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Comparative analysis of spike glycoprotein in coronaviruses among different animal species

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Abstract
Coronaviruses are a diverse group of viruses known to infect a wide range of animal species, including mammals and birds. The spike glycoprotein plays a pivotal role in viral entry and host cell recognition, making it a key determinant of host specificity and pathogenicity. Understanding the relationship and variations in the spike glycoprotein among different animal species is crucial for elucidating the zoonotic potential and interspecies transmission dynamics of coronaviruses. In this comparative analysis, we investigate the spike glycoprotein sequences of various coronaviruses from diverse animal hosts. Through phylogenetic and sequence similarity analyses, we identify evolutionary relationships within the spike glycoprotein. Our findings shed light on the cross-species transmission potential and the adaptive mechanisms of coronaviruses, contributing to the broader understanding of emerging viral threats and guiding strategies for zoonotic disease surveillance and control.

Keywords: Coronavirus, phylogenetic tree, spike glycoprotein

Introduction
Coronaviruses (CoVs) are enclosed viruses with single-stranded, positive-sense RNA genomes. They are the largest known RNA viruses, with genome sizes ranging from 26 to 32 kilobases. CoVs have been shown to infect humans as well as a wide range of animal species. All coronaviruses' structure and activity are essentially dictated by four key structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N), as well as non-structural proteins cleaved from the orf1a/b polyprotein. RNA-dependent RNA polymerase, protease, and endoRNAse are examples of non-structural proteins (Pal *et al*., 2020) [6].
The spike (S) protein is one of these structural proteins that is essential for attachment to and fusion with the host cell. The identified host receptor in both SARS-CoV and SARS-CoV-2 is Angiotensin-converting enzyme 2 (ACE2) (Hoffman, 2020). The nucleocapsid (N) protein attaches to the CoV genome and aids in its packaging into the ribonucleoprotein complex, resulting in the formation of the capsid. The membrane (M) protein is in charge of shaping the viral envelope and organising viral assembly via interactions with other structural proteins. Although it is the smallest of the structural proteins, the envelope (E) protein is integrated into the virion envelope, albeit in a modest proportion of the total expressed envelope protein (Jackson *et al*., 2022) [3].
The S protein has become one of the most crucial targets for the development of *SARS* vaccines and treatments, particularly for SARS-CoV, due to its crucial role in receptor identification, viral attachment, and entry. Genomic investigation of the SARS-CoV-2 (COVID-19) Coronavirus showed 89% sequence similarity to the Coronavirus from bats and 82% similarity to the human SARS-CoV (Du *et al*., 2009) [2]. This study aims to investigate how the spike gene behaves and manifests in various animal species. In order to create vaccines and treatments, it is important to have a better understanding of potential cross-species transmission.

Materials and Methods
The nucleotide sequences of spike glycoprotein of different animal species were downloaded from NCBI in FASTA file format. The sequences of enlisted are in table 1.

Table 1: Details of the sequences

S. No.	Species	Accession no.
1.	Bat coronavirus isolate 160228	KY783851.1
2.	Pangolin coronavirus isolate cDNA31-S	MT799526.1
3.	SARS coronavirus civet014	AY572036.1
4.	Middle East respiratory syndrome-related coronavirus isolate MERS-CoV-Jeddah-Camel /1227/2016	MK910259.1
5.	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/MLI/Wuhan-Hu-1-154/2021	OP173217.1
6.	Turkey coronavirus strain TCoV/TX-R/98 isolate	GU213202.1
7.	Bovine coronavirus isolate KWD10	AY935646.1
8.	SARS coronavirus isolate CUHKtc55NS	DQ412629.1
9.	Canine respiratory coronavirus	AY150272.1
10.	Porcine epidemic diarrhea virus isolate	OP374017.1
11.	Feline coronavirus isolate F21071412-2	OQ351918.1

These sequences were aligned using MEGA11 (Molecular Evolutionary Genetics Analysis) software. Multiple sequence alignment was done by using the MUSCLE program. The aligned sequences were saved in MEGA file format. This file was then used for the construction of a phylogenetic tree. For phylogenetic analysis, the Maximum Likelihood method was used with 100 bootstrap replicates with the Tamura-nei model.

Results and Discussion

Figure 1 depicts the evolutionary relationships of the SARS-CoV-2 Spike (S) glycoprotein with other coronaviruses as a phylogenetic tree. The SARS-CoV-2 S protein is closely related to the SARS-CoV and Bat coronaviruses, implying that these viruses are its closest relatives and likely sources. Additionally, the MERS coronavirus shows a next proximal relation to SARS-CoV-2. Notably, the spike glycoprotein of SARS-CoV-2 exhibits a close relationship with the Pangolin coronavirus, indicating that SARS-CoV-2 may have acquired its receptor from the Pangolin coronavirus. This connection is particularly significant as the recently discovered *Pangolin*-CoV 2019 shares nearly identical amino acid residues in its gene with SARS-CoV-2, as reported by Narh *et al*. in 2020 [4].

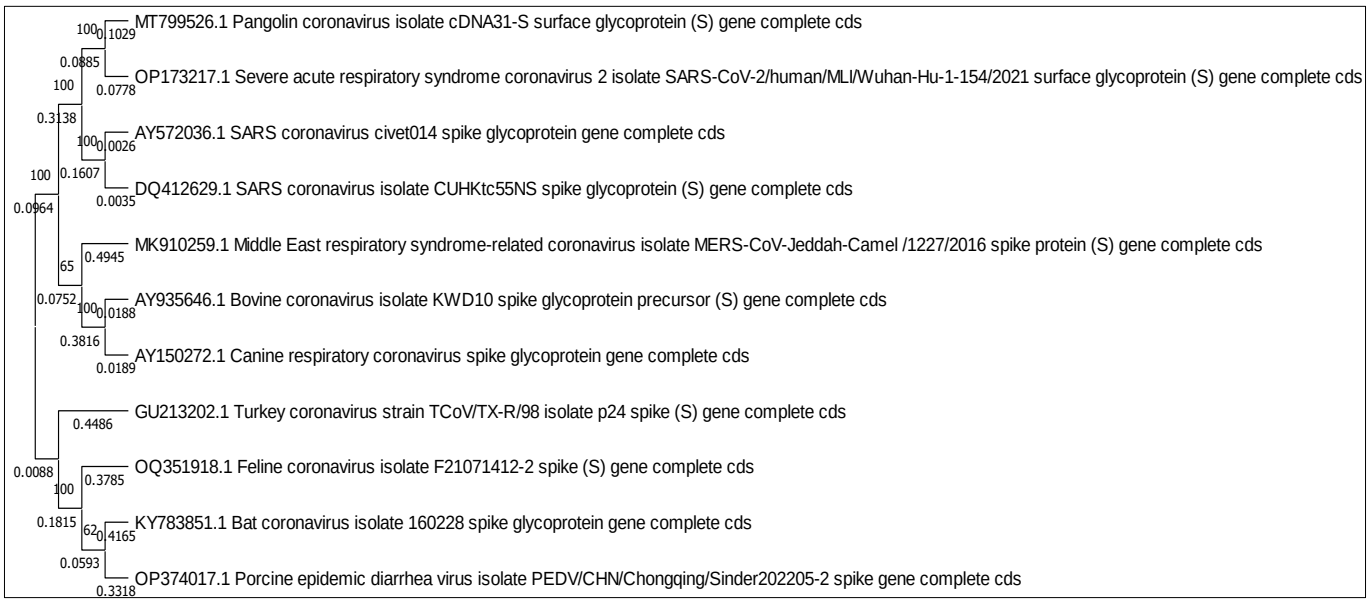

Fig 1: Phylogenetic tree of Spike glycoprotein of Coronavirus of different species

Civet CoV (Civet Coronavirus) and SARS-CoV (Severe Acute Respiratory Syndrome Coronavirus) are linked in that SARS-CoV is believed to have originated from civet cats, which acted as an intermediate host for the transmission of the virus to humans during the 2002-2003 SARS outbreak, as documented by Zhu *et al*. in 2020 [8]. While SARS-CoV is believed to have originated in bats, civet cats, often found in live animal markets, played a crucial role in facilitating the transmission of the virus from bats to humans. Genetic changes likely occurred in the virus while infecting civet cats, enhancing its ability to infect humans effectively. Civet CoV refers specifically to a strain of Coronavirus found in civet cats, which shares similarities with SARS-CoV but is distinct from it. Civets and dromedary camels are thought to be the intermediary hosts of SARS and MERS-CoV, from which they were transferred to humans (Dhama *et al*., 2020) [1].

The next most closely related virus is the MERS coronavirus. The principal animal reservoir for MERS-CoV has been identified as camels. The virus is thought to be spread to humans through intimate contact with infected camels or by consuming raw camel products (Omrani *et al*., 2015) [5]. As a result, the spike protein of a Coronavirus that primarily infects bats, for example, may not have the same affinity for receptors in bovines, dogs, or birds, and therefore, these animals are not susceptible to infection by human coronaviruses. Also, turkey CoV, and feline Cov are outgroups. Canine CoV is believed to have come originally from pigs, while another, more recent, Canine Respiratory Coronavirus (CRCoV) may have jumped from cows. It's important to note that while some coronaviruses can infect both animals and humans (zoonotic transmission), the ability to infect and cause disease in different species depends on

various factors, including the specific virus strain, the receptor-binding affinity of the spike protein, and the immune response of the host.

Sequence similarity between bat coronaviruses and pig coronaviruses in the spike gene suggests a common evolutionary origin or shared genetic ancestry. However, it does not necessarily mean that pig coronaviruses can directly infect humans or that they pose the same level of risk as some bat coronaviruses. The ability of a virus to infect and cause disease in a specific host depends on various factors, including the virus's adaptation to the host's cellular receptors and immune responses (Banerjee *et al*., 2019) [9].

Because medications discovered for closely related viruses (SARS-CoV, MERS) may be functionally repurposed as potential anti-SARS-CoV-2 agents, phylogenetic analysis is useful in understanding Coronavirus illness and potential therapeutics.

Conclusion

In summary, the phylogenetic relationships depicted in this article provide a comprehensive framework for unraveling the intricate connections between different coronaviruses, shedding light on their origins, transmission dynamics, and potential therapeutic interventions.

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