MOLECULAR-BASED DETECTION ON CORONAVIRUS IN BATS OF THE UNIVERSITY OF LAMPUNG CAMPUS

Undergraduate Thesis

Written by

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ABSTRACT

MOLECULAR-BASED DETECTION ON CORONAVIRUS IN BATS OF THE UNIVERSITY OF LAMPUNG CAMPUS

Elfita Nova Yunior

The presence of bats in urban environments can have both a positive impact, such as seed dispersal and a negative impact due to its the potential for zoonosis that can be transmitted from animals to humans. The acute respiratory disease pandemic occurred in December 2019 caused by a coronavirus associated with a virus in bats. In Myanmar, coronavirus was found in insect-eating bats and in Indonesia, coronavirus has been found in bats in Gorontalo. Research on the detection of coronavirus in bats in Indonesia has never been conducted on the island of Sumatra. This study aims to identify bat species and detect the presence of coronavirus in bat oral swab samples using the predict protocols method, in the Department of Mathematics area, University of Lampung. Bat life trapping and oral swab sampling of bats were carried out in the back site area of the Mathematics Department, Faculty of Mathematics and Natural Sciences, University of Lampung as part of the urban area. Detection using the predict protocol method has been conducted at the Biotechnology Laboratory, Lampung Disease Investigation Center including the stages of RNA extraction, cDNA synthesis, predict protocol, coronavirus amplification, and electrophoresis. Of six individual bats, two fruit bat species were found, five are Cynopterus *brachyotis* and one *Cynopterus sphinx*. All bat oral swab samples obtained were *predictively negative*, indicating that the six individual bats caught were not infected with coronavirus.

Keywords: Bats, Coronavirus, Predict protocol, University of Lampung, Zoonosis

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LETTER OF AUTHENTICITY OF UNDERGRADUATE THESIS

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Hereby I declare that my undergraduate thesis title

"Molecular-Based Detection on Coronavirus In Bats of The University of Lampung Campus"

In truly and entirely my own work, created in accordance with the applicable norms and academic ethics. Furthermore, I do not object to the use of some or all of the data from this undergraduate thesis by faculty members and/or the study program for publication purposes, provided that my name is mentioned.

If it is later proven that my statement is untrue, I am willing to accept academic sanctions, including the revocation of my bachelor's degree, as well as legal consequences.

Bandar Lampung, June 12 2025

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BIOGRAPHY

The author was born in Central Lampung, Lampung on November 21, 2002, as a the oldest of three siblings, from Mr. Yuni Iswanto and Mrs. Sri Wahyuningsih. The author started his first education at the Integrated Islamic Kindergarten Insan Kamil in 2008 to 2009. Elementary school at SDIT Insan Kamil in 2009 - 2015, junior high school at SMPN 1 Terbanggi Besar from 2015 to 2018 and senior high school at MAN 1 Lampung Tengah from 2018 to 2021. The author was officially accepted as an undergraduate student in the Study Program of Biology, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung in 2021 through the Joint Selection for Entry to Colleges.

During her time as an undergraduate student, she participated as an laboratory practice assistant of some courses i.g. Ecology, AMDAL, Cell Biology, Molecular Biology Techniques, and Genetic Engineering. In 2023, she and her team was granted the Student Creativity Program of Community Service done in Liman Benawi, Lampung Tengah. In 2024, for her internship course (Praktik Kerja Lapangan), scientific articles with the title **Molecular Detection of Lumpy Skin Disease in Cattle Samples *(Bos taurus)* at Lampung Disease Investigation Center** were published**.** In the same year, as part of a practical course (Kuliah Kerja Nyata) she became a national delegate of the University of Lampung in Maluku. Results of this research was presented in the International Conference on Medical Science and Health (ICOMESH) and is an ongoing process as part of its chapter book.

PRAYER

Bismillahirahmanirrahim

By giving thanks to Allah SWT, I dedicate my work, which I have done with love and affection to:

My father, mother, and family, who always support me and give me encouragement and prayers at every step of the way.

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My friends from college who supported each other.

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MOTTO

"It may be that you dislike something even though it is good for you, and it may be that you like something even though it is bad for you. Allah knows, but you do not know."

(Q.S. Al-Baqarah: 216)

"If you cannot bear the fatigue of studying, then you must be able to bear the pain of ignorance."

(Imam Syafi'i)

"If you were given the opportunity to change your life, would you stay the same or go for something better?"

(Qiz Balqiz)

"Fall down 7 times, stand up 8"

(Mark Lee)

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All the praises are to Allâh, the Lord of the 'Alamîn. I give thanks to Allah SWT. on His grace and guidance have given the author the ability to complete this final assignment and send prayers and greetings to the Prophet Muhammad, SAW.

Thesis with the title **"Molecular based Detection on coronaviruses in bats of the University of Lampung campus"** which has been implemented from February 2024 to February 2025 funded by PIU HETI University Lampung and in collaboration with the Lampung Disease Investigation Center.

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I realizes that in the process of writing this thesis there are many shortcomings and errors. Therefore; suggestions are welcome. Hopefully this thesis can be useful for all of us.

Bandar Lampung, June 12 2025

Elfita Nova Yunior

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I. INTRODUCTION

1.1 Background

Indonesia is one of the countries that has high biodiversity. According to National Geographic Indonesia (2019), Indonesia ranks second in the world for its highest biodiversity after Brazil. Biodiversity is the diversity of all types of plants, animals and microorganisms (microorganisms), as well as the processes that occur in the ecosystem and its environment. In terms of species diversity, Indonesia has the world's largest palm diversity, namely more than 400 types of wood Dipterocarps which is the largest type of commercial wood in Southeast Asia and more than 25 thousand types of flowering plants and various fauna. Indonesia's mammal richness is the highest in the world with 515 species (36% endemic), 121 species of swallowtail butterflies (44%), the world's highest of reptiles with more than 600 species, including bats (Ministry of Environment and Forestry, 2003).

Bats are the only mammals that can fly, their upper body parts are modified into wings, an extension of the body that forms from elastic membranes. The function of these wings is to help fly and cover his body (Lekagul and McNeely, 1977). There are two types of bats in Indonesia, fruit-eating bats, frugivorous and insect-eating bats, insectivorous bats. Frugivorous bats help disperse seeds from the fruits they eat, while insect-eating bats as biological control, maintain the balance of the ecosystem by eating insects that disturb plants. The bats habitat includes caves, forests, hollow trees and fruit trees such as oil palm (*Elaeis guineensis*), jackfruit (*Artocarpus heterophyllus*), and

breadfruit (*Artocarpus altilis*). The existence of trees is very important for the life of bats (Prasetyo *et al.,* 2011). Apart from forests, bats also live in urban settlements.

Bats are hosts for many viruses and diseases. The interaction between bats and humans allow disease-causing microorganism enter the human body (Wijayanti, 2021). Bats are known as reservoirs of viruses that threaten human health. Recently, the acute respiratory disease pandemic in December 2019 was caused by coronaviruses and is associated with a virus that exists in bats. Valitutto *et al.* (2020) stated that coronavirus was found in bats that eat insects in Myanmar. In Indonesia, coronavirus in bats was found in Gorontalo, a virus discovered through nucleotide sequencing analysis in *Pteropus alecto* bat confirmed to be coronavirus (Febriani *et al.,* 2018).

Research on the detection of coronavirus in bats on Sumatra Island has never been done. Predict Protocol based detection can be done to determine the presence of coronavirus in bats in urban settlements, especially Bandar Lampung, Lampung. The Predict Protocol is a molecular analysis technique that groups viruses including influenza viruses, paramyxoviruses, and coronaviruses. Common Predict Protocol methods used to detect the presence of infectious diseases and to provide understanding of potential pathogens and how they are transmitted to humans or animals (Goldstein *et al.,* 2016; Rustiati *et al*., 2024). One of the techniques that can be used to detect coronavirus is through oral swab samples.

1.2 Research Objectives

This research has the following objectives.

1. Identification of bat species in the Department of Mathematics, Faculty of Mathematics Natural Sciences, University of Lampung;
2. Detection of coronavirus in bat oral swab samples on campus University of Lampung with Predict Protocol technique.

1.3 Use of Research

This research is to obtain confirmation of the existence of coronavirus in bat oral swab samples on the University of Lampung campus.

1.4 Theoretical Framework

Bats are one of the animals that have an important role in the environment, seed dispersal from fruits that are eaten and also as a pest control. Fruit bats helps the pollination process through eating activities, insects eating bats act as biological controllers. Bat habitats are in caves, forests, hollow trees, and fruit trees. In addition to the forest, many bats are found in urban areas. Bat is a host for many viruses and diseases. Myanmar finds virus identical to SARS CoV-2 in bod bats. In Indonesia, the virus has been found in bats in Gorontalo.

The virus found was confirmed to be a coronavirus. Human activities tend to reduce the proportion of land as natural habitat bats, as a result humans and bats live in the same space and allow interaction between humans and bats. Detection of coronavirus in bats in urban settlements is necessary as an effort to detect the presence of coronavirus in bats early. One of the the location chosen for sampling was in the Department of Mathematics area in Faculty of Mathematics and Natural Sciences, University of Lampung. This location was chosen due to its natural landscape as a habitat for bats. There are oil palm trees (*Elaeis guineensis*), jackfruit (*Artocarpus heterophyllus*), and breadfruit (*Artocarpus altilis*). It has the potential for interaction between humans and bats. Bat life trapping was carried out by mist net. The method for detecting coronavirus is through oral swab samples analyzed molecularly with Predict Protocol technique in the Biotechnology Laboratory, Lampung Disease Investigation Center. It is to confirm the presence of coronavirus in bats in University of Lampung campus area.

II. LITERATUR REVIEW

2.2 Bats

2.2.1 Bats Biology

Bats are the only mammals that can fly. Their wings bats are formed from the extension of the second and fifth fingers which covered by a flying membrane, a patagium. The patagium is formed between the forelegs and hind legs, while between the hind legs and tail forms an interfemoral or sac. In female bats, the patagium functions to hold the young newborn bats (Wijayanti, 2021).

There are two types of bats found in Indonesia, insectivorous or insect-eating bats (Figure 1) and frugivorous or fruit-eating bats (Figure 2). These bats have an important role in maintaining balance in the ecosystem. Fruit bats help disperse seeds from fruit eaten and insectivorous bats eat insects which disturbs plants (Prasetyo *et al*., 2011).

Figure 1. Insectivorous bat (Source: National Geographic, 2015).

Figure 2. Fruit bat (Source: National Geographic, 2015).

Insectivorous bats and fruit-eating bats have morphological differences. Insectivorous bats have a smaller body and eyes than fruit bats, as well as various facial shapes. Insectivorous bats have tragus rounded at the tip. The tragus serves to help insect-eating bats that rely on their abilities hearing at night to look for prey, echolocation capabilities. Fruit bats have larger body size, eyes, and a snout like a dogs (Fitria *et al.*, 2021).

The classification of bats is as follows:

Kingdom : Animalia

Phylum : Chordata

Class : Mammalia

Order : Chiroptera

(Corbert and Hill, 1992).

There are 21 families in Chiroptera, nine are found in Indonesia, Pteropodidae, Megadermatidae, Nycteridae, Vespertilionidae, Rhinolophidae, Hipposideridae, Emballonuridae, Rhinopomatidae, dan Molossidae. Thera are 10 genus in Pteropodidae, include Pteropus, Acerodon, Cynopterus, Eonycteris, Macroglossus, Nyctimene, Rousettus, Dobsonia, Balionyteris, and Harpionycteris (Suyanto, 2001).

2.1.2 Habitat and Behavior

The natural habitats of bats are diverse, including caves, forests and plantations, especially in shaded environments. Bats sleep in hanging up site down. The fruit-eating bat species sleeps in large trees and some insect-eating bats take shelter in holes trees, bamboo gaps, or house ceilings in settlements (Cobert and Hill, 1992). Bats are nocturnal animals, active at night. sunset to sunrise. This behavior is caused by its wings has a thin skin membrane and is very vulnerable against sunlight, so more heat is absorbed than that is released. Bats have adaptations in the form of senses that are very helpful for movement at night, reducing competition with animals that are active during the day, such as birds. During the day, bat hangs with its legs upside down, hiding its body with wings when it is cold, and flaps its wings when it is hot air (Prasetyo *et al.*, 2011). Bats also perform this behavior echolocation using echo information to understand space, control the direction of movement, and

adjust navigation strategies. flights. The presence of tragus in the insectivorous bat's ear helps for echolocation (Teshima *et al.,* 2022).

2.1.3 Ecological Status

The existence of bats is threatened by human activities, such as hunting for meat, destruction of the natural habitat of bats, and climate change that makes it difficult for bats to adapt. The International Union for Conservation of Nature (IUCN) states twenty-three species of bats are in the critically endangered (CR), facing long-term extinction risks, 85 species bats in the endangered category (EN), 113 species of bats vulnerable (VU), and 236 bat species are "data deficient". It shows that bats need attention for conservation efforts (Bat Conservation International, 2024).

2.2 University of Lampung

The University of Lampung is one of the universities in Lampung. It was founded on September 23, 1965 based on the Decree of the Minister of Higher Education and Science (PTIP) No. 195 of 1965 with two faculties, namely economics and law. The University of Lampung area is a representative living space which is shared, both with humans and wild animals, because its natural landscape is quite supportive as a natural habitat for wildlife including bats. The Department of Mathematics, Faculty of Mathematics and Natural Sciences, University of Lampung has a landscape with many trees that have the potential to be a habitat for bats. The habitat of bats is in trees or on the roofs of buildings. This is allows direct interaction between bats and humans that have the potential to cause zoonotic effects.

2.2 Zoonosis

High human population in urban areas requires wider space. Space cannot be expanded, so there is a change in land use that tends to reduce the proportion of land as natural habitat wildlife and resulting in the use of human and wildlife living together. This allows for direct and indirect interaction directly between humans and wildlife. One of the effects of human interaction with wildlife in the environment is zoonosis (Wijayanti, 2021). According to the World Health Organization (2020), zoonosis is an infectious disease that transfers from animals to humans. Zoonotic pathogens can be in the form of bacteria, viruses, or parasites. Zoonoses can spread from animals to humans directly through food, water, or the environment. The development of zoonoses in recent years shows that diseases transmitted from animals to humans are increasing (Khairiyah, 2011).

2.3 Coronavirus

Coronavirus comes from the Coronavidae Family, Orthocoronavirinae Subfamily, and the Order Nidovirales. Coronaviruses are wrapped in a capsule with a genome positive-sense single-stranded RNA that has the ability to encode mRNA (messenger RNA) and protein, as well as symmetrical nucleocapsids helical. The coronavirus genome has a size ranging between 26 and 32 kb. Coronavirus is a group of viruses that can cause disease in humans, birds, poultry, and fish. Coronavirus infections in humans generally mild, can cause respiratory tract illness. Rare types of coronavirus infections in humans, including cervix acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), or pneumonia (Wasito and Waryastuti, 2020).

Coronavirus can mutate a million times higher than its host because it is an RNA virus that has a high mutation rate (Duffy, 2018). One of the reasons coronavirus has a high mutation rate is relatively high is because it has a very long RNA strand and increasing the possibility of mutation. Coronavirus undergoes recombination with high genetics, parts of its genomic RNA are

exchanged for other viral RNA from the same or different species (Beltz, 2022).

SARS-CoV-2 is a coronavirus that causes coronavirus disease (Covid-19) which caused a pandemic in December 2019. This virus infects many people and is very life threatening, especially physical and mental health (Susilo *et al.*, 2022). KawalCovid19 (2023) stated that until January 2023 in Indonesia there were 6.72 million cases confirmed and 160,777 deaths due to the coronavirus pandemic. Bats are a reservoir for many viruses such as ebola, rabies, nipah, and coronavirus. In China, a virus was found in bats that is similar to with SARS-CoV. The proportion of antibodies against bat SARS-CoV in bats studied reached 84% (Calisher *et al.*, 2006).

2.4 Predict Protocol Technique

The Predict Protocol method is a technique of the Emerging Pandemic Program Threats (EPT) managed by the US Agency for International Development (USAID). The Predict Protocol technique has contributed as a global early warning system that can detect and mitigate the impacts from new zoonotic diseases that jump from animals to humans. Samples were collected from a variety of animals, including bats, rodents, primates, and livestock (Predict Consortium, 2020).

The focus of the Predict Protocol is to build an evidence base for the emergence of zoonotic diseases from wildlife sources that will provide information to reduce, control and prevent emerging infectious diseases (EID). The Predict Protocol method aims to reduce the potential for danger for wildlife populations by simply implementing life trapping and implementing conservation ethics in all activities. Method approach Predict Protocol should achieve detection of unknown viruses but potential to emerge. Sampling was conducted near the community humans who are susceptible to zoonoses (Johnson *et al.*, 2015; Gilardi *et al.*, 2018).

In identifying and describing DNA and RNA viruses in wildlife samples, Predict Protocol implements consensus polymerase chain reaction (cPCR) and high-throughput sequencing (HTS). This method allows detection of viruses that may be present at low levels in surveyed populations such as alphaviruses, arenaviruses, astroviruses, bunyaviruses, coronavirus, filovirus, flavivirus, herpesvirus, orthomyxovirus, paramyxovirus, poxvirus, reovirus, retrovirus, and rhabdovirus (Gilardi *et al.*, 2018).

III. RESEARCH METHOD

3.1 Research Time and Location

Research project on "Molecular-Based Coronavirus Molecular Detection Study on Bats on the University of Lampung Campus" is under the research project of Dra. Elly Lestari Rustiati, M.Sc. Detection of Potential Existence Emerging Infectious Disease (EID) in Bats in Lampung based on Predict Protocol funded by PIU HETI University of Lampung and in collaboration with the Lampung Disease Investigation Center. Bat life trapping and oral swab sampling was carried out in the Department of Mathematics area, Faculty of Mathematics and Natural Sciences, University of Lampung in February 2024. Molecular analysis was performed in January-February 2025 in the Biotechnology Laboratory, Lampung Disease Investigation Center.

3.2 Tools and Materials

The tools used are mist net, calico bag, caliper, pesola, PCR WorkStation, biosafety cabinet (BSC) class II, centrifuge, vortex, micropipette with filter tips, microtube, optical plate, laminar air flow, collection tube along with spin column, thermo cycler, chamber, Qubit Fluorometer, and agarose gel electrophoresis.

The materials used are rubber gloves, masks, tissues, kits from QIAGEN (*QIAamp® DNA Mini Kit* (250) cat. no. 51306) which consists of AVL

buffer , carrier RNA, AW1 buffer, AW2 buffer , AVE buffer , collection tube 2 ml, invitrogen kit (QubitTM RNA BR Assay Kit), kit from Tetro kit cDNA synthesis (BIO 6201), forward primer and reverse primer (Table 1), and Nuclease Free Water.

Table 1. Primer sequences for RNA-Dependent RNA Polymerase gene markers (Watanabe et al., 2010).

Primer	Primary sequence
Round 1: Forward	5'GGTTGGGAYTAYCCHAARTGTGA'3
Round 2: Reverse	5'CCATCATCASWYRAATCATCATA '3
Round 1: Forward	5'GAYTAYCCHAARTGTGAYAGAGC'3
Round 2: Reverse	5'CCATCATCASWYRAATCATCATA'3

3.3 Procedures

3.3.1 Bat Life Trapping

Selection of bat capture and sampling locations based on preliminary survey of the existence and activity of bats. The Department of Mathematics, Faculty of Mathematics and Natural Sciences Natural Science was chosen because there are active bats and potential sources of food, oil palm trees (*Elaeis guineensis*), jackfruit (*Artocarpus heterophyllus*), and breadfruit (*Artocarpus altilis*) which is the habitat of bats. Preparations for capturing bats were at 17.00 WIB by installing a mist net 4-6 meters high. Bat catching was carried out at 18.30 WIB at sunset, because bats are sensitive to light.

Bats trapped in mist nets were handled carefully to releasing it from the mist net. The bat handling process is carried out at 18.48 WIB from the capture of the first bat individual. Bats are placed in numbered calico bags for further identification of species.

3.3.2 Identification of Bat Species

Identification of bat species is done by morphology and morphometry. Morphological observations are carried out by observing hair color, face shape, gender, and status reproduction. Morphometric observations are carried out by measuring forearm length, calf length, ear length, and body weight bats (Figure 3). The process of identifying bat species refers to the *Field Guide Series book: Bats in Indonesia* (Suyanto, 2001). Bat trapping and identification assisted by Krisantus Endra Unggul Kusuma, S.Si.

Figure 3. Identification of bat species by morphology and morphometry

3.3.3 Bat Oral Swab Sampling

In bat oral swab sampling, handling techniques (handling) and restraint (animal control with aids) bats are done to facilitate sampling. The technique handling and restraint of bats must take into account their welfare. In addition to making it easier to take samples, it also maintains animals remain safe in the sampling position. Handling techniques performed using the pinch grip method (clamping) in aseptic conditions using rubber gloves by clamping the membrane part and bat's neck (Figure 4). The thumb and middle finger clamp the membrane bat towards the middle while the index finger presses the neck bats. Before being released, the bats are given a drop of water using a damp tissue. The bat release was carried out at bat capture location.

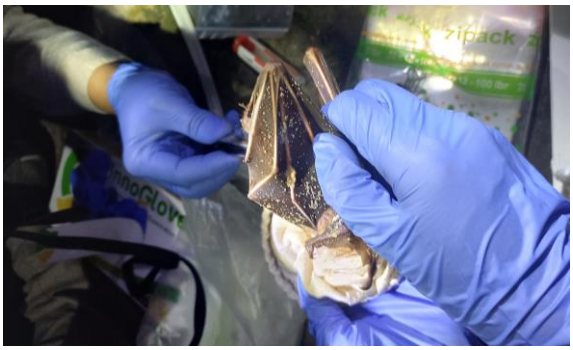

Figure 4. Hand position when handling and restraining bats.

Oral swab samples were taken by swabbing the mouth of the bat thoroughly gently using a small size cotton swab (Figure 5) then the cotton swab is inserted into a VTM tube (Figure 6) and stored in a cool box. The samples obtained are then taken into the cool box and then taken to Lampung Disease Investigation Center Biotechnology Laboratory and stored in the freezer. Sample preparation and molecular detection coronavirus was conducted at the Biotechnology Laboratory, Lampung Disease Investigation Center. Oral swab samples were placed in a sample rack and vortexed before extraction to maintain sample quality.

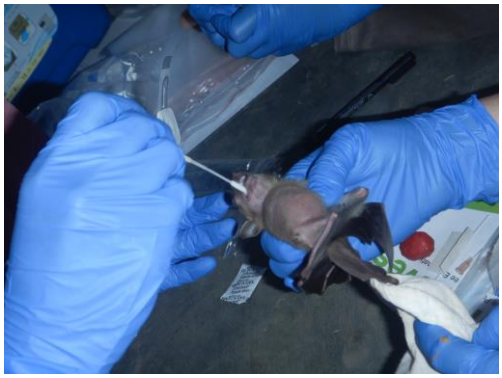

Figure 5. Bat oral swab sampling

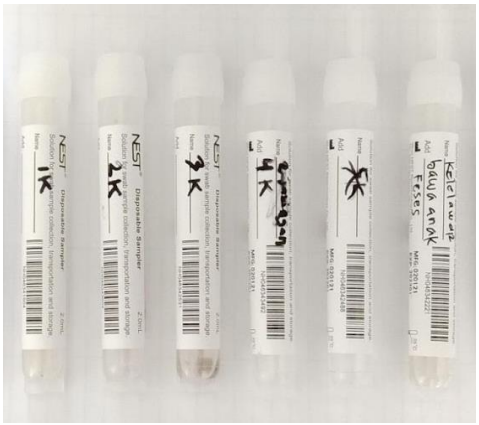

Figure 6. Results of bat oral swabs in VTM tubes

3.3.4 RNA extraction

Extraction was carried out using a DNA extraction kit inside biosafety cabinet. RNA extraction was carried out based on the protocol from QIAamp® RNA Mini Kit (250) consisting of three core stages, lysis, binding, and purification. The first stage is the lysis process which carried out by adding 5.6 µl of carrier RNA and 560 µl of buffer AVL into a microtube containing 140 µl of sample. The solution that contained the sample, carrier RNA, and AV buffer were homogenized using a vortex for 15 seconds and incubated for 10 minutes at room temperature.

Then the second stage is binding. The solution that has been incubated 560 µl of absolute alcohol was added and homogenized by vortexing for 15 seconds. A total of 630 µl of the homogeneous solution transferred into a spin column and centrifuged for one minute at a speed of 10000 rpm at a temperature of 4 – 8ºC. The centrifugation process serves to form a supernatant at the bottom of the collection tube. The supernatant is discarded and the collection tube is replaced. The remaining solution is transferred to the viral spin column and centrifuged again at 10,000 rpm at a temperature of 4 – 8ºC. The function of absolute alcohol in solution is to bind compounds to the silica membrane in the spin column.

The third stage, namely purification using buffer AW1 and buffer AW2. The first step is 500 µl of AW1 buffer added to the spin column and centrifuged for one minute at a speed of 10000 rpm at a temperature of 4 – 8ºC. The supernatant is discarded and replaced with the collection tube. The second step is as much as 500 µl of buffer AW2 was added to the spin column and centrifuged for three minutes at a speed of 14000 rpm at a temperature of 4 – 8ºC. After that, the supernatant was discarded and replaced with a new collection tube, and was done centrifugation for 1 minute at 14000 rpm in temperature 4 – 8ºC.

All purification processes are complete, then the spin column transferred to a microtube for the elution stage. A total of 60 µl of buffer AVE is added to the spin column which is inside microtube which was then centrifuged for one minute with speed of 10000 rpm at a temperature of 4 – 8ºC. The spin column is discarded and microtubes containing extracted RNA are stored in a cupboard freezer at -20ºC.

3.3.5 cDNA synthesis

The extraction results in the form of DNA sample are then continued to the process cDNA synthesis. cDNA synthesis is carried out because of the RNA in the virus is fragile, easily degraded in a short time, so it needs to be changed into cDNA as it is more stable. The cDNA synthesis process is carried out through two stages, master mix and amplification using Tetro kit cDNA synthesis (BIO 6201). Master mix is a reagent that is used in the RNA amplification process. Making a master mix begins with the process of mixing 1.5 ml of RNA sample, 2 ml of RT buffer, 0.5 ml RNase, 0.5 ml dNTP, 0.5 ml Oligo (dt), 0.5 ml Tetro RT, and 4.5 ml Depc water. The DNA amplification process is a process DNA chain copying using Polymerase Chain Reaction with the help of enzymes at certain times and certain cycles using the ABI 7500 PCR ThermoCycler. DNA amplification consists of into three stages,

stage 1 (denaturation) at a temperature of 48ºC for 20 minutes, stage 2 annealing at a temperature of 95ºC for two minutes, and stage 3 extension at a temperature of 72ºC for 10 minutes. cDNA synthesis is carried out to prevent RNA degradation. The conversion of RNA to cDNA synthesis is carried out using RT-PCR (reverse transcriptase Polymerase Chain Reaction) method with the help of the reverse transcriptase enzyme which will make mRNA as a template to produce a copy of DNA complementary (Widowati, 2013; Al Amin *et al.,* 2022).

This method allows detection of coronaviruses that may be present in low levels in the surveyed population such as alphaviruses, arenavirus, astrovirus, bunyavirus, coronavirus, filovirus, flavivirus, herpesvirus, orthomyxovirus, paramyxovirus, poxvirus, reovirus, retroviruses, and rhabdoviruses (Gilardi *et al.,* 2018).

3.3.6 Amplification of the Coronavirus Predict Protocol

The coronavirus Predict Protocol amplification process is carried out by heminested Polymerase Chain Reaction (PCR) with marker primers RNA-Dependent RNA Polymerase (Rdrp) gene and was performed twice PCR repetition, round 1 and round 2. PCR round 1 stage begins by mixing 13 µl of master mix, 1.4 µ Nuclease Free Water, 0.8 µl of each encoding primer Rdrp forward and reverse genes , and 5 µl of cDNA template. Next In the PCR round 2 stage, the template cDNA was replaced with 5 µl of the results. round 1 amplification. The stages of round 1 and 2 amplification go through five the same stages, pre denaturation (94°C) for 2 minutes, denaturation (94°C) for 20 seconds, annealing (50°C) for 30 seconds, extension (72°C) for 30 seconds, and post extension (72°C) for 7 minutes. The denaturation, annealing and extension processes were carried out for 35 times.

Predict Protocol amplification is carried out with heminested Polymerase Chain Reaction using RNA-Dependent RNA gene marker primers Polymerase (Rdrp). Heminested PCR amplification is a technique which is used to detect coronaviruses, including SARS-CoV-2 by increasing the sensitivity and specificity of the PCR method. In this technique is done in two rounds of PCR. In the second round using primer set located within the amplified sequence from the first round. The heminested PCR technique produces more products. short compared to the amplification of the first round which increases specificity due to reducing non-specific amplification (Green and Sambrook, 2019). The advantages of using heminested PCR are can detect target virus RNA molecules with a large sample size low and makes it more sensitive than conventional PCR. (Yip *et al.,* 2020; Sirakov *et al.,* 2022).

3.3.7 Electrophoresis

The electrophoresis process is carried out with the aim of separating DNA/RNA based on its electrical charge applied to the agarose gel and given an electric current. The electrophoresis process begins with making agarose gel. One gram of agarose powder is put into in an erlenmeyer flask, 100 mL of TAE buffer was added and heated in the oven for 3 minutes. SYBR Safe DNA dye as much as 10 µl is put into an erlenmeyer flask containing agar solution and homogenized slowly by shaking aligned horizontally. After that, the agar solution is inserted into the chamber that has been fitted with a comb and wait until the gel solution harden, then the comb is removed from the agar mold. The agar that has hardened then dropped into the chamber for the electrophoresis process. The marker is inserted into the first well, followed by insert 5 µl of sample (PCR amplicon results) into wells in order to be sequential. The power supply cable is connected to chamber in the cathode and anode sections. The electrophoresis process is regulated with a voltage of 100 V and a current of 400 A for 25 minutes.

3.3.8 Interpretation of Results

Based on the primers used, the electrophoresis results of the sample can be it is said to be positive if it reaches a size of 440bp. Predict Protocol is a screening method for early detection the presence of coronavirus. A positive PCR result cannot identify a positive sample. The sample should be further tested by sequencing.

3.3.9 Data Analysis

Data analysis was carried out in a qualitative descriptive manner which is displayed with figures and tables that include the proportion of positive and negative results negative of all samples tested.

3.3.10. Flowchart

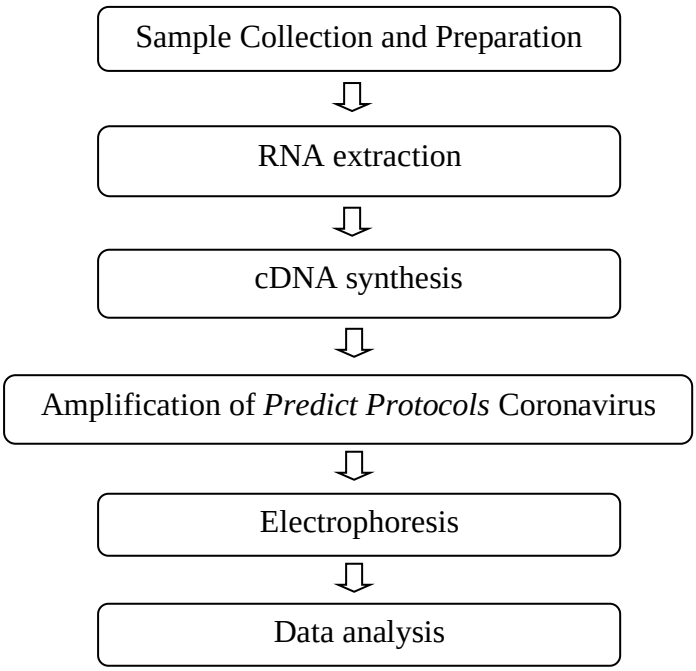

Figure 7. Flowchart of molecular-based coronavirus detection study in the University of Lampung campus area.

V. CONCLUSION AND SUGGESTIONS

5.1 Conclusion

In conclusion of the study "Molecular-Based Coronavirus Detection Study on Bats in the University of Lampung Campus Area" is as following:

1. Bat species identification in the Department of Mathematics, Faculty of Mathematics and Natural Sciences, University of Lampung, include two types of fruit-eating bats *Cynopterus brachyotis* and *Cynopterus sphinx*.
2. Coronavirus detection in bats in the University campus area Lampung is predicted negative which shows that six the individual bats caught were not infected with coronavirus.

5.2 Suggestions

Further studies on other virus families from oral swab samples bats with the Predict Protocol method with more samples is recommended.

REFERENCES

Al Amin, TR, Bimantara, ARIF, Pi, S., Biotech, M., Annisa Khumaira, S. P., & Biotech, M. (2022). Semi-Nested RT-PCR as a Method for Detecting Tilapia Lake Virus (Tilv) in Tilapia (*Oreochromis niloticus*). *(Doctoral dissertation, Aisyiyah University Yogyakarta).*

Alves, R.S., do Canto Olegário, J., Weber, M.N., da Silva, M.S., Canova, R., Sauthier, J.T., ... & Canal, C.W. (2022). Detection of Coronavirus In Vampire Bats (*Desmodus rotundus*) In Southern Brazil. *Transboundary and Emerging Diseases*, 69(4), 2384-2389.

Anam, K., Cahyadi, W., Azmi, I., Senjarini, K., & Oktarianti, R. (2021). Analysis of DNA Electrophoresis Results with Image Processing Using the Gaussian Filter Method. IJEIS *(Indonesian Journal of Electronics and Instrumentation Systems)*, 11(1), 37.

Bat Conservation International. (2024). https://www.batcon.org/about-bats/bats-101/ accessed on May 28, 2024 at 23.56 WIB.

Beltz, L.A. (2022). *Pathogenic Coronaviruses of Humans and Animals: SARS, MERS, COVID-19, and Animal Coronaviruses with Zoonotic Potential.* Elsevier.

Calisher, C.H., Childs, J.E., Field, H.E., Holmes, K.V., & Schountz, T. (2006). Bats: Important Reservoir Hosts of Emerging Viruses. *Clinical Microbiology Reviews*, 19(3), 531-545.

Corbet, G. B., & Hill, J. E. (1992). *The Mammals of the Indomalayan Region: A Systematic Reviews.* Oxford: Oxford University Press.

Food Security, Food Crops, and Horticulture Service of Lampung Province. (2023). https://dinastph.lampungprov.go.id/detail-post/pembangunan-dan-pengembangan-taman-keanekaragaman-hayati-provinsi-lampung accessed on May 8, 2024 at 19.52 WIB.

Dobson, A. P. (2005). What Links Bats to Emerging Infectious Diseases?. *Science*, 310(5748), 628-629.

Duffy, S. (2018). Why are RNA Virus Mutation Rates So Damn High?. *PLOS biology*, 16(8).

Fahlevi, MR, Bakti, D., Sitepu, SF, & Prasetyo, AE (2018). Characterization Molecular Elaeidobius kamerunicus Faust.(Coleoptera: Curculionidae) Origin of North Sumatra Using. *Journal of Agroecotechnology (Joa)-Faculty of Agriculture Usu*, 6(2), 259-270.

Febriani, WD, Saepuloh, U., Ayuningsih, ED, Saputra, RS, Purbatrapsila, A., Nangoy, MJ, ... & Pamungkas, J. (2018). Bat Coronavirus of Pteropus alecto from Gorontalo Province, Indonesia. *The International Journal of Tropical Veterinary and Biomedical Research*, 3(2), 36-42.

Fitria, A., Nurrismawati, A., Haiqal, MRN, Aulianisa, T., Suryanda, A., & Dewahrani, YR (2021). Morphometry and Description of Microchiroptera Roosting Site At the Bodogol Nature Education and Conservation Center. *Proceedings of Biology Education*, 4(1), 160-170.

Genetica Education. (2023). *Common Issues in DNA/ RNA Gel Electrophoresis and Troubleshooting.* https://geneticeducation.co.in/common-issues-in-dna- rna-gel-electrophoresis-and-troubleshooting/#google_vignette accessed on March 7, 2025 at 14:09 WIB.

Gilardi, K. V., & Mazet, J. A. (2018). The United States Agency for International Development Emerging Pandemic Threats PREDICT Project—Global Detection of Emerging Wildlife Viral Zoonoses. *Fowler's Zoo and Wild Animal Medicine Current Therapy*, 9(110).

Goldstein, T., Rejmanek, D., Tremeau-Bravard, A., Liang, E., Navarrete, I., Lipkin, I. , Anthony, S. J. (2016). Laboratory Protocols for PREDICT II. *Surveillance*. 3(4).

Green, M.R., & Sambrook, J. (2019). Nested Polymerase Chain Reaction (PCR). *Cold Spring Harbor Protocols*, 2019(2).

KawalCovid19. *Monitor Information About COVID-19 Precisely and Accurately*. https://kawalcovid19.id/ accessed on September 15, 2024 at 21.54 WIB.

Ministry of Environment and Forestry. (2003). http://perpustakaan.menlhk.go.id/pustaka/home/index.php?page=ebook&co de=ka&view=yes&id=1 accessed on May 8, 2024 at 18.53 WIB.

Kornienko, IV, Aramova, OY, Tishchenko, AA, Rudoy, DV, & Chikindas, M.L. (2024). RNA Stability: A Review of the Role of Structural Features and Environmental Conditions. *Molecules*, 29(24), 5978.

Khairiyah, K. (2011). Zoonosis and Prevention Efforts (Case of Sumatra North). Journal of Agricultural Research and Development, 30(3), 117-124.

Lekagul, B., and McNeely, J. A. (1977). *Mammals of Thailand*. Bangkok: Association for the Conservation of Wildlife.

Marlinda, D., Meliani, C., & Kamal, S. (2022). Identification of Bat Species (Chiroptera) in the Uin Ar-Raniry Banda Aceh Campus Area. In Proceedings of the National Seminar on Biology, *Technology and Education*, 9(1).

Mattioli, R., Maggiore, A., Di Risola, D., Federico, R., D'Erme, M., Francioso, A., & Mosca, L. (2023). Natural Deep Eutectic Solvents Protect RNA from Thermal-Induced Degradation. *Archives of Biochemistry and Biophysics*, 745, 109714.

National Geographic. (2015). https://www.nationalgeographic.com/animals/mammals/facts/bats accessed on November 12, 2024 at 18.32 WIB

National Geographic Indonesia. (2019). https://nationalgeographic.grid.id/read/131833161/kepunahan-biodiversitas-tertinggi-indonesia-peringkat-ke-6 accessed on May 8, 2024 at 18.35 WIB.

Prasetyo, PN, Noerfahmy, S., & Tata, HL (2011). *Typical Bat Species of Sumatran Agroforest.* Bogor: World Agroforestry Centre–ICRAF.

Predict Consortium. (2020). *Predict Standard Operating Procedures for One Health Surveillance*. One Health Institute, University of California, Davis.

Rustiati, EL, Priyambodo, Azmi, SN, Pratiwi, DN, Yunior, EN, Kurniasih, V., Saswiyanti, E., & Srihanto, EA (2024). Applying Predict Protocols for Detection of the Potential Presence of Coronaviruses on Bats in Lampung, Indonesia. *African Journal of Biological Science*, 6(13).

Saiful, M., Febrina, H. S., Fauzan, M., Maisa, D. R. V., Maolani, A., Suryanda, A., & Dewahrani, Y. R. (2021). Studi Literatur Perbandingan Keanekaragaman Kelelawar di Pulau Kalimantan dan Jawa. *BIO-EDU: Jurnal Pendidikan Biologi*, 6(3), 185-197.

Sendow, I., Field, H., Adjid, R.A., Syafriati, T., Darminto, D., Morrissy, C., & Daniels, P. (2008). Seroepidemiology of Nipah Virus in Flying Foxes and Pigs in Several Regions in Indonesia. *Indonesian Journal of Biology*, 5(1), 82179.

Setyaningtijas, AS, Rahma, A., Hanadhita, D., Tarigan, R. Maheswari, H., Mayasari, NLPI, and Agungpriyono, S. (2024). *The Secret Behind the Uniqueness of Bats as Virus Reservoirs.* Bogor: IPB Press.

Sirakov, I., Popova-Ilinkina, R., Ivanova, D., Rusenova, N., Mladenov, H., Mihova, K., & Mitov, I. (2022). Development of Nested PCR for SARS-Cov-2 Detection and Its Application for Diagnosis of Active Infection In Cats. *Veterinary Sciences*, 9(6), 272.

Subudhi, S., Rapin, N., & Misra, V. (2019). Immune System Modulation and Viral Persistence In Bats: Understanding Viral Spillover. *Viruses*, 11(2), 192.

Suyanto, A. (2001). *Field Guide Series: Bats in Indonesia*. Bogor: LIPI Research and Development Center.

Suyanto, A. (2003). *Fruit-Eating Bats from Mount Bromo National Park. Halimun.* Bogor: LIPI Research and Development Center.

Tamam, MB, Ramadani, AH, & Mahbubillah, MA (2022). Detection Coronavirus in Bats in Lamongan Regency. *Livestock and Animal Research*, 20(1), 83-90.

Valitutto, M.T., Aung, O., Tun, KYN, Vodzak, M.E., Zimmerman, D., Yu, J. H., ... & Mazet, J. (2020). Detection of Novel Coronaviruses In Bats In Myanmar. *PloS one*, 15(4).

Wasiati, AR, Bimantara, ARIF, Pi, S., Biotech, M., Anindita, NS, Pt, S., & Biotech, M. (2022). Optimization and Sensitivity Test of Polymerase Chain Reaction (PCR) Primers of RNA-Dependent RNA Polymerase (Rdrp) GEN SARS-Cov-2 Originating from Indonesia *(Doctoral dissertation, Aisyiyah University Yogyakarta).*

Wasito and Waryastuti, H. (2020). *Coronavirus*. Yogyakarta: Lily Publisher

Widiyanti, NLPM, Maryam, S., Parwata, IP, & Mulyadiharja, S. (2014). Comparison of DNA (Deoxyribonucleic Acid) Marker Band Appearance Standard and Determination of Y-Chromosome DNA Length Isolated from Human Blood in Separation Using Different Media. *In Proceedings of the National Seminar on Mathematics and Natural Sciences.*

Widowati, EW (2013). Design of Cytochrome B (Cyt B) Primers as One of the PCR (Polymerase Chain Reaction) Components for Detecting Pig DNA.

Wijayanti, F. (2021). *Bats in Urban Ecosystems*. Depok: Rajawali Printing.

World Health Organzation (WHO). (2020). *Zoonoses*. https://www.who.int/news-room/fact-sheets/detail/zoonoses accessed on May 30 2024 pukul 20.39 WIB.

Yip, CCY, Sridhar, S., Leung, KH, Ng, ACK, Chan, KH, Chan, JF W., ... & To, KKW (2020). Development and Evaluation of Novels and Highly Sensitive Single-Tube Nested Real-Time RT-PCR Assays For SARS-Cov-2 Detection. *International Journal Of Molecular Sciences*, 21(16), 5674.