Detection of Emerging Infectious Disease in *Cynopterus brachyotis* and *Rhinolopus boorneensis* as Reservoirs of Zoonotic Diseases in Indonesia

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Abstract

Bats are mammal species that have the highest diversity. During the last two decades, various types of infections caused by microorganisms have again become epidemic in many countries, this phenomenon is known as a new infectious disease that arises and re-emerges or Re-Emerging Infectious Diseases (EID/ REID). The purpose of this study was to identify EID in bats in the karst area of South Malang. The research method of bat sampling is conducted in the karst area of South Malang, bats are identified by species, taken by blood, prepared and carried out by NGS (next generation sequencing). From the results of the research conducted, obtained 2 types of bats namely fruit-eating bats (*Chiroptera brachjatis*) and insect bats (*Rhinolopus boorneensis*). From the results of the NGS, we get specific viruses and bacteria in bats. The conclusion of this research is that bat in South Malang Karst has potential as a carrier of EID and zoonotic disease.

Keywords: *bat, emerging infectious disease, South Malang karst, zoonotic diseases*

Introduction

Bats or known as *Chiroptera* have order-level taxon namely Kingdom *Animalia*, Phylum *Chordata*, Mammalian Class, and Superordo *Laurasiatheria*. Bats or Chiroptera are divided into two sub-orders, namely Micro Chiroptera which are generally insectivor or known as insect-eating bats and Mega Chiroptera which are generally frugivores or known as fruit-eating bats[1]. In general, bats live in a colony but some live solitary[2,3]. In the world there are 18 families, 192 genera and more than 977 species of bats[4]. In Indonesia, there are 8 families and 72 species of members of the Suborder Megachiroptera and 133 species of members of the Micro Chiroptera Sub-order that more than 50% live in caves[3,5].

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Bats carry many diseases that can infect humans, most of which are viruses. In Asia, the Nipah virus, SARS-corona virus, Ebola Reston virus, and the lyssa virus, some are zoonotic viruses that have very high fatality rates, for example 40-80% of humans infected with the Nipah virus have died. Bats infected by the Nipah virus are around 10-30% in each colony, showing signs of previous Nipah infection, and the virus can be excreted through infected bats' saliva and urine. Research results on bats also found that Lyssa virus (a rabies-related virus) was excreted in saliva, transmitted by bite, and found in bats from Thailand. In addition, evidence of the Ebola Reston virus has been found in bats from the Philippines and Bangladesh, Corona virus related to SARS has also been found in Rhinophid and other bats in China to the rest of the world and many other viruses that have recently been found in bats, but the effect is unknown yet on human health[6]. The purpose of this study was to identify EID in bats in the karst area of South Malang, Indonesia.

Materials and Methods

GPS Garmin 64s SEA, Bat trap, Hanet to catch bats, Caliper, Scalpel blades, DSLR Camera Nikkon D3000, Flakon Bottle, as a preservation place for Syring, Calico bags, Canoe drums, Strorage boxes, Formalin 10%, Alcohol 70% , chloroform, for bat anesthesia, DNA and RNA extraction reagents, PCR reagents, NGS (Next Generation Sequencing).

Sample Preparation

The study was conducted in the karst area of South Malang and the Integrated Lab of UIN Sunan Ampel Surabaya, Indonesia. The research period is 6 months from May 2019. 200 microliters of the original sample stored at -70 °C were thawed three times and then centrifuged for 10 minutes. OmniCleave Endonuclease (250U; Madison Epicenter Distributor, WI USA, OC7850K) was added to the supernatant and added 2.5 mM MgCl2, incubated for 1 hour at 37 °C. The genome was extracted from the sample using NucliSENS easy-MAG.

Preparation of NGS

Agilent RNA 6000 Nano Kit (Life Technologies, Waldbronn, Germany) was used to evaluate the total quality of RNA. RNA concentrations were measured using the BR Assay (Life Technologies) Qubit RNA kit. NGS data were processed for NGS sample preparation using the Stranded RNA-Seq Kits SMART (Clontech, Takara Bio Company, CA, USA), Construction for Transcriptom Analysis on the Illumina Platform. The Agilent 2100 Bioanalyzer DNA kit (Life Technologies, Waldbronn, Germany) is used to measure and control the quality of DNA sequencing. NGS was performed using the MiSeq v2 kit (500 cycles) (Illumina, San Diego, CA). After automated clustering in MiSeq, sequencing is processed and genomic sequence readings are obtained.

Computational Analysis of NGS Data

Bowtie 2.032 software is used to filter readings that map the genomes of viruses and bacteria from bats. Data compared to data in the NCBI bank, confirmed again using blastn and blastx, with default parameters.

Results

Table 1. NGS results in the *Chiroptera brachjatis.*

Name	Sequence Read	Total Number of Reads	Average Lenght	Standart Deviation Lenght	Average Quality Score	Standart Deviation Quality Score
Nipah Virus	12.300	18.236	251	125.9	33.8 %	8.6
Hendra Virus	10.850	18.234	741	120.6	32.9 %	8.4
Rabies Virus	9950	11.923	534	135.7	32.8 %	8.2
Japanese Enchephalitis	9475	10.997	1500	124.6	30.9 %	8.1
Alpha Herpes Virus	112.450	149.459	920	112.8	30.8 %	7.9
Bat Coronavirus	20.500	27.164	422	123.5	32.7 %	8.0
Polyomavirus	5,999,600	7,732,040	857	124.8	32.1 %	8.5
Bufavirus	4765	4765	4189	132.9	34.9 %	8.2
Rubulavirus	13.200	15378	1729	130.8	33.8 %	8.3
Australian Lysavirus	10300	11822	5344	133.9	30.7 %	8.4
Bat Reovirus	3,131	1,054,029	418	121.7	30.8 %	8.1
Lagos Bat Virus	10.609	11.930	458	109.8	31.9 %	8.5
Duvenhage Virus	9.875	11.976	479	107.6	32.9 %	7.6
E. coli	3,550,675	4,614,635	363	130.3	33.1 %	8.0

Cont... **Table 1. NGS results in the *Chiroptera brachjatis.***

Salmonella spp	4,200,350	4,857,450	3921	129.5	32.8 %	7.7
Enterobacter spp	4,100,550	4,278,063	1635	101.9	30.5 %	7.9
Klebsiella spp	4,900,345	5,231,007	1331	106.8	30.7 %	7.8
Serratia spp	5,150,355	5,241,455	1815	109.4	30.5 %	8.0
Citrobacter spp	287.689	296,175	1880	124.8	30.6 %	8.1
Pseudomonas spp	5,500,457	6,518,993	770	132.0	30.4 %	8.2
Clostridium septicum	3,175,876	3,266,706	1263	131.6	30.8 %	8.3
Micrococcus spp	3,450,235	3,862,811	1525	130.9	30.9 %	8.4
Staphylococcus spp	2,767,675	2,831,587	804	127.8	30.7 %	8.2

Table 2. NGS results in *Rhinolopus boorneensis*.

Name	Sequence Read	Total Number of Reads	Average Lenght	Standart Deviation Lenght	Average Quality Score	Standart Deviation Quality Score
Nipah Virus	12.300	18.236	251	125.9	33.8 %	8.6
Hendra Virus	10.850	18.234	741	120.6	32.9 %	8.4
Rabies Virus	9950	11.923	534	135.7	32.8 %	8.2
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Micrococcus spp	3.450.235	3.862.811	1525	130.9	30.9 %	8.4
Staphylococcus spp	2.767.675	2831587	804	127.8	30.7 %	8.2

Lenght: bp (base pair)

Discussion

Bat identification refers to the identification key Payne *et. al.* and Suyanto. The first thing to do is catch bats with mistnet, handnet and hanet. Catching this bat is done when the bat returns from foraging. Some of the bats that are caught will be used as samples and the next steps will be taken, while some will be released again after the species has been identified, especially endemic species[2,3]. Bats used as samples were anesthetized with chloroform in a calico bag until dead for 5 minutes (Suhardjono, 1999). Morphometry identification is done based on body measurements. From the results of the study found *Chiroptera brachjatis* fruit-eating bats and insect-eating bats *Rhinolopus boorneensis*[7].

Rhinolophus borneensis or prok-bruk Kalimantan has rather small body characteristics with forearm length of 40-44 mm and there is antitragus. Species of the *Rhinolophus* Genus are characterized by complex nasal leaves, posterior nasal leaves which are triangular in shape, whereas intermediate and anterior nasal leaves are horseshoe shaped[3]. *Rhinolophus borneensis* has grayish brown hair color. Morphometric measurements on captured samples included Weight (6 gr), Head and Body (48.16 mm), Tail (24.11 mm), Ear (20.62 mm), Hind Foot (5.08 mm), Forearm (39.96 mm), Tibia (15.76 mm). Rhinolophus borneensis is distributed in Java, Kalimantan, Sarawak and Sabah, and Asia (Suyanto, 2001). IUCN status Rhinolophus borneensis is a bat species recorded at IUCN with a Least Concern or LC status[8]. In this study also used NGS (next generation sequencing) in analyzing the EID of the two bat species found. The results of the NGS analysis on *Chiroptera brachjatis* and *Rhinolopus boorneensis* can be seen in Tables 1 and 2.

Emerging and Re-Emerging Infectious Diseases (EID/REID) are complex interactions between disease-causing agents, humans, and the environment. In addition, genetic and biological factors from disease-causing agents and humans as hosts are the main contributors to the emergence of EID/REID. Social, political and economic factors also have a role in the process of the emergence of infectious diseases, more specifically, these factors are behavior, demographic status, technological and industrial progress, economic development, land use, trade, travel between countries, and declining quality of the public health system[9,10].

Some countries, especially in the Southeast Asia region are countries that are vulnerable to the emergence of EID/REID[6]. About 60-75% of EID/REID cases are zoonoses, which are diseases transmitted from animals to humans, both livestock or wild animals[5]. Wild animals are considered as a reservoir of several pathogens that were previously unknown to cause infection in humans, one of the wild animals that has the potential to cause zoonosis is bats[11].

Genetic analysis shows that the bat's immune system evolved along with its flight ability. Bat body temperature when flying can reach temperatures of 41 ºC, the same as experienced by mammals when a fever, which is 38-41 °C, conversely, hibernation in bats that live in areas with four seasons has been shown to inhibit the replication of pathogenic viruses and cause some viruses to participate in hibernation. Changes in body temperature is a natural selection of viruses that are thought to increase the diversity of viruses in bats[12]. This results in the co-evolution of viruses and bats which results in the virus being able to survive over a wide temperature range[13,14].

Based on several research results that the number of zoonotic viruses found per bat species is more than rodentia[15]. Diversity of virus species in bats is also related to the tendency of different bat species to live in the same ecosystem (sympatry) thereby increasing interaction with each other[11,15]. More than 248 new viruses have been successfully isolated or detected in bats over the past 10 years and some of these viruses have considerable zoonotic potential. In addition, based on the results of research conducted by Sendow *et al*. found that Henipahvirus in bats on Java and Sumatra[16], Wada *et al*. found gammaherpes virus in fruit bats[17], Sasaki *et al*. found alphaherpesviruses[18]. In this study, found more than one virus in both bats using NGS (Next Generation Sequencing) (table 1 and table 2).

In the study also found bacteria in both bats namely *Chiroptera brachjatis* fruit-eating bats and Rhinolopus Boorneensis-eating bats. Bacteria found in *Chiroptera brachjatis* fruit-eating bats and insect-eating bats *Rhinolopus boorneensis* are more than 1 species and have properties that can be transmitted to humans (*E. coli*, *Salmonella*)[19]. Based on the results of research conducted by Aminollah found *E. coli* and *Salmonella* bacteria in the Pongangan cave in Gresik. Another data published that *Sterptobacillus*, *Streptococcus*, *Staphylococcus*, *Shigella* and *Bacillus* in the Menoreh Karst region. The results of this study, the detection of EID in bats in karst of South Malang, Indonesia is

a new discovery and is a source of information both in Indonesia and in the world.

Conclusion

In sum, *Chiroptera brachjatis* fruit-eating bats and insect-eating bats *Rhinolopus boorneensis* were obtained. In addition, EID is obtained in bats, so it needs further study, especially on EID in non-bat host (humans and other animals).

Conflict of Interest: The authors declare that they have no conflict of interest.

Source of Funding: This study funded by the Ministry of Religion of the Republic of Indonesia.

Acknowledgement: We thank Arif Nur Muhammad Ansori for editing the manuscript.

Ethical Approval: This study was approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

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