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RNA Isolation from Oral Swab Sample On Bats of Rural Area, Braja Harjosari, East Lampung

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Abstract

Background. Bats are considered animals that carry infectious diseases, are known to be reservoirs of viruses, and are suspected of causing disease outbreaks. The World Health Organization stated that this disease outbreak is caused by Coronavirus Disease 2019 (COVID-19), which is caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2).

Aims. Under the Research Innovation and Collaboration Program - Higher Education for Technology and Innovation Project (HETI) and in collaboration with the Lampung Disease Investigation Center, a preliminary study on EID detection on bats based on Predict Protocols was done to identify the presence of coronavirus on bats, including rural areas, Braja Harjosari, East Lampung. It is located next to Way Kambas National Park.

Methods. Oral swab samples were taken from ten bats, 3 species: *Cynopterus brachyotis* and *Cynopterus horsfieldi,* and *Scotophilus kuhlii*. RNA extraction was conducted by Predict Protocols, which has four stages: lysis, binding, washing, and elution.

Conclusion. Two samples showed thin-band luminescence. While in the quantity test, there are three samples of good concentration results between 1.8 and 2.0.

Keywords: Bats, Braja Harjosari, Coronavirus, RNA Isolation,

INTRODUCTION

Bats belong to the order Mammalia and have the ability to fly by using their wings. Bats are classified in the order Chiroptera, which means “hand wings”. This name comes from its distinctive feature: forelimbs that are converted into wings. This makes bats the only mammals

capable of flight (Fitria *et al*., 2021). The diversity of bats in Indonesia itself is quite large. Indonesia is home to 205 species of bats, representing 21% of all bat species worldwide. This number is divided into 72 species of bats in fruit-eating bats, consisting of 1 family, Pteropodidae, and 133 species of bats in insectivorous bats, consisting of the Rhinolophidae, Hipposideridae, Emballonuridae, Megadermatidae, Nycteridae, Vespertilionidae, Rhinopomatidae, and Molossidae families (Saputra *et al*., 2017).

Bats have a variety of resting places. It starts in homogeneous forests, then occurs in heterogeneous forests, plantations, residential areas, and most often in caves. The presence of bats in rural settlements allows direct contact with humans or other animals. Wijayanti (2021). This physical contact has the potential to cause zoonosis. According to the World Health Organization (WHO), zoonoses are diseases or infections that are transmitted naturally from vertebrate animals to humans. Bats are believed to be reservoir mammals for several zoonotic viruses that infect humans, livestock, and wildlife. According to Akbar *et al*. (2020) CoV is believed to be transmitted from bats and snakes obtained from a multispecies market in Wuhan, China. Although SARS-CoV-2 is believed to have originated from bats, transmission is not thought to have occurred directly. Research on the presence of coronavirus in bats in Indonesia has been done before, precisely in Olibuu-Gorontalo Province. However, the detection of coronavirus in bats in Lampung has never been conducted. One of the areas where bats can be found is at Braja Harjosari, East Lampung, the buffer zone next to Way Kambas National Park, their natural habitat.

The initial stage of coronavirus detection using the predict protocol is RNA isolation. RNA isolation is carried out in four stages: lysis, binding, washing, and elution. The basic principle of RNA isolation is to obtain a cell extract containing tissue cells, DNA, and RNA after the tissue is broken down and extracted. This cell extract will later be purified to obtain cell pellets containing total DNA/RNA (Faatih, 2009). The next step taken in this test is to confirm the purity of nucleic acids in the sample. The results of RNA isolation will be tested quantitatively and qualitatively using agarose gel electrophoresis and Qubit Fluorometer.

This study is novel because it represents an initial molecular investigation of RNA isolation from bat oral swab samples in Braja Harjosari, East Lampung, a rural buffer area adjacent to Way Kambas National Park. By generating preliminary RNA quality and quantity

data, this research contributes baseline information for future coronavirus detection and emerging infectious disease surveillance in bats from Lampung.

METHOD

Sampling of bat oral swabs was conducted in rural areas of Braja Harjosari village, East Lampung, and molecular detection was carried out in collaboration with the Biotechnology Laboratory at the Lampung Disease Investigation Center. This research was conducted under the supervision of Dr. Elly Lestari Rustiati, M.Sc., funded by PIU HETI University of Lampung and in collaboration with Lampung Disease Investigation Center.

Life trapping and sample collection

Bat trapping is conducted prior to sampling. At this stage, the mist net is stretched on the active path of the bat. Bats that are caught are then taken orally using a cotton swab by inserting a small cotton swab into the bat's mouth approximately 2 cm and gently swabbing along the bat's mouth. Then, the cotton swab is inserted into a VTM tube and stored at a cool temperature.

RNA isolation.

RNA isolation is carried out in four stages: lysis, binding, washing, and elution. The lysis phase was carried out by adding 5.6 µl carrier RNA, 560 µl AVL buffer, and 140 µl of the sample to the microtube. The solution that has been added to the sample is then vortexed, followed by incubation at room temperature for 10 minutes. Binding was performed by adding 560 µl of absolute alcohol and homogenizing by vortexing for 15 seconds. The solution was transferred to the spin column with 630 µl and centrifuged at 10,000 rpm for 1 minute at 4-8°C. Discard the supernatant and replace the collection tubes, then transfer the remaining solution to the viral spin column and centrifuge again at 10,000 rpm for 1 minute at 4-8°C. This step was carried out by adding 60 µl of AVE buffer into the spin column and centrifuging at 10000 rpm at 4-8ºC for one minute. The spin column was discarded, and the microtube containing the extracted RNA was stored in a refrigerator at -20ºC. RNA purity testing was performed using a Qubit fluorometer. The kit used was the Invitrogen Kit (QubitTM RNA BR Assay Kit). A working solution was added, and the sample was placed in a special microtube and tested step by step on the Qubit fluorometer. The purity test results can be checked on the layer.

DISCUSSION

Oral swab samples were taken from ten bats, 3 species: fruit-eating bats of Cynopterus brachyotis (n=7) and Cynopterus horsfieldi (n=2), and an insectivorous bat of *Scotophilus kuhlii* (n=1). Agarose gel electrophoresis was performed to qualitatively assess the presence of RNA in the isolates by molecular weight. RNA quality test results (Figure 1, Table 1) showed the luminescence of two thin bands. RNA quality can be declared good if the 28S/18S ribosomal band is very clearly visible on the agarose gel with the thickness of the 28S band more clearly visible than the 18S band (Schroeder et al., 2006; Felipe *et al*., 2023).

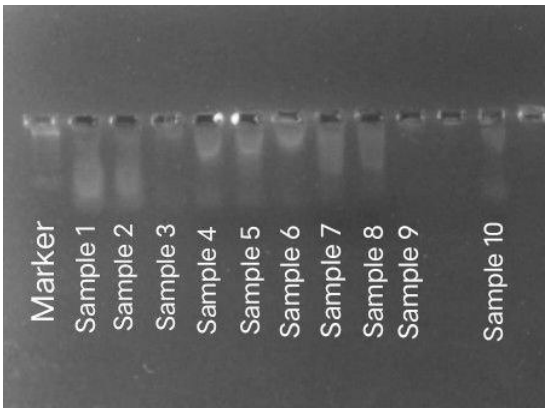

Figure 1. RNA quality test results

Table 1. RNA quantity test results using Qubit Fuorometer

Sample	Concentration (ng/ µl)
Sample 1	1,91
Sample 2	1,99
Sample 3	1,60
Sample 4	1,59
Sample 5	1,29
Sample 6	1,64
Sample 7	2,08
Sample 8	1,83
Sample 9	0,870
Sample 10	1,79

Isolation results in a good DNA purity level of about 1.8 to 2.0. RNA quantification usually measures purity, protein concentration, RNA concentration, and uptake. The purity level of RNA is directly proportional and positively correlated with the uptake value. An uptake value of 2.0 indicates the sample is uncontaminated or pure. In the results of measuring this quantity (Table 1), samples 1, 2, and 8 showed concentration results between 1.8 and 2.0. Proteins are macromolecules composed of several amino acids with specific functions. The RNA isolation process requires removing proteins from the sample, as they can cause RNA contamination. RNA concentration is the amount of RNA (μg) in a sample (ml). Qubit Fluorometry is a quantitative method that uses the principle of fluorescent dyes. Qubit fluorescence is a sensitive and more accurate method for measuring nucleic acids (Pratiwi & Widodo, 2020).

Aspect	Description
State of the Art	Bats are widely recognized as important reservoirs of zoonotic viruses, including coronaviruses. Previous studies have reported coronavirus detection in bats in several Indonesian regions, but molecular surveillance in rural buffer zones near conservation areas remains limited. RNA isolation from oral swab samples is an essential preliminary step for molecular detection using protocols such as PREDICT, followed by RNA quality and quantity assessment through agarose gel electrophoresis and Qubit fluorometry.
Research Gap	Although coronavirus-related bat studies have been conducted in Indonesia, there is still limited information on RNA isolation and preliminary molecular surveillance of bats in Lampung, especially in Braja Harjosari, East Lampung, a rural area located near Way Kambas National Park. The lack of baseline molecular data from bats in this human–wildlife interface area creates a gap in early zoonotic disease detection.
Novelty	This study provides preliminary molecular evidence from oral swab samples of bats in Braja Harjosari, East Lampung. The novelty lies in applying RNA isolation using PREDICT-based procedures to bat samples from a rural buffer zone near Way Kambas National Park, generating initial RNA quality and quantity data as a foundation for future coronavirus or emerging infectious disease surveillance in Lampung.

CONCLUSIONS

In the RNA quality test, two samples showed thin-band luminescence. While in the quantity test, there are three samples of good concentration results between 1.8-2.0.

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