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PHYLOGENETIC ANALYSIS AND MUTATION OF SARS-COV-2 IN BATS IN KARST MALANG CITY, INDONESIA

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Abstract

Introduction: *A group of people in China were hospitalized with an initial diagnosis of pneumonia of unknown cause. The patients were linked to a wholesale wet seafood and animal market in Wuhan, Hubei Province, China. The disease has spread to other provinces in China, Thailand, Japan, and South Korea in less than a month. SARS-CoV-2 was found to originate from bats. Therefore, this research aims to analyze SARS-CoV-2 mutation in bats in Malang Karst, Indonesia.* ***Methods:*** *Other bat body parts used as research samples include the brain, liver, kidneys, intestines, pancreas, fetus, blood, lungs, and ectoparasites. The samples were taken separately and placed in a container containing 10% PBF. For further analysis, we used RNA Extraction, Real-Time PCR, Sequencing, and CoV Gisaid mutation analysis software to analyze the sequencing data. Then, EMBL software will be used to analyze the phylogenetically.* ***Results and Discussion:*** *There was 1 sample that showed a positive result for Covid-19, namely the intestine of the Cynoptera brachyotis species. There were differences between SARS-CoV-2 in bats in Malang Karst in Indonesia compared to SARS-CoV from 2000 to 2019. The spike protein's receptor binding domain (RBD) is the most variable part of the coronavirus genome.* ***Conclusion:*** *From the research results, one positive sample was obtained using Real-Time PCR, and based on mutation analysis, mutations were found in SARS-CoV-2 against the SARS-CoV virus from 2000-2019. Further research is needed, especially regarding SARS-CoV-2 as a vaccine.*

INTRODUCTION

The coronavirus is one of the main pathogens that attacks the human respiratory system. Previously, there were outbreaks of coronaviruses, including Severe Acute Respiratory Syndrome (SARS)-CoV and Middle East Respiratory Syndrome (MERS)-CoV. Both outbreaks pose an extraordinary threat to public health (1). It was observed that around 2019, at the end of December, several patients diagnosed with pneumonia were being treated in hospitals. However, after further investigation, the cause of pneumonia was unknown. Epidemiologically, these patients are related to the Wuhan, Hubei Province, China's seafood and animal wet wholesale market. On January 7, 2020, since then it has continued to spread rapidly to more than 190 countries and regions (2).

It was recorded that from 31 December 2019 to 3 January 2020, 44 cases were reported, and it can be said that this has increased rapidly. It didn't take long (less than 1 month) for this disease to spread to other provinces in China, Thailand, Japan, and South Korea. Initially, this disease was identified as a novel coronavirus (2019-nCoV). Still, on February 11, 2020, the WHO announced a new name: Corona Virus Disease (Covid19), which is caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). The lack of information and the level of public knowledge regarding the condition of Covid19 has caused the spread of cases to become more widespread. There were ±86 initial cases reported in several countries, including Taiwan, Thailand, Vietnam, Nepal, Sri Lanka, Japan, Singapore, Malaysia, Cambodia, Saudi Arabia, South Korea, and Indonesia. Ultimately, WHO officially declared Covid19 a pandemic on March

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12, 2020. It was reported that as many as 634,835 cases and 33,106 deaths worldwide as of March 29, 2020. In Indonesia itself, as of September 2020, there were 203 thousand positive cases of Covid19 and 8,336 victims who died. Indonesia is in fourth place in the number of confirmed cases, with 440,549 cases, as well as third in Asia and 21st in the world in the number of deaths (3). If the epidemic continues, it could threaten major hub countries in Asia. The potential for human-to-human transmission needs to be a major concern so that public health officials continue to be vigilant, especially at export locations (4).

Coronavirus (CoV) is a single-stranded positive-sense RNA virus that belongs to the Coronaviridae family. Based on the genome organization and phylogenetic relationships, the coronavirus has been classified into the Coronavirinae subfamily consisting of the four genera Alphacoronavirus (αCoV), Betacoronavirus (βCoV), Gammacoronavirus (γCoV), and Deltacoronavirus (δCoV) (5). In 2002-2003, SARS-CoV emerged in China with 8000 clinical cases and 800 deaths. Since 2012, MERS-CoV has caused persistent epidemics on the Arabian Peninsula. Both viruses have been found to have originated in bats and were then transmitted to intermediate mammalian ferrets in the SARS-CoV case and camels in the MERS-CoV case and eventually infected humans (6).

In Indonesia, bats and other animals are widely traded in several live animal markets. Collectors catch bats in forests or caves and sell them to traders at animal markets and restaurant owners (7). Until now, there has been a lot of hunting for bats for human consumption. This is because some people in Indonesia still believe in traditional medicine, such as consuming bats to cure asthma (8). Apart from that, there are eight points based on which bats will represent excellent bioindicators. This consists of relative taxonomic stability, large geographic areas, rich trophic diversity, provision of key ecosystem services, gradual response to environmental changes correlated with other components of biodiversity (such as insects), rapid population decline due to slow population growth, the possibility of measuring several variables (population size, feeding activity, etc.), and the role of bats as reservoirs of novel infectious diseases whose epidemiology may reflect environmental pressures (9).

Based on the explanation above, this research aims to analyze SARS-CoV-2 in bats in Malang Karst, Indonesia. The research was carried out in Malang Karst because this place has very minimal light intensity and is mainly covered by trees, making it a comfortable habitat for bats (10). Bats are mostly forest mammals. Many species roost and/or forage in forests or use forest patches and corridors as migratory stopovers and stops (9). Apart from that, socio-demographically, there are several settlements around this location. People who live close to bat habitats are more at risk of contracting disease because they are used to coming into contact with bats (11).

METHODS

Sample Collection

The research was conducted in a cave in the Malang Karst area, Indonesia. This research used the following materials: 70% alcohol and cotton. These materials assist the anesthesia process and preserve bat samples from which specimens will be taken later. Species samples were captured using the mist net trap method. The bat sampling process uses tools including mist nets, ropes, poles (2.5 – 3 m long) to attach mist nets, long-stemmed nets, calico bags, and scissors. Then, the personnel tasked with catching bats use personal protective equipment such as masks and gloves. Once captured, samples are taken using long tweezers. Captured samples were identified using identification books and cameras.

Researchers chose to catch the bats in the afternoon because that is when they start their activities. The captured bats are then identified to determine their species. The bats we caught in 2019 included *Cynoptera brachyotis, Hipposideros diadema, Hipposideros larvatus, Rhinolopus bornaensis, Rhinolopus affinis, Rhinolopus australis* and *Miniopetrus schreibersii*. Then, in 2020, we caught 6 types of bat species, including *Hipposideros larvatus, Hipposideros bicolor, Hipposideros diadema, Miniopetrus Australis, Hipposiseros ater,* and *Rhinolopus pusillus.* We caught bats in 2019 because, at the end of that year, the first case of Covid19 was found in China, but it had not yet reached Indonesia. We want to see whether the SARS-CoV-2 virus is present in bats in Indonesia. Then we caught bats again in 2020 because, in that year, Covid19 cases started to enter Indonesia and were declared a pandemic by WHO.

The bat is placed on its side (dorsal recumbency) on the styrofoam. The bat wings were fastened proximally and distally using safety pins. After that, a ventral laparotomy operation was performed to open the abdominal cavity. Other bat body parts used as research samples include the brain, liver, kidneys, intestines, pancreas, fetus, blood, and lungs. We also collected ectoparasites found in the bodies of bats as part of research sampling. Most people consume these organs because they have been proven effective in treating various diseases, such as asthma and kidney disease (12). Apart from that, we also used blood as one of the

research samples because the virus can enter humans through direct contact with infected animal body fluids such as blood, secretions, and mucus. Bats can be carriers of the virus, where transmission to other animals and humans occurs due to consuming contaminated body tissue. Several viruses (other than SARS-Cov-2) can be detected in the blood, including the Hendra virus, Equine encephalitis virus, and Madariaga virus (13). Then, we also examined ectoparasites in the body because ectoparasites are disease agents in animals usually found outside the human body, including on the skin's surface and between the hair. Ectoparasites also include parasites that do not remain in the host's body but come and go into the host's body (14). Therefore, we chose these organs and ectoparasites as research samples. The samples were taken separately and placed in a container containing 10% PBF.

RNA Extraction

RNA extraction was carried out on bat organ samples with the MagNA Pure 96 system (Roche, Penzberg, Germany). In addition, RNA was also extracted from cell culture supernates with a mini RNA virus kit (QIAGEN, Hilden, Germany).

Real-Time PCR

The 25 µL reaction contains 5 µL RNA, 12.5 µL 2 × reaction buffer provided with Superscript III one-step RT-PCR system with Platinum Taq Polymerase (Invitrogen, Darmstadt, Germany; contains 0.4 mM each of Deoxyribo-nucleoside triphosphate (dNTP) and 3.2 mM magnesium sulfate), 1 µL reverse transcriptase / Taq mixture from the kit, 0.4 µL 50 mM magnesium sulfate solution (Invitrogen), and 1 µg nonacetylated bovine serum albumin. Thermal cycling was carried out in 4 processes, the first at 55°C for 10 minutes for reverse transcription. Next, it was carried out at a temperature of 95°C for 3 minutes and then 45 cycles at a temperature of 95°C for 15 seconds. Then, the final step was carried out at 58°C for 30 seconds.

Sequencing

DNA sequencing was obtained from amplification results using RT-PCR. The sequencing reaction uses dye terminator disclose enzymatically. Purification is carried out before sequencing the PCR product with the aim of cleaning the PCR product from residual buffer and other reaction components. This is done to obtain maximum sequencing results.

Sequencing Result Data Analysis

The sequencing data were analyzed using the CoV Gisaid mutation analysis software. Phylogenetic analysis was analyzed using EMBL (European Molecular Biology Laboratory) software because it is the most widely used and useful for DNA sequence analysis. The tools facilities owned by EMBL software are complete, especially for analyzing DNA (Clustal, etc.) and proteins.

RESULTS

Bats Identification

Based on Table 1, in 2019, there were 7 types of bats in the research sample, including *Cynoptera brachyotis, Hipposideros diadema, Hipposideros larvatus, Rhinolopus bornaensis, Rhinolopus affinis, Rhinolopus australis,* and *Miniopetrus schreibersii*. Several organs were taken from the seven bats for research, including the brain, liver, kidneys, intestines, pancreas, and blood. From the results of the examination using Real-Time PCR, there was 1 sample that showed a positive result for Covid19, namely the intestine of the *Cynoptera brachyotis* species.

Table 1. Data on Bats in Karst Malang City

Bats Species	Bats of 2019								
	Organs Collected								
	Brain	Liver	Kidney	Intestine	Pancreas	Fetus	Blood	Lung	Ectoparasites
Cynopterus brachyotis	X	X	X	X*	X	-	-	-	-
Hipposideros diadema	-	X	X	X	-	-	X	-	-
Hipposideros larvatus	X	X	X	X	-	-	X	-	-
Rhinolopus bornaensis	X	X	X	X	X	-	-	-	-
Rhinolopus affinis	X	X	X	X	-	-	X	-	-
Rhinolopus australis	X	X	X	X	-	-	-	-	-
Miniopterus schreibersii	X	X	X	X	-	-	-	-	-
Bats Species	**Bats of 2020**								
Hipposideros larvatus	-	-	X	X	-	-	X	X	-
Hipposideros bicolor	-	-	X	X	-	-	X	X	-
Hipposideros diadema	-	-	X	X	-	-	X	X	-
Miniopterus australis	-	-	X	X	-	-	X	X	-
Hipposideros ater	-	-	X	X	-	-	X	X	-
Rhinolopus pusillus	-	-	X	X	-	-	X	X	-

**Positive Covid 19 using Real Time PCR*

In 2020, 6 species of bats were sampled for research, including *Hipposideros larvatus, Hipposideros bicolor, Hipposideros diadema, Miniopetrus Australis, Hipposiseros ater,* and *Rhinolopus pusillus*. Several organs were taken from the six bats for research on the kidneys, intestines, pancreas, blood, and lungs. From the Real-Time PCR examination, all organ samples showed negative results for Covid19.

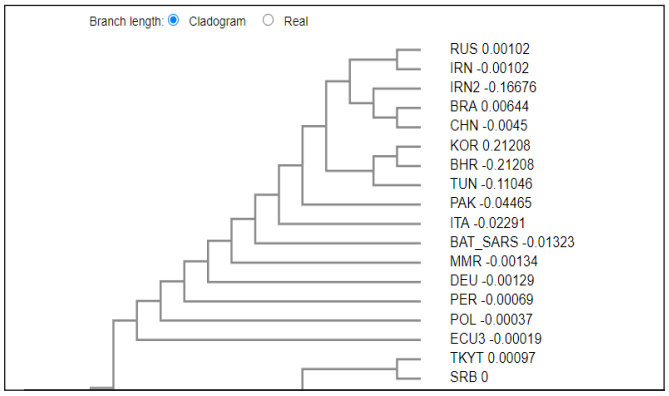

Figure 1. Phylogenetic Analysis of SARS-CoV-2 from Bats with Circulating SARS-CoV-2

SARS-CoV-2 Mutations in Bats

Based on Table 2, the research results found differences between SARS CoV 2 in bats in Malang Karst in Indonesia compared to SARS CoV from 2000 to 2019. The spike protein's receptor binding domain (RBD) is the most variable part of the coronavirus genome. Six RBD amino acids have been shown to be important for binding to the ACE2 receptor and determining the host range of SARS-CoV-like viruses. With coordinates based on SARS-CoV, namely Y442, L472, N479, D480, T487 and Y4911, which correspond to L455, F486, Q493, S494, N501 and Y505 in SARS-CoV-2. Five of these six residues differ between SARSCoV-2 and SARS-CoV. The following figure shows the mutated SARS-CoV-2.

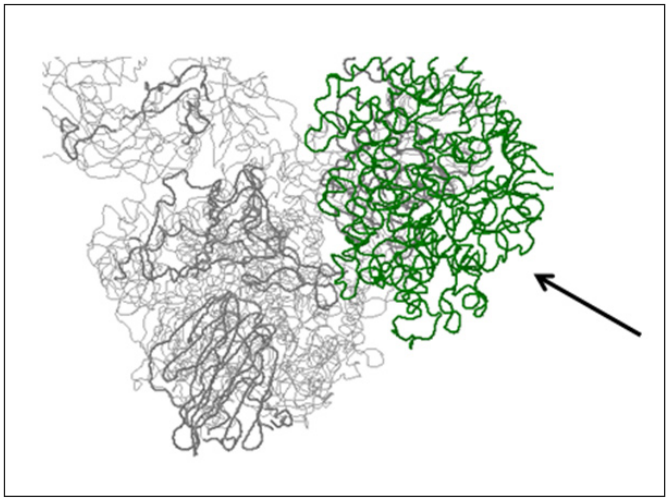

Figure 2. Location of SARS-CoV-2 Mutations in Malang Karst Bats in Indonesia

Table 2. Analysis of SARS-CoV-2 Mutations in Bats from Karst Malang Indonesia Against SARS CoV

SARS CoV	SARS CoV-2 from Bats, Karst Malang Indonesia			
	Id	Coverage	Number of Mutations	Mutation Location
NSP1 hCoV-19-like/bat/Yunnan/RaTG13/2013	100%	15.6%	0	No mutations
NSP2 hCoV-19-like/bat/Yunnan/RaTG13/2013	95.8%	11.1%	3	P543S, K544R, E587D
NSP14 hCoV-19-like/bat/Yunnan/RaTG13/2013	95.7%	4.4%	1	K212R
NSP1 hCoV-19/Wuhan/WIV04/2019	100%	15.6%	0	No mutations
NSP2 hCoV-19/Wuhan/WIV04/2019	98.6%	11.1%	1	E587D
NSP14 hCoV-19/Wuhan/WIV04/2019	100%	4.4%	0	No mutations
NSP1 hCoV-19-like/pangolin/Guangdong/1/2019	100%	15.6%	0	No mutations
NSP2 hCoV-19-like/pangolin/Guangdong/1/2019	88.7%	11.1%	8	V523L, I530V, A531T, P543S, V560I, I577V, E582D, E587D
NSP14 hCoV-19-like/pangolin/Guangdong/1/2019	100%	4.4%	0	No mutations
NSP1 SARS-like/Bat/Nanjing/SL-CoVZXC21/2015	96.4%	15.6%	1	C24R
NSP2 SARS-like/Bat/Nanjing/SL-CoVZXC21/2015	94.4%	11.1%	4	R542K, L551M, V582D, E587D
NSP14 SARS-like/Bat/Nanjing/SL-CoVZXC21/2015	95.7%	4.4%	1	K212R
NSP1 SARS-like/Bat/Nanjing/SL-CoVZC45/2017	92.9%	15.6%	2	G14V, P15Q
NSP2 SARS-like/Bat/Nanjing/SL-CoVZC45/2017	94.4%	11.1%	4	R542K, L551M, V582D, E587D
N SARS-like/Bat/Nanjing/SL-CoVZC45/2017	100%	5.5%	0	No mutations
NSP1 SARS-like/Bat/Nanjing/SL-CoVZC45/2017	92.9%	15.6%	2	G14V, P15Q
NSP2 SARS-like/Bat/Nanjing/SL-CoVZC45/2017	94.4%	11.1%	4	R542K, L551M, V582D, E587D
NSP14 SARS-like/Bat/Nanjing/SL-CoVZC45/2017	95.7%	4.4%	1	K212R
NSP1 SARS/Toronto/Tor2/2004	92.9%	15.6%	2	L6P, V8F
NSP2 SARS/Toronto/Tor2/2004	59.2%	11.1%	29	R521K, S522A, V528T, I530V, A531T, Q532H, Q539K, I541V, R542K, G543S, K544R, Q546E, L547T, Q548G, V559I, T560I, D565E, S566T, H567L, D568P, V570E, L571V, T572L, S573T, N580T, E582D, E584Q, A585P, E587D
NSP14 SARS/Toronto/Tor2/2004	95.7%	4.4%	1	K212R
N SARS/Toronto/Tor2/2004	86.4%	5.2%	3	G193N, N194S, N206T
NSP14 MERS/Netherlands/HCoV-EMC/2014	50.0%	5.7%	15	C199V, K203P, Q205R, K206T, M209L, N211D, A215T, A216C, Y217F, S219D, P220R, L221V, Q222Y, Y224E, A225Q

SARS CoV	SARS CoV-2 from Bats, Karst Malang Indonesia			
	Id	Coverage	Number of Mutations	Mutation Location
NSP14 OC43/USA/VR759/2019	44.0%	4.8%	14	R195K, A198V, V200I, R202P, I204R, S205T, N207C, V208L, T210D, K211R, V215C, Y216F, N217S, S218D
NSP14 HKU1/Hong_Kong/HKU1/2005	48.0%	4.8%	13	R195K, A198V, L200I, R202P, L204R, N205T, D207C, V208L, P210D, N211R, Y216F, N217S, S218D
229E/Wuerzburg/229E/2000	0 %	0 %	0	No mutations
NL63/Amsterdam/NL63/2004	0 %	0 %	0	No mutations

DISCUSSION

Bats as A Reservoir for Viruses

Bats are the second largest order of mammals, with about 1400 known species. In addition to their ability to fly, various biological characteristics make bats unique among mammals. Therefore, bats make a significant contribution to the diversity of the class, as well as to local mammalian fauna in tropical and temperate regions. Even the number of species and genera and the boundaries between taxa are still not completely clear. Recent events show that knowledge about bat diseases and their role as vectors of pathogens is also highly fragmented. (15). New virus sequences are continually being discovered over a wide geographic area and in an ever-increasing number of bat species (16). The release of zoonotic viruses from bat populations can vary greatly in space and time (17). Several viruses, such as Ebola, Marburg virus, Nipah virus, Hendra virus, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory coronavirus (MERS-CoV), and SARS-CoV-2, have infected humans for the last 50 years and mentioned again with various species of bats (16).

In the past few decades, no research said bats could spread the virus to humans. However, research on bats and viruses in recent decades has strengthened the notion that bats are 'special' as reservoir hosts for new viruses (18). This is because the free radicals bats produce are relatively low, but the anti-oxidant content is high, making bats resistant to oxidative stress. The high resistance of bats to oxidative stress has implications for their longevity, their role as virus reservoirs and also their potential as model animals (19). For example short-nosed fruit bats have the potential to be infected with 5 (29%) recorded viruses, including Influenza A virus, Issyk-Kul, Japanese encephalitis, Kyasanur forest disease, and Nipah virus. (20).

The coronavirus genome, which ranges from 26 to 32 kilobases in length, includes a variable number of open reading frames (ORFs). The SARS-CoV-2 genome is reported to have 14 ORFs coding for 27 proteins. The spike surface glycoprotein plays an important role in binding to receptors on host cells. It is important for determining host tropism and transmission capacity, mediating receptor binding, and membrane fusion. In general, the coronavirus spike protein is functionally divided into the S1 domain, responsible for receptor binding, and the S2 domain, responsible for cell membrane fusion. Eight additional proteins (3a, 3b, p6, 7a, 7b, 8b, 9b, and orf14) and four major structural proteins, including spike surface glycoprotein (S), small envelope protein (E), matrix protein (M), and protein nucleocapsid (N), located at the 3rd end of the SARS-CoV-2 genome (5).

When the researchers compared SARS-CoV-2 with SARS-CoV in terms of amino acid levels, they found that SARS-CoV-2 was very similar to SARS-CoV, but there were some important differences in the 8a, 8b, and 3b proteins. When researchers compared SARS-CoV-2 with MERS-CoV, they found that SARS-CoV-2 was completely unrelated to MERS-CoV. From the phylogenetic tree based on the whole genome, SARS-CoV-2 is parallel to the SARS-like bat CoV, while SARS-CoV is a descendant of the SARS-like bat CoV lineage, thus indicating that SARS-CoV-2 is closer. Genomic analysis of nine patient samples also confirmed that SARS-CoV-2 is more like two SARS-like bat CoVs from Zhoushan in eastern China, bat-SL-CoVZC45 and bat-SL-CoVZXC21, than SARS-CoV and MERS-CoV. At the whole genome level, SARS-CoV-2 shares 87.99% sequence identity with bat-SL-CoVZC45 and 87.23% sequence identity with bat-SL-CoVZXC2, which is genetically less like SARS-CoV (approx. 79%) and MERS -CoV (approximately 50%). At the protein level, the lengths of most of the proteins encoded by SARS-CoV-2, bat-SL-CoVZC45, and bat-SL-CoVZXC21 are similar, with only minor insertions or deletions. Although SARS-CoV-2 is more similar to bat-SL-CoVZC45 and bat-SL-CoVZXC21 at the whole genome level, the SARS-CoV-2 receptor binding domain located in row B is closer to it than to SARS-CoV (21).

It was reported that 27 of the first 41 infected patients had been exposed to the Huanan Seafood Market. Therefore, it is believed that this new coronavirus originated from the Huanan Seafood Market in Wuhan

and spread from animals to humans through the process of trading, transporting, slaughtering, and trading wild animals (22). Bats have the most types of coronavirus in their bodies and are hosts for various types of coronavirus, such as SARS-CoV and MERS-CoV. SARS-CoV and MERS-CoV are considered highly pathogenic, and SARS-CoV was likely transmitted from bats to civets and MERS-CoV was transmitted from bats to dromedary camels and ultimately to humans (23). There is a close relationship between human, animal, and environmental health as zoonotic pathogens emerge and re-emerge in bats (16).

SARS-CoV-2 Mutations in Bats

SARS-CoV-2 and Hipposideros bat CoV have high sequence similarity and are similar to SARS in China. So it can be concluded that the natural lighting of SARS-CoV-2 is most likely Hipposideros. The finding that the pangolin coronavirus genome shares an 85.5% to 92.4% sequence similarity to SARS-CoV-2 suggests that pangolins should be considered as possible hosts in the emergence of SARS-CoV-2 (24). Viral host shifts are generally associated with new adaptations to exploit the cells of the new host species optimally. However, SARS-CoV-2 has required little or no significant adaptation to humans since the start of the Corona Virus Disease 2019 (Covid19) pandemic until October 2020. This is in line with the ancestors of SARS-CoV-2, who were non-human and required little or no new adaptations to infect humans successfully. However, no model can detect all signs of historical genomic adaptation. The mutations that allow SARS-CoV-2 to infect humans could have arisen due to genetic deviations in the host reservoir before exposure to humans (25).

A virus from bats closely related to SARS-Cov-2, namely Sarbecovirus BANAL-236, can infect human cells, even though it does not have a furin cleavage site on its spike protein. BANAL-236 replicates efficiently and asymptomatically in humanized mice and macaques, whose enteric tropism differs greatly from SARS-CoV-2. BANAL-236 infection causes protection against superinfection by virulent strains. Therefore, the origin of SARS-CoV-2 should be evaluated, including the presence of spike-bearing arboviruses with furin cleavage sites in bats (26).

Studies show that SARS-CoV-2 is likely a new recombinant virus with a genome like the coronavirus originating from horseshoe bats, namely SARSr-Ra-BatCoV-RaTG13. Meanwhile, the RBD is closest to pangolin-SARSr CoV/MP789/Guangdong/2019, which comes from smuggled pangolins in Guangzhou (27). Natural selection occurring on Sarbecovirus in horseshoe bats versus early evolution of SARS-CoV-2 in humans. Although there is moderate evidence of diversifying positive selection for SARS-CoV-2 in humans, this was limited to the early phases of the pandemic, and purifying selection was much weaker on SARS-CoV-2 than on the related bat Sarbecovirus (25). The results show that a new bat sarbecovirus appears to have the same potential to infect humans as the initial strain of SARS-CoV-2. However, preliminary studies show that BANAL-236 replicates in primate VeroE6 cells with a small plaque phenotype compared to SARS-CoV-2 (28).

Based on structural and biochemical studies, SARS-CoV-2 appears to have RBD binding with high affinity to ACE2 from humans, ferrets, cats, and other species with high receptor homology. Whereas the above analysis shows that SARS-CoV-2 can bind to high-affinity human ACE2, the computational analysis predicts that the interaction is not ideal and that the RBD sequence differs from that shown in SARS-CoV for optimal receptor binding. Thus, the high affinity of the SARS-CoV-2 spike protein binding for humans, ACE2 is most likely the result of natural selection in humans or human-like ACE2, which allowed other optimal binding solutions to emerge. Studies show that 2019-nCoV uses the same ACE2 reverse enzyme cell entry receptor as SARS-CoV (29-30).

The second important feature of SARS-CoV-2 is the polybase cleavage site (RRAR) at the junction of S1 and S2, the two subunits of the spike. This makes effective cleavage of furin and other proteases possible and plays a role in determining the infectivity of the virus and host. In addition, leading proline was inserted on this site in SARS-CoV-2, so, the order inserted is PRRA. The bends created by proline are thought to result in the addition of O-linked glycans to S673, T678, and S686, which flank the cleavage site and are unique to SARS-CoV-2. Polybasic cleavage sites were not observed in the 'B lineage' associated betacoronaviruses, although other human betacoronaviruses, including HKU1 (lineage A), had these sites and predicted O-linked glycans. Given the rate of genetic variation in the spike, it is likely that SARS-CoV-2 viruses with partial or complete polybasic cleavage sites will be found in other species. The functional consequences of the polybasic cleavage site in SARS-CoV-2 are unknown, and it is important to determine their impact on transmission and pathogenesis in animal models. Experiments with SARS-CoV have shown that inserting furin cleavage sites at the S1-S2 junction improves cell fusion without affecting viral entry. In addition, the efficient cleavage of the MERS-CoV spike activates coronaviruses such as MERS from bats to infect human cells. The function of the glycans' predicted

O-links is unclear, but they could create a 'mucin-like domain' that protects the epitope or key residue in the SARS-CoV-2 protein spike. Some viruses use mucin-like domains as glycan shields that involve immunoevasion. Although the prediction of O-linked glycosylation is strong, experimental studies are needed to determine whether this site is used in SARS-CoV-2 (31-32).

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CONCLUSION

Based on the research results, it can be concluded that bats are positive for Covid19 in Malang Karst. SARS-CoV-2 was found in the intestinal organs of *Cynoptera brachyotis* bats. Based on mutation analysis in the SARS-CoV-2 genome, there are differences in mutations in the SARS-CoV virus from 2000 to 2019. However, from the research that has been carried out, we cannot confirm the protein structure. Therefore, further research is needed on the structure of the SARS-CoV-2 protein for vaccine purposes.

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