Coronaviruses of synantropic bats: an unexplored threat

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Abstract. More than 60% of human viral pathogens are of zoonotic origin, resulting from accidental or frequent animal infections. Bats are reservoirs of various viruses, pathogens in humans of varying severity from mild asymptomatic forms to severe lethal outcomes. And are associated with the spread of various viruses (Marburg virus, Ebola virus) and, above all, coronaviruses. Since the 2000s, three independent outbreaks of coronaviruses with the emergence of new zooanthroponotic human coronaviruses (Betacoronavirus) with epidemic and pandemic potential have been registered. The first outbreak was reported in 2002 (SARS), caused by SARS-CoV, in the PRC (Guangdong Province). The second outbreak occurred in 2012 (MERS, MERS), associated with MERS-CoV, Saudi Arabia. The third occurred in 2019 PRC (Hubei Province), this case evolved into a COVID-19 pandemic caused by SARS-CoV-2. These cases seem to be independent, but the literature reports that the emergence of all these viruses is related to evolutionary processes driven by environmental and genetic aspects. It is assumed that the coronavirus causative agent of severe acute respiratory syndrome-2 (SARS-CoV-2) originated from a coronavirus-infected bat of the genus Rhinolophus. After initial emergence, due to the host vector. SARS-CoV-2 is now transmitted worldwide through human-to-human transmission. Data from experimental studies show that animal species such as cats, ferrets, raccoon dogs, Javanese macaques, rhesus macaques, white-tailed deer, rabbits, Egyptian fruit bats and Syrian hamsters are susceptible to SARS-CoV-2 infection. However, natural SARS-CoV-2 infections have only been reported in domestic dogs and cats, tigers, lions, snow leopards, cougars and gorillas in zoos, and farm-raised mink and ferrets. Although human-to-animal transmission has been reported in several cases, animal-to-human transmission of SARS-CoV-2 has been reported only from mink to humans on mink farms.

1 Introduction

The first records of coronavirus infection in animals appeared in the late 1920s, when an acute respiratory infection of domestic chickens appeared in North America [1]. The causative agent was identified as a virus in 1933. In 1936, the disease and virus were recognized as unique from other viral diseases. They became known as infectious bronchitis

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virus (IBV) but were later officially renamed avian coronavirus. In the late 1940s, two more animal coronaviruses were discovered: JHM, which causes brain disease (murine encephalitis), and mouse hepatitis virus (MHV), which causes hepatitis in mice. Human coronaviruses were discovered in the 1960s [2]. In 1961, the virus was obtained from a schoolboy in Epsom, England, who was suffering from the common cold. The specimen, designated B814, was confirmed as a new virus in 1965. In 1966, new cold viruses (designated 229E) collected from medical students at the University of Chicago were also reported. Using electron microscopy in 1967, it was shown that the three viruses were morphologically related by their common shape and characteristic club-like spikes similar to the solar corona on their surface. This new group of viruses was named coronaviruses after their characteristic morphological appearance [3].

Coronaviridae (CoV) – is a family of human and animal viruses that cause diseases of the respiratory, gastrointestinal, and nervous systems. The CoV family includes two subfamilies: the Coronavirinae, which is subdivided into four genera (*Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*), and the Torovirinae, which consists of two genera (*Torovirus* and *Bafinivirus*) [6]. In addition to bats and humans, coronaviruses can infect a wide range of domestic and wild animals, including pigs, cattle, mice, cats, dogs, chickens, deer and hedgehogs [7, 8].

Despite the discovery of animal coronaviruses associated with SARS-CoV-2, the evolutionary origin of this virus has not been established. The consequences of the COVID-19 pandemic affect all aspects of human activity, including animal health, and have a serious adverse socioeconomic impact worldwide [9]. Patient Zero SARS-CoV-2 is believed to have been infected at the Huanan South China seafood market, a live animal market in Wuhan, China. There are many species of mammals in this seafood and wildlife market [10]. Such live-animal markets provide ideal conditions conducive to frequent interspecies contact between wild and domestic species, and humans. After transmission to an intermediate host, SARS-CoV-2 may have undergone adaptive genetic recombination. To prevent further spread of infection and the fourth coronavirus outbreak, it is necessary to identify those animal species that are susceptible to infection with SARS-like CoV because they can act as intermediate hosts or reservoirs of the virus and transmit the infection to humans. SARS-CoV-2 is already widespread in the population. Thus, detection of this virus in domestic or wild animals may not confirm their role as reservoirs or intermediate hosts. This may be due to the fact that these cases may result from the spread of the disease from humans to animals [11].

The purpose of this paper is to consider the most likely source of infection, the bat, and to analyze the pros and cons of direct transmission of the SARS-CoV-2 virus to humans.

2 Structure of the virus

Coronaviruses contain single-stranded positively charged RNA and have one of the largest genomes of all RNA viruses. Two-thirds of the coronavirus genome at the 5'-end encodes viral proteins involved in viral RNA transcription and replication, and one-third at the 3'-end encodes structural and group-specific accessory proteins. The major structural proteins of coronaviruses are: S (spike), E (envelope), M (membrane), and N (nucleocapsid). The S protein includes the receptor-binding domain (RBD) of the viral spikes, which helps bind to the angiotensin-converting enzyme-2 (ACE-2) cell receptor [12]. RBD SARS-CoV-2 may play a role in interspecies transmission mechanisms. Genetic analysis of genome sequence, ACE-2 receptor homology indicates that bats are a natural reservoir for these viruses, but based on ACE-2 homology studies, direct transmission of SARS-CoV-2 from bats to humans is unlikely [13]. An insertion of the unique peptide PRRA in the human SARS-CoV-2 virus at the S1 and S2 junction of the spike protein (S) was also identified. This insertion may be

involved in the proteolytic cleavage of protein S and may affect the host range as well as the transmissibility of the virus. According to studies by Zhai et al. among the 20 amino acids present in the ACE-2 receptor that come in contact with viral protein S, ACE-2 can still act as the SARS-CoV-2 receptor even if seven amino acids are replaced [14].

SARS-CoV-2 penetrates the host cell through the interaction of its S-protein (RBD) with the host ACE-2 receptor [15].

Only a few mammals are considered to have CoV associated with SARS-CoV-2 - these are bats, pangolins, deer, and mink. SARS-CoV-2 has the greatest homology with the bat coronavirus isolate RaTG13 from Rhinolophus affinis (intermediate horseshoe bat) in the entire genome (93.7%), S-protein (92.86%), ORF1ab (96.5%) and nucleocapsid protein (96.9%) genes [16].

Damas et al. studied the ACE-2 sequences of 410 vertebrate species (252 mammals, 72 birds, 65 fish, 17 reptiles, and four amphibians) to predict their ability to bind RBD SARS-CoV-2. The interaction between ACE-2 and RBD was classified into five categories: very high, high, medium, low, and very low. A total of 18 animal species fell into the "very high" category, all of which belonged to Old World primates and great apes that showed complete similarity to humans on all 25 ACE-2 binding residues. Another 28 species fell into the "high" category (high probability of RBD binding). This includes cetacean species (12), rodent species (7), deer species (3), lemuriform primates (3), and giant anteater (Myrmecophaga tridactyla), southern tamandua (Tamandua tetradactyla), and Angolan colobus (Colobus angolensis) [17].

3 The Bats

Bats are the only mammals capable of flying, which gives them a great migratory advantage over other terrestrial mammals. Also, bats are the second largest group of mammals and have an extensive distribution on all continents except Antarctica. [18].

The geographic localization of coronavirus outbreaks associated with bats can be estimated from their habitats. Bats are a reservoir of α-CoV and β-CoV and have been found in bat colonies in Africa, North and South America, Australia, Asia, and Europe. According to the data, bats belonging to different families but inhabiting the same cave contain the same coronavirus species. For example, Hipposideros lekaguli has been reported to contain Miniopterus coronavirus in group 1 and Rhinolophus shameli in group 5. Studies also demonstrate the versatility and adaptability of CoV to bats at the interspecies and intraspecies levels. Bats have physiological and behavioral traits, such as a high metabolic rate and resulting high body temperature due to flight have led to changes that have been reflected in the increased tolerance of the immune system and the achievement of evolutionary commensalism with CoV. The constant increase in contacts of bats with other animals plays a key role in the extensive interspecific transmission of CoV. The tolerant immune system of bats leads not only to long-term carrier, but also to recombination, mutation, and evolution of CoV. Bats are also rapidly adapting to new conditions created by human actions, which increases the risks associated with changes in the natural landscape and the risk of new virus outbreaks. For example, the rate of deforestation in Southeast Asia is the highest among tropical regions of the world: 1.2% of the forest area is lost annually; thereby fragmenting the natural environment, forcing bats to live closer to humans [19].

Because of their species diversity and ubiquitous distribution, bats are considered optimal reservoir hosts for CoV. Researchers have also suggested several possible events that may have led to the successful interspecies jumping of CoVs over the past decade. For example, bats are used as food in southern China and other Southeast Asian countries. Live bats can be found in markets and wildlife restaurants in South China, making it much easier for bats to interact with various species they would not encounter in the wild and for humans to have

direct contact with bats. Bat coronaviruses are able to use molecules present on the surface of human or other animal cells as a receptor for interspecies transmission, thus helping to overcome the interspecies barrier. With the rise of global economic development, increased urbanization, extensive farming and climate change, there is a gradual invasion of "wild lands," which increases human contact with wild animals.

Analysis of the SARS-CoV-2 genome revealed multiple recombinations. When different viral strains infect the same host, genetic recombination is possible, creating new viral genomes. Based on this concept, SARS-CoV-2 could hypothetically be the result of recombination of bat and pangolin viruses in the same host of unknown identity.

Brintnell et al. performed a detailed phylogenetic analysis, hereditary sequence reconstruction, and in situ molecular dynamics simulations to study the functional evolution of Spike-RBD and found that a common hereditary virus with RaTG13, had a high ability to bind to the human ACE2 receptor. It was also found that SARS-CoV-2 has a high affinity for human ACE-2, which was fully acquired about 7-50 years ago. In 2021, another group of scientists found that SARS-CoV-2 evolved long before the pandemic, several decades ago (95% HPD: 1930-2000), seeing RaTG13 as the virus closest to SARS-CoV-2. They also found that SARS-CoV has the same divergence time as SARS-CoV-2, 40-70 years, using known existing bat virus lines. Starr et al. analyzed the evolutionary history of ACE-2 binding to various sarbekoviruses and found that this virus evolves quite easily, a hereditary trait [20].

4 Conclusion

The danger of bat coronaviruses lies in their ubiquitous spread, carriage of infection without clinical signs, and interspecies transmission. But the greatest epidemiological danger of coronavirus infection is its ability to replicate and recombine. Also, humans themselves, by trespassing into nature and violating the boundaries of the wild range, contribute to the spread of coronaviruses, thereby creating new human-animal contacts that would not occur under natural conditions.

At the moment, it is not clear through which "animal" the virus evolved and was transmitted from bats to humans, but there is no data either confirming or absolutely refuting direct transmission from bats.

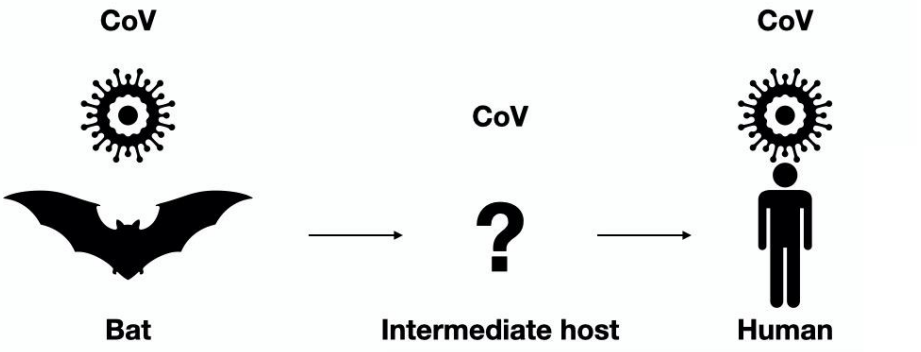

Fig. 1. The unknown intermediate host of bat coronaviruses.

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