Reverse transcription-polymerase chain reaction (RT-PCR) method is used to identify the RNA of the virus. The primers used in this study are as shown in the table 2 below;

Table 2: List of primers used in RT-PCR

Oligonucleotide	**Sequences (5' – 3')**	**Expected size**	**References**
BunS+	AGTAGTGTACTCCAC	900bp	
Bun ns-	CCCCTACCACCCACCC		
Orthobunya_F	GGGTAGCACTAGCATTTATCCA	200 bp	(Perot *et al*., 2021)
Orthobunya_R	TGTAGACACCCACAAACGTATC		

RT-PCR was conducted using thermal cycler T100 (Biorad, USA) based on the following steps in table 3 below;

Table 3: Steps of RT-PCR

Cycle step	Temperature (ºC)	Time	Cycle
Initial denaturation	94	2 minutes	1
Denaturation	94	30 seconds	40
Annealing	47-53	1 minute	
Extension	72	30 seconds	
Final extension	72	5 minutes	1

7.6: Agarose Gel Electrophoresis

Agarose gel electrophoresis was used to analyse the PCR results. This agarose gel was prepared by adding the agarose agar with Tris-Borate-EDTA buffer (TBE) in a beaker and heated for 2-3 minutes in the microwave to fully dissolve the solution. The solution is let cooled at room temperature until the temperature of the solution reduces until 50˚C. One µL of Midori Green DNA stain was added into the solution and the beaker was swirled gently. The solution was poured in the mould with 20 well comb and left for 20 to 30 minutes under room temperature until it solidified.

Five µL of samples were diluted with one µL Midori Green loading dye (Nippon Genetic, Germany) and incorporated into the well by using the pipette. The electrophoresis is conducted for 40 minutes at 100 Volt. The result was observed and analysed by using a UV transilluminator (ImageQuant LAS 500, USA).

8.0 Results

Table 4 shows some of the results of the microscopic examinations of the identified bat flies detected through direct microscopy using light microscope at 40x magnification, respectively.

Table 4: The ectoparasites taken from bats in Sekayu, Terengganu & Gunung Reng, Kelantan characterized based on its family

Bat ID	The appearance of ectoparasites under the light microscope	Suspected ectoparasite
S53 (*Rhinolophus affinis*)		Bat flies *Streblidae*.
R3A (*Eonycteris spelaea*)		Bat flies *Nycteribiidae*

**Suspected ectoparasites family and genus were identified and compared with other studies*

A total of flies taken from bats from different species were identified based on its morphology. The family of the bat flies were classified based on their morphology of having wing or wingless respectively to identify its family either *Strebliidae* or *Nycteribiidae*, respectively.

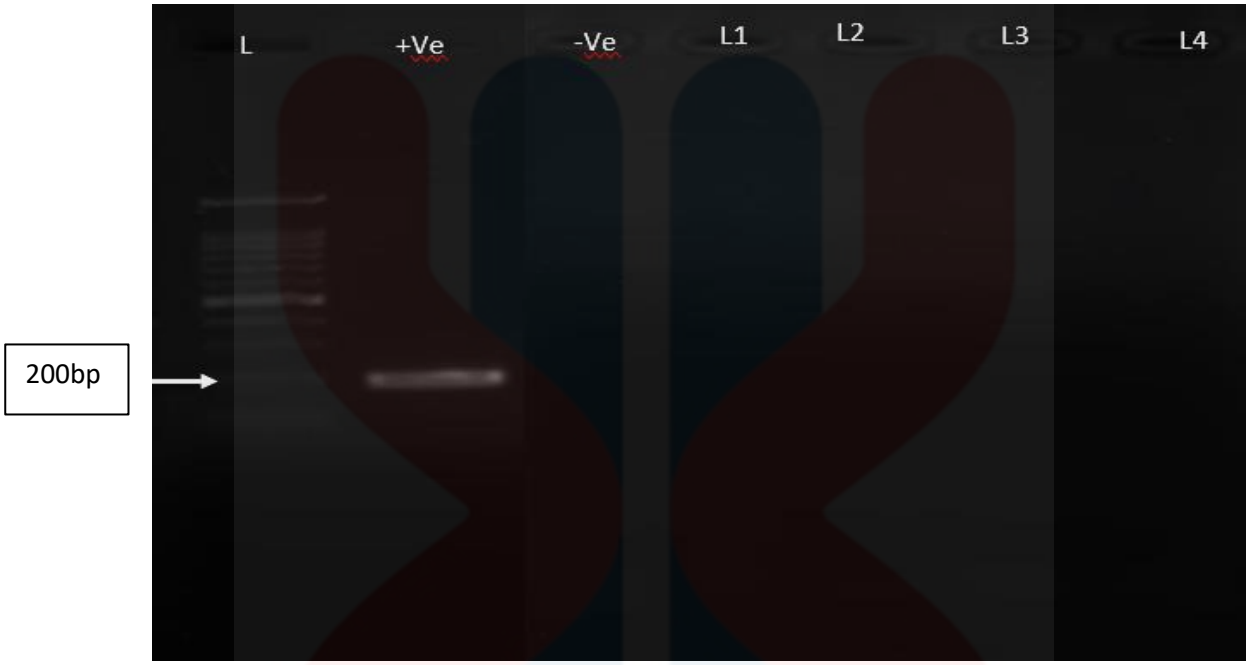
A total of 35 pooled samples were homogenized for RNA extraction and proceeded with Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). The first RT-PCR was conducted on the samples using published universal primers forward (5' AGTAGTGTACTCCAC 3') and reverse (5' CCCCTACCACCCACC 3') based on the Small (S) gene of Bunyavirus with the expected size at 900bp. From the results in Figure 1, all samples tested are negative on RT-PCR with no band appear at 900bp. The positive control failed to appear on the targeted band (900bp).

Figure 1: Electrophoresis gel of RT-PCR of 12 samples

Lane L: DNA ladder size 100bp; Lane +Ve: Positive control (Kedah Fatal Kidney Syndrome Virus); Lane L1-L12: Ectoparasites sample (batflies) RE4, RE11, RA1, RE3, SR2, SR1, RE2, SD1, RE9, RE6, RE8, RE7 respectively; red letter fonts: Samples with unexpected size bands; Lane -Ve: Negative control.

RT-PCR was conducted again using S gene specific forward (5'GGGTAGCACTAGCATTTATCCA3') and reverse (5'TGTAGACACCCCACAAACGTATC 3') primers (Palya et al., 2019), the expected band of the product is 200 bp. The results showed that all of the samples in Figure 2 were negative. The positive control appeared on the expected band size (200bp).

Figure 2: Electrophoresis gel of RT-PCR



Lane L: DNA ladder size 100bp; Lane +Ve: Positive control (Kedah Fatal Kidney Syndrome Virus); Lane -Ve: Negative control; Lane L1-L4: Batflies samples 1-4; The expected size is 200bp

9.0 Discussion

The result showed that the majority of the bats in Sekayu, Terengganu and in Gunung Reng, Kelantan were infested with ectoparasites of bat flies under the *Nycteribiidae* and *Streblidae* family. This finding was an expected finding as it is similar to a study done previously, in which bat flies in *Nycteribiidae* and *Streblidae* are the most common bat ectoparasites found on bats (Patterson et al., 2009). However, this result may also occur due to limitations of the equipment used in sample collection which limit the amount and variation of ectoparasites collected especially for the collection of main vectors of bunyavirus; ticks and mosquitoes. For instance, a mosquito trap is unavailable to catch the mosquitoes and unfortunately, none of the tick was observed on the studied bat samples.

From the RT-PCR result using also universal primers of S gene the electrophoresis gel shows presence of a faint, non-specific band in five of the samples. The usage of universal primer which is shorter in sequence (18 nucleotides) causes bindings or annealing of the primer on more nucleotide regions in the samples. Hence, various agents can be detected, may it be of a Bunyavirus or other agent, which results in presence of non-specific band on gel electrophoresis. Furthermore, the Bunyavirus family has many genera and serotypes. For example, in the case of Orthobunyavirus, there are more than 170 viruses in this genus which are further divided into 18 serogroups; namely Anopheles A, Anopheles B, Bakau, Bunyamwera, Bwamba, Capim, California and Gamboa (Maizan PhD thesis, 2007). All these genera have different sizes of S segment which might further explain the presence of many non-specific bands in this study, which is in agreement with previous study (Maizan PhD

thesis, 2007). The positive control used failed to appear on the expected bp size (900bp) in this procedure. The failure of the positive control used in this study to produce the expected band size is due to the positive control sample used, in which it is from a novel local strain of Bunyavirus, Kedah fatal kidney syndrome virus (KFKSV) in which its RNA template may not match with the sequences of the primers used. Hence, the 5' end of the primer strand could not anneal to the 3' end of the DNA template of the positive control, thus inhibit the synthesis of the desired copy of DNA.

The second RT-PCR result for the samples that employed the primer set of S gene (Palya *et al*., 2019) were all negatives. This may happen due to several reasons; the primer mismatch as the primer used is a S gene specific primer, thus limiting the chance of binding to the complement DNA nucleotide presence in the samples during RT-PCR. This finding is further supported with the previous findings of KFKSV sequencing which shows low nucleotide sequence identities of KFKSV isolates with the closest Orthobunyavirus (Umbre virus) where the large, medium and small segments have 78%, 77%, and 86% sequences identities, respectively. (Palya *et al*., 2019)

Moreover, the sample of ectoparasites used in this study were all bat flies in which the chances to detect Bunyavirus from this ectoparasite is generally low as Bunyavirus is commonly transmitted by mosquitoes and ticks (CDC Works 24/7, 2022) and only rarely in bat flies. The first Orthobunyavirus, Wolkberg virus isolated from bat flies, Eucampsipoda africana (Diptera: Nycteribiidae) was obtained from Egyptian fruit bats (Rousettus aegyptiacus) found in the wild in South Africa. (Jansen Van Vuren et al., 2017) Furthermore, there is no reported case of Bunyavirus transmitted by bats' ectoparasites in Malaysia till this date.

10.0 Conclusion

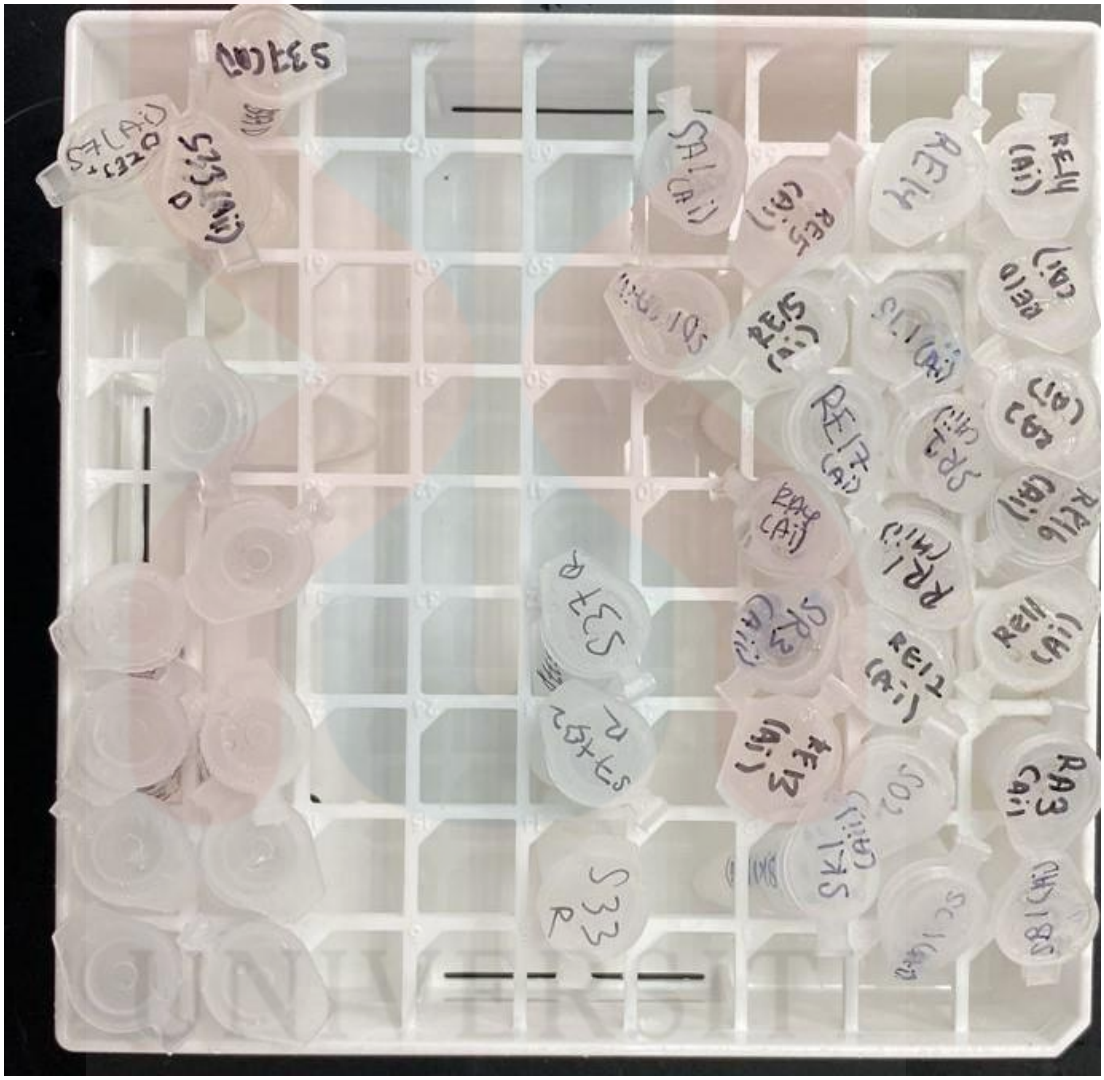
In conclusion, bat flies may act as vectors for Bunyavirus transmission to and from bats to other species. However, based on the study, the involvement of bat flies as a vector for Bunyavirus transmission to and from bats to other species could not be fully identified due to the limitation in the number, family variation of the ectoparasites collected and sequences variations of the viruses which limit the detection of the virus using these 2 sets of the primers.

11.0 Recommendations and future work

Several limitations were noted in this study. For future study, it is wise to increase the sample size (e.g., n=100) to produce meaningful results to observe the presence and the prevalence of Bunyavirus in ectoparasites of bats. Secondly, the study can be improved by using a variety of ectoparasites, from different orders. In this case, the study was constricted as the ectoparasite samples used were mainly bat flies which is the most common ectoparasite seen on bats but rarely act as the vector for this virus in comparison with ticks and mosquitoes. Next, the results show presence of non-specific bands from gel electrophoresis, which may happen due to short sequences of the primers used in this case as well as nucleotide size variation in different genus and serogroups of Bunyavirus. Hence, sequencing for the non-specific bands is required for further identification of the virus to further confirm the identity of the band whether they belong to Bunyavirus or other organisms.

Appendix A

Appendix A.1: Sample labelling



Labelling of the samples based on the location it was collected (S= Sekayu; RE= Gunung Reng), bat identification number (ID), family of ectoparasites (Ai= *Nycteribiidae*, Aii= *Strebliidae*) and the sample number.

References

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