

TOPICS ON MOLECULAR CHARACTERIZATION OF THE NOVEL CORONAVIRUS SPECIES SARS-CoV-2

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DOI: 10.4025/rcvsp.v7i1.55497

ABSTRACT

Since December 2019, a new coronavirus species named SARS-CoV-2 has been related to thousands of cases of severe respiratory disease worldwide, been considered a public health issue. Molecular comparisons between isolates from SARS-CoV-2 and other coronavirus species showed identity levels around 79% with the human strain SARS-CoV. However, sequence homology analysis showed that the most closely related known viruses with SARS-CoV-2 are two bat SL-CoVs (~89%), revealing similar evolutionary relationships and evidences that bats can act as reservoirs of SARS-CoV-2. Despite this, viral RNA has been detected in two dogs and two cats belonging to SARS-CoV-2 infected owners, in Hong Kong and Belgium, and in one tiger maintained at the Bronx Zoo in New York City. Additionally, ferrets and cats are found to be highly susceptible to SARS-CoV-2 in an experiment carried out in a controlled environment. However, there is no evidence of these animals acting as reservoirs of the virus. Despite the high genetic identity found among SARS-CoV-2 strains, mutations have been identified, mostly in the structural protein S gene, but until now, there is no enough evidence to relate specific mutation in the viral genome to a higher number of infected patients or death.

KEYWORDS: Bats; Dogs; Cats; Molecular analysis; SARS-CoV-2.

INTRODUCTION

Coronaviruses (CoVs) are enveloped single-strand RNA virus that belong to *Coronaviridae* family and are commonly associated with mild respiratory and enteric disease in avian and mammalian species worldwide (WOO et al., 2009).

During December 2019, five patients over forty years old were admitted to hospitals with an initial diagnosis of pneumonia of an unknown etiology in Wuhan, Hubei Province, China (LU et al., 2020a). Through molecular diagnosis techniques, it was possible to identify a new Coronavirus strain (2019 novel coronavirus or SARS-CoV-2) related with the etiology of the disease named COVID-19 (REN et al., 2020).

Due its potential for rapid horizontal spread, SARS-CoV-2 has emerged and infected thousands of humans in several countries, causing a pandemic scenario and a lot of deaths (BENVENUTO et al., 2020; HAIDER et al., 2020). At the time of this manuscript preparation,

COVID-19 has been resulted in over 16.114.449 confirmed cases and over 646.641 deaths worldwide (OPAS/OMS).

Because of the severity of the disease and in order to provide information for the development of vaccines and other forms of viral control and prevention, lots of studies are being developed to enable the understanding of the evolutionary origin, molecular and antigenic characteristics of this virus.

DEVELOPMENT

Coronaviruses strains are classified into four genera based on their genetic properties: *Alphacoronaviruses* (α), *Betacoronaviruses* (β), *Gammacoronaviruses* (γ), and *Deltacoronaviruses* (δ) (WOO et al., 2009). Before the emergency of SARS-CoV-2, there were six CoVs known to infect humans, two α -CoVs (229E and NL63) and four β -CoVs (OC43, HKU1, Severe Acute Respiratory Syndrome (SARS)-CoV, and Middle East Respiratory Syndrome (MERS)-CoV), all of them with confirmed potential of zoonotic transmission (PERLMAN and NETLAND, 2009; ZAKI et al., 2012).

SARS-CoV and MERS-CoV emerged in China 2002 and Saudi Arabia 2012, respectively (ZHONG et al., 2003; ZAKI et al., 2012). Both species were associated with severe acute respiratory syndrome in humans and were detected in about 27 countries (WHOa; WHOb). Several studies showed that bats are the reservoir of a wide variety of coronaviruses, including SARS-CoV-like and MERS-CoV-like viruses (DREXLER et al., 2014). However, there are evidences that other animals can also act as reservoirs or incidental hosts from these viruses, contributing for its environment maintenance (GUAN et al., 2003; WANG et al., 2006; REUSKEN et al., 2013).

To determine the origin, evolution and antigenic resemblance of SARS-CoV-2, molecular studies regarding nucleotide and protein sequences from the virus has been performed since its discovery. Comparisons between isolates from the novel coronavirus and SARS-CoV showed identity levels around 79%. When compared with MERS-CoV sequences, identity range was about 50% (LU et al., 2020b; REN et al., 2020). Despite the similarity with SARS-CoV, phylogenetic analysis revealed that SARS-CoV-2 fell within the genus *Betacoronavirus* but in a different clade from SARS-CoV, which was sufficiently to be considered a new human-infecting betacoronavirus species (LU et al., 2020b; REN et al., 2020).

Additionally, phylogenetic analysis was performed considering genome sequences from betacoronavirus identified from rats, cattle and bats (LU et al., 2020b; REN et al., 2020). Sequence homology analysis showed that the most closely related known viruses with SARS-CoV-2 are two bat SL-CoVs (bat-SL-CoVZC45 and bat-SL-CoVZXC21) identified in 2005 in Zhoushan, Zhejiang, China, presenting 73-89% nucleotide identities (WONG et al., 2019; LU et al., 2020b; REN et al., 2020). In phylogenetic analysis both bats species clustered in the same clade that SARS-CoV-2, revealing similar evolutionary relationships and evidences that bats can act as reservoirs of SARS-CoV-2 (LU et al., 2020b).

To clarify more details about possible SARS-CoV-2 hosts and reservoirs, Ji et al. (2020) performed phylogenetic analysis using 271 SARS-CoV-2 genome sequences available on GenBank databases and relative synonymous codon usage (RSCU) using available coding sequences of the SARS-Cov-2 genome (1CDS's, 9672 codons), bat-SLCoVZC45 genome and of wildlife species as snakes, hedgehog, marmot, manis and bird genomes. Phylogenetic analysis revealed the high identity with bat SL-CoVs, as mentioned earlier. In turn, RSCU bias showed that SARS-CoV-2, bat-SL-CoVZC45, and snakes from China have similar synonymous codon usage bias, however SARS-CoV-2 and snakes have the highest similarity, suggesting that snakes and bats may act as the virus hosts or reservoirs. Since there was no contact between the first SARS-CoV-2 positive humans with bats and most of them had a

history of exposure to the virus at the Huanan Seafood Wholesale Market where poultry, snakes, marmots, birds, frogs, and hedgehogs are sold, the existence of other hosts or reservoirs cannot be discarded (LU et al., 2020a; JI et al., 2020).

Viral zoonotic transmission is the most accepted hypothesis for the first SARS-CoV-2 positive cases. Although SARS-CoV-2 shares high nucleotide identity with bat coronavirus, it has not been detected in these or other animals before the pandemic. However, in the last few months, viral RNA has been detected in two dogs and two cats belonging to SARS-CoV-2 infected owners, in Hong Kong and Belgium (PRO/AH/EDR). In this four cases it was possible to observe that all the animals were asymptomatic, low viral title was detected, there was no transmission for an animal to another and the viral sequences obtained from one of the dogs and its owner were identical, suggesting that transmission occurred from the owner to the animal (PRO/AH/EDR; LEROY et al., 2020). All these findings contribute to the statement that there is no evidence of development of a productive and infectious infection in dogs and cats.

Besides dogs and cats infection, SARS-CoV-2 RNA genome was also found in a four-year-old tiger maintained at the Bronx Zoo in New York City, named Nadia, presenting a dry cough and decrease in appetite. Nadia's sister Azul, two Amur tigers, and three African lions also developed the same clinical signs, however they have not been tested. According to the Zoo officials the cats were probably infected by a person caring for them who was positive for the virus (JAMES, 2020).

In addition to the mentioned above, Shi et al. (2020) developed an experiment in a controlled environment and found that ferrets and cats are highly susceptible to SARS-CoV-2, dogs have low susceptibility, and pigs, chickens, and ducks are not susceptible to the virus. This susceptibility are related with the molecular characteristics of the viral structural protein S, an envelope glycoprotein which is involved in the attachment of the virus to the host cell that can undergo genetic recombination and extend the number of virus hosts (LI et al., 2016). Moreover, although in their study it was possible to detect viral transmission between cats, once the conditions were non-natural, more studies are needed to elucidate the risk of pets infection from humans as well as the role of infected cats as infections sources of other pets and humans.

Coronaviruses possess the biggest RNA single-strand positive sense genome within virus (Su et al., 2016). Genetic mutations and recombination are common between this virus family and generally occurs during their replication cycle (Su et al., 2016). Although genetic analysis showed that the firsts SARS-CoV-2 strains detected in China were almost identical (99,9%), suggesting that they originated from a same source, lots of new genome sequences were already obtained from strains of different countries, and despite the high genetic identity found among them, mutations have been identified, mostly in the structural protein S gene (LU et al., 2020b; ZEHENDER et al., 2020; Castillo et al., 2020). Recently, based on these genetic differences between SARS-Cov-2 strains, Tang et al. (2020) and GISAID database (www.gisaid.org/CoV2020) proposed their classification in lineages/subtypes.

The high rate of viral dissemination and the environmental pressure may contribute to genome mutation and the emergence of new SARS-CoV-2 subtypes. Until now, there is not enough evidence to relate specific mutation in the viral genome to a higher number of infected patients or death (Castillo et al., 2020). However, constant surveillance of mutations arising is needed.

CONCLUSION

Given the gravity of COVID-19 pandemic, despite all these recent discoveries and the advances in control and prophylaxis programs, a lot of issues related with the evolutionary origin, molecular and antigenic characteristics of SARS-CoV-2 remains unclear and more studies are needed for better understanding and adoption of effective preventive measures.

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