Genetics & Biodiversity Journal

Journal homepage: https://journals.univ tlemcen.dz/GABJ/index.php/GABJ

ISSN: 2588-185X

OPEN ACCESS

Original Research Paper

In silico study of the DNA sequences of the Coronavirus in several animal species and human.

Derouiche L.[1*], Bouguelil F. Z.[2], Zelelif I.[2]

[1]Higher School of Food Sciences & Agri-food Industries Algiers, Algeria.
[2]Nature and Life Sciences Faculty, Saad Dahlab University of Blida (USDB 1), Blida, Algeria.

Corresponding Author: Derouiche Louiza, Higher School of Food Sciences & Agri-food Industries Algiers, Algeria; **E-mail:** derouiche@essaia.dz

Article history: Received: October 9, 2024; Revised: November 22, 2024; Accepted: November 30, 2024

Abstract

Coronaviruses are positive-sense single-stranded RNA viruses, and their name derives from their crown-like appearance. In this study, we inventoried the animal species affected by Coronaviruses, and then we performed a comparative phylogenetic analysis based on DNA sequences among the different viral strains of Coronaviruses that have affected these animals as well as humans. To carry out this work, we used MEGA software version 11 to analyze the DNA sequences extracted from GenBank of the S gene. We classified the viral strains of this gene according to the infected animal species and the geographical area in which they were found. We worked on 33 nucleotide sequences of the S gene from different strains, such as SARS-CoV-2 and MERS-CoV affecting humans, Bat-CoV affecting bats, and PEDV and PDCoV affecting pigs, across several countries including China, Saudi Arabia, the United States, Japan, and Spain. We found that the S gene is characterized by an accumulation of mutations among the different strains, with certain nucleotide changes that may consequently be responsible for the transmission of the virus from animals to humans. Moreover, our results show that the S gene exhibits a very high genetic diversity among the Coronavirus strains, whether they infect animals or humans in different countries.

Keywords: Coronavirus, animals, humans, S gene, phylogeny.

الخلاصة

فيروسات كورونا هي فيروسات الحمض النووي الريبوزي أحادية السلسلة، واسمها يرجع إلى مظهر الإكليل الشمسي. قمنا في هذه الدراسة بحصر الأنواع الحيوانية المصابة بفيروس كورونا، ثم قمنا بإجراء مقارنة عن طريق تحليل التطور الوراثي بناءً على تسلسل الحمض النووي بين سلالات فيروس كورونا المختلفة التي أثرت على الحيوانات وكذلك البشر. لتنفيذ هذا العمل، استخدمنا الإصدار 11 من برنامج التحليل التطوري للوراثة الجزيئية لتحليل تسلسل الحمض النووي المستخرج من بنك الجينات لجين سبايك. وقمنا بتصنيف السلالات الفيروسية لهذا الجين حسب أنواع الحيوانات المصابة والمنطقة الجغرافية التي تواجد فيها. لقد عملنا على 33 تسلسلًا من النيوكليوتيدات لجين سبايك من سلالات مختلفة، مثل السلالات التي تصيب البشر، والخفافيش، والخنازير، عبر عدة دول مثل الصين، والمملكة العربية السعودية، والولايات المتحدة، واليابان، وإسبانيا. ووجدنا أن هذا الجين يتميز بتراكم الطفرات بين السلالات المختلفة مع تغير معين في النيوكليوتيدات وبالتالي يمكن أن يكون سببا في انتقال الفيروس من الحيوان إلى الإنسان. بالإضافة إلى ذلك، تظهر نتائجنا أن جين سبايك يقدم تنوعًا جينيًا عاليًا جدًا بين سلالات فيروس كورونا، سواء للسلالات التي تصيب الحيوانات أو تلك التي تصيب الإنسان في بلدان مختلفة

الكلمات الرئيسية: فيروس كورونا، الحيوانات، الإنسان، جين سبايك، السلالات.

Introduction

It is now well established that the Coronavirus is one of the oldest, most famous, and widely spread viruses worldwide. It is considered the causative agent of various respiratory and digestive diseases. The Coronaviridae family comprises a group of 11 viruses that infect vertebrates (Siddell et *al*., 1983). According to the International Committee on Taxonomy of Viruses, Coronaviruses are classified under the order Nidovirales, family Coronaviridae, and subfamily Coronavirinae. Based on early serological evidence and subsequent genomic data, Coronavirinae is divided into four genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus* (Fung and Liu, 2019). Each genus is further divided into several viral strains, such as BCoV in cattle, CCoV in dogs, and IBV in birds (Alluwaimi et *al*., 2020).

In 2003, there were only 10 Coronaviruses with complete genomes available; subsequently, an additional 16 Coronaviruses with sequenced complete genomes were added.

These include two human Coronaviruses (human Coronavirus NL63, human Coronavirus HKU1), 10 other Coronaviruses from mammals (SARS Coronavirus from bats; Bat-CoV HKU2, Bat-CoV HKU4, Bat-CoV HKU5, Bat-CoV HKU8, Bat-CoV HKU9, Bat-CoV 512/2005, Bat-CoV 1A; equine Coronavirus; beluga Coronavirus), and four avian Coronaviruses (turkey Coronavirus, bulbul Coronavirus HKU11, thrush Coronavirus HKU12, munia Coronavirus HKU13) (Woo et *al*., 2012).

It seems that mutations in the S gene over time lead to the emergence of different viral strains affecting various species in diverse geographical areas. In this context, we focused on reconstructing evolutionary trees or phylogenies using a bioinformatics approach to trace the evolutionary history of mutations in the S protein genes of Coronaviruses in different animal species as well as in humans. This will allow us to understand the behavior of Coronaviruses and provide an estimate as close as possible to the structure of a phylogeny based on their distribution across countries and species.

Material and methods

DNA sequences were extracted from a molecular biology database known as GenBank to trace the phylogenetic tree and make comparisons between the DNA of several Coronavirus strains that have affected different animals as well as humans.

Data extraction from GenBank

All the DNA sequences studied in our work were downloaded from GenBank, which is a database constructed and distributed by the National Center for Biotechnology Information (NCBI). It is a collection of all publicly available nucleotide and protein sequences, along with bibliographic and biological annotations, accessible at the URL (https://www.ncbi.nlm.nih.gov/genbank/) (Sayers et *al*., 2020). These records are generated from direct communications with the original authors' DNA sequence databases, who provide their records to make the data available to everyone or as part of the publication process (Clark et *al*., 2016).

Phylogenetic analysis

For processing DNA sequences, there are several software programs available to generate phylogenetic trees, among which is the MEGA software that we have already used to study SARS-CoV-2 in other works (Derouiche et *al*., 2022; 2023). Molecular Evolutionary Genetics Analysis (MEGA) is a program that allows for the exploration and analysis of nucleotide and protein sequences (Tamura et *al*., 2011). We used MEGA version 11 to perform the phylogenetic analysis and obtain the phylogenetic trees (Kuhls and Mauricio, 2019) after several processing and analysis steps, which can be summarized as follows: inputting the DNA sequences in FASTA format; aligning these sequences using ClustalW; selecting the method for constructing the phylogenetic tree and adjusting the parameters of the chosen method. In our case, we used the UPGMA method (Unweighted Pair Group Method with Arithmetic Mean). This simple method allows for the transformation of a distance matrix (between different organisms, populations, or nucleotide sequences) into a rooted tree based on the similarities between pairs of sequences (Thompson et *al*., 1994).

Results and discussion

Results from GenBank

We analyzed over 1,273,329 DNA sequences from different Coronavirus strains published in GenBank, but we only selected the nucleotide sequences that include the Spike gene "S" (a structural gene) common to several infected animal species (Li, 2016). Among these sequences, we eliminated very short or repeated sequences; noting that there are multiple sequences presenting the same strain and country but of different sizes (nucleotide base pairs), we chose the sequence with the largest size. The 33 DNA sequences used in this study, representing viral strains of the Coronavirus classified according to the affected animal species across different countries as well as the strains that have infected humans, are listed in table I.

The phylogenetic tree (Figure 1) was constructed based on the S gene of different viral strains of the Coronavirus that have affected several animal species and humans, with sizes varying between 4374 bp and 1992 bp. According to the taxonomy of the Coronaviridae family, it is divided into four genera, which are represented in the phylogenetic tree obtained by four clades: *Alphacoronavirus,*

Deltacoronavirus, Gammacoronavirus, and *Betacoronavirus*. The clades *Alphacoronavirus* and *Betacoronavirus* are further divided into several sub-clades representing the lineages or viral strains.

Table I. Nucleotide sequences of different strains of Coronaviruses extracted from GenBank.

Animal	Species	Region	Virus	Size in bp	Locus
Bat	*Tylonycteris pachypus*	China	Bat-CoV	4059	MW218379
Camel	*Camelus dromedarius*	Ethiopia	DcCoV	4101	MN514969
		Morocco	DcCoV	4092	MN514971
Bovine	Bovine	China	BCoV	4092	MN982181
		South Korea	BCoV	4092	HM573326
Dog	*Canis lupus familiaris*	China	CCoV	4374	LC190907
Civet	*Civette palmiste*	China	SARS-CoV-1	4236	DQ514532
	Civette palmiste	China	SARS-CoV-1	3768	AY627047
Turkey	*Meleagris gallopavo*	USA	TCoV	3627	KF652218
Guinea fowl	Guineafowl	France	GfCoV	3951	MG765535
Ferret	*Melogale moschata*	China	FeCoV	4374	EF192156
Human	*Homo sapiens*	France	HCoV-OC43	4077	KF963240
	Homo sapiens	Netherlands	HCoV-HKU1	4071	MN488637
	Homo sapiens	China	HCoV-HKU1	4071	DQ437613
	Homo sapiens	Saudi Arabia	MERS-CoV	4062	KT806016
	Homo sapiens	USA	SARS-CoV-2	3822	MZ027646
	Homo sapiens	Iran	SARS-CoV-2	3822	MW136267
Pig	Pig	China	TGEV	4344	MH167923
	Pig	Canada	PEDV	4271	KM189366
	Pig	Mexico	PEDV	4200	KY828999
	Pig	Japan	PEDV	4161	KY619734
	Sus scrofa	China	PEDV	4161	MN508818
	Pig	Thailand	PEDV	4161	KX981900
	Pig	Vietnam	PEDV	4158	KX708901
	Pig	Spain	PRCV	3678	M94097.1
	Pig	China	PDCoV	3480	KX534090
	Pig	USA	PDCoV	3483	KP995356
	Sus scrofa	Mexico	PDCoV	3483	MK478381
Yak	Yak	China	YakCoV	4092	MH810162
Chicken	Chicken	China	PDCoV	3480	MK248485
Rat	*Bandicota savilei*	Laos	RtBs-CoV	3435	MT085176
Cat	*Felis catus*	China	FCoV	4359	MK987175
	Domestic cat	China	FCoV	1992	MT181985

Phylogenetic relationships within the S gene tree

Our phylogenetic tree consists of four groups: *Alphacoronavirus, Betacoronavirus, Gammacoronavirus,* and *Deltacoronavirus*, which contain various viral strains that infect different animal species. According to Woo et *al.* (2012), *Alphacoronaviruses* and *Betacoronaviruses* only infect mammals, while *Gammacoronaviruses* and *Deltacoronaviruses* infect birds, although some of them can also infect mammals.

Cowley et *al.* (2011) published that the canine Coronavirus (CCoV), which infects dogs, is closely related to the transmissible gastroenteritis virus (TGEV) of pigs, the ferret Coronavirus, and the feline Coronavirus (FCoV). In our tree, the three Coronaviruses from China, namely CCoV, FCoV, and FeCoV, which infect the dog, cat, and ferret respectively, as well as PRCV from Spain and TGEV from China, which infect the pig, are all grouped together in the same Alpha subgroup (αa). The position of the two strains CCoV (HCM47/2015) and FeCoV (DM95/2003) on the same branch is explained by the work of Dung et *al*. (2017), where they found through phylogenetic analysis of the complete S gene that CoV/HCM47/2015 from China was similar to CoV/DM95/2003, sharing 95.9% similarity. Additionally, according to Yuan et *al.* (2018), the porcine respiratory Coronavirus (PRCV) is a

respiratory variant of TGEV, with the S protein coded by PRCV lacking about 200 amino acids in the N-terminal region that contain determinants related to the enteropathogenicity of TGEV. Therefore, the TGEV strain from China and PRCV from Spain are in the same branch.

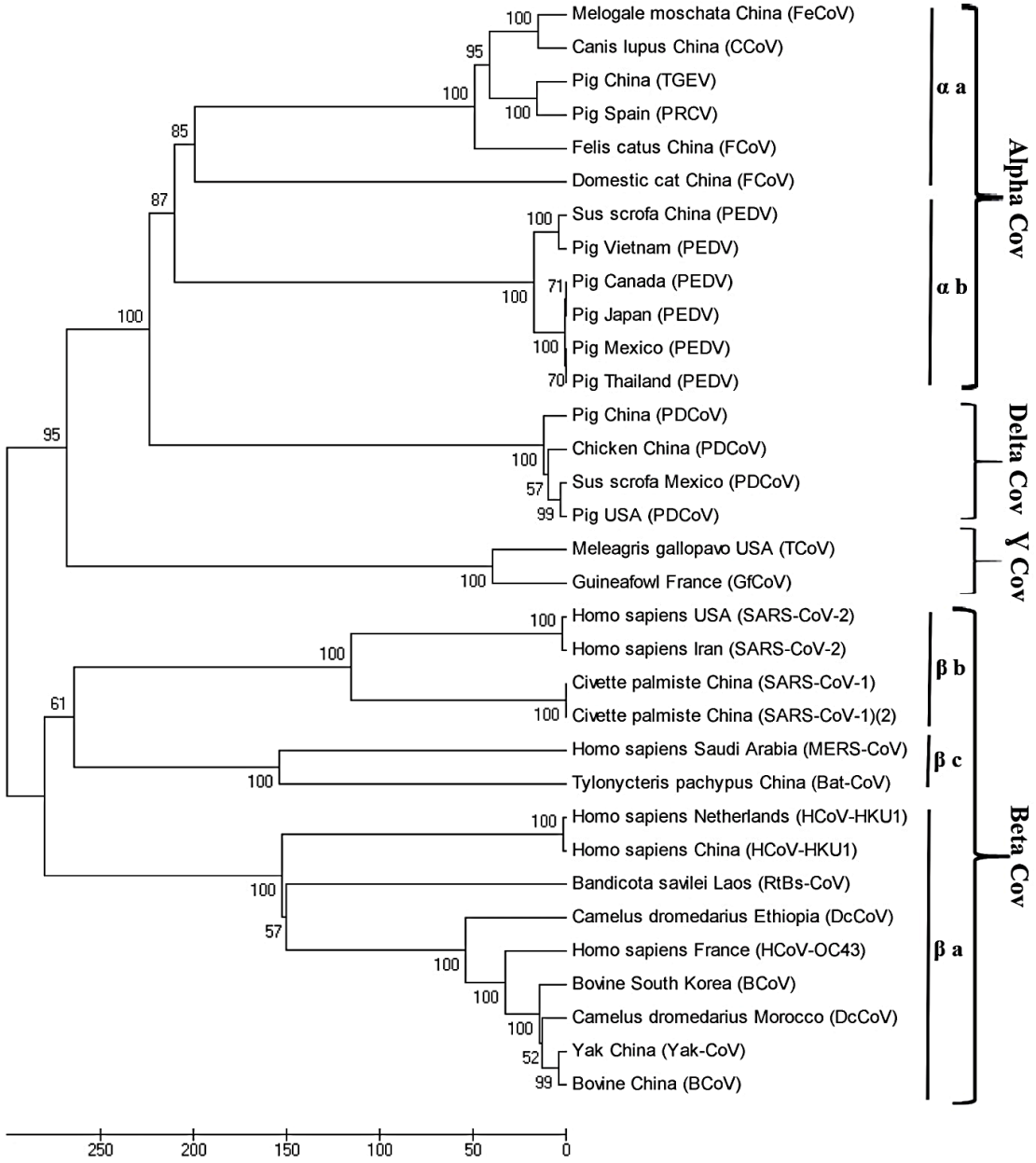

Fig. 1. Phylogenetic tree of the S gene representing different strains of Coronaviruses.

Within our phylogenetic tree, the PEDVs from Japan (14JM-01), Canada (ON-007), Thailand (P1915-NPF-071511A), Mexico (PEDV/MEX/MICH/01/2013), China (SHpd/2012), and Vietnam (HUA-PED88), which infect pigs, belong to the same subgroup (αb). Nguyen et *al.* (2018) demonstrated that the strain from Japan (14JM-01) is a North American strain with 100% identity in the nucleotide sequence of the S gene with the Canadian strain (ON-007). They also noted that the strain P1915-NPF-071511A is the first strain of the emergence of an American-type PEDV in Thailand, with the S gene sequence sharing 100% similarity with American strains KUIDL-PED-2014-002 and USA/Ohio60/2013, which share 99.81% similarity with the strain from Mexico (PEDV/MEX/MICH/01/2013). These findings confirm our results for the four previous strains that were in the same branch. Meanwhile, Than et *al.* (2020) showed that the HUA-PED88 strain from Vietnam (2015) is genetically related to the circulating PEDV strains in China (SHpd/2012).

According to the work of Qingqing et *al.* (2019), potential interspecies transmission of PDCoV may exist between domestic pigs and birds, indicating that the pig Coronavirus PDCoV CH/GD03/2015 infected chickens (PDCoV CC-HNZK), and that PDCoV CC-HNZK-02 exhibited two mutations in the S gene: Asp became His at position 138, and Gln was replaced by Lys at position 641, which explains the close position of PDCoV CC-HNZK to PDCoV CH/GD03/2015 in our tree. Additionally, Pérez et *al.* (2019) noted that the Mexican PDCoV strain is phylogenetically closer to PDCoV strains reported in the United States, with an analysis of S gene sequences obtained from Mexican isolates sharing 99.6% nucleotide identity with North American strains.

The GfCoV/FR/2011 Coronavirus from France and the TCoV from the USA are in the same branch. According to Bouwman et *al.* (2019), the Guinea fowl Coronavirus GfCoV/FR/2011 from France is closely associated with the TCoV from India (IN/421/10), showing a similarity of 86.44%.

SARS-CoV-2, first reported in China in December 2019, quickly became a pandemic with devastating effects (Chen et *al.*, 2020). According to Manuel and Cardozo (2020), the variant carrying the D614G Spike mutation, which is the transition from aspartate (D) at position 614 frequently found in Chinese strains to glycine (G), was spread worldwide by travelers; Korber et *al.* (2020) suggested that these G614 variants were likely introduced and reintroduced in different locations. The Asian outbreak was predominantly D614 until February. By early April 2020, G614 had become the dominant form of the pandemic, which explains the position of the strains SARS-CoV-2/human/IRN/K1r-112/2020 from Iran and SARS-CoV-2/human/USA/TG701123/2020 from the United States in the same branching, as both collected after April 2020 contain the G614 mutation.

The two SARSr-CoV-1 strains from civets in China are in the same branch and share 99.42% similarity. According to Guan et *al.* (2003), after the emergence of severe acute respiratory syndrome human Coronavirus (SARS-CoV-2) in southern China in 2002/2003, SARS-Like (SARSr-CoV-1) strains carried by palm civets from live animal markets in Guangdong were found, with their genomic sequences showing 99.8% identity with that of human SARS-CoV. They were considered an intermediate host that facilitated the passage of the virus to humans.

According to Guangwen et *al.* (2013), MERS-CoV belongs to lineage C of *Betacoronaviruses*, being closely related to Tylonycteris Bat-CoV HKU4 (Ty-Bat-CoV HKU4) in the S gene (66.8% to 67.4%). Viral gene fragments that are identical or closely similar to those of MERS-CoV have also been recovered from bats, raising again the possibility that bats act as a natural reservoir for MERS-CoV. Chen et *al.* (2015) state that most of the recombination and sequence diversity is found in the S gene, and these changes can affect the structural conformation of the receptor-binding domain (RBD) (with positively selected sites located in the RBD of the S protein). The RBD shows two regions: a binding region and a central region, both crucial for the virus to recognize and enter host cells and interact with related human receptors; MERS-CoV uses the DPP4 receptor to penetrate host cells. Mutations have been observed at two sites in the RBD binding region, suggesting that amino acid substitutions could enhance MERS-CoV's ability to bind to different host cells and thus facilitate its transmission between species (Zhang et *al.*, 2016). The strong similarities between the DPP4 receptors of humans, camels, horses, and bats allow MERS-CoV to infect them (Folegatti et *al.*, 2020). This justifies the position of MERS-CoV strains from Saudi Arabia and Tylonycteris Bat-CoV HKU4 from China (Macao) in the same branch of our tree.

So et *al.* (2019) noted that the DcCoV-HKU23 from Morocco, which affected camels, differed from BCoV that infected cattle by a range of 0.0249 to 0.0300 substitutions per site, and from HCoV-OC43 of humans by a range of 0.0445 to 0.0468 substitutions per site. Similarly, the species most closely related to HCoV-OC43 remains BCoV rather than DcCoV-HKU23, knowing that BCoV and HCoV-OC43 share an overall nucleotide identity of 96% in their S gene. These data justify the position of the BCoV strain from South Korea, which is closest to the human species strain OC43 (HCoV-OC43) from France in our tree. The results of So et *al.* (2019) highlighted active recombination of Coronaviruses circulating in dromedaries and are also relevant to the emergence and evolution of other *Betacoronaviruses*, which explains the position of DcCoV from *Camelus dromedarius* in Ethiopia and Morocco in our tree. The same phenomenon explains the position of BCoV from South Korea and China.

The work of Kin et *al*. (2016) on the genetic comparison of BCoV and HCoV-OC43 reveals that HCoV-OC43 results from zoonotic transmission from cattle to humans, as these two viruses are extremely close in terms of genetic and epidemiological characteristics.

Conclusion

The significance of our study is based on the links between Coronavirus strains in different geographical areas; we utilized phylogeny, a useful method in population genetics and epidemiology. It is an essential component in biology for studying the relationships and evolutionary history of organisms, as well as their molecular and morphological diversification. For this work, we used a bioinformatics tool for phylogenetic analysis and the construction of the phylogenetic tree, specifically the MEGA 11 software. The analyses were applied to 33 sequences of the S gene to classify the viral strains of Coronavirus from each genus according to the affected species and the geographical areas where they were found. The results demonstrated that the DNA sequences of the spike protein gene S are divided into four groups represented by four different clades: *Alphacoronavirus, Betacoronavirus, Gammacoronavirus,* and *Deltacoronavirus*. The significant number of animals infected by this virus, as well as humans, is due to a high genetic diversity among Coronavirus strains, which reflects the instability of the S gene, expressed by its very high mutation rate.

Author's Contributions

Author 1: Data analysis, drafting the article.

Author 2: Data analysis.

Author 3: Data analysis.

References

Alluwaimi A. M., Alshubaith I. H., Alali A. M., Abohlaïka S., 2020. The Coronaviruses of Animals and Birds: Their Zoonosis, Vaccines, and Models for SARS-CoV and SARS-CoV2. Front. Vet. Sci. 7 : 582287.

Bouwman K. M., Delpont M., Broszeit F., Berger R, Weerts E. A., Lucas M. N., Delverdier M., Belkasmi S., Papanikolaou A., Boons G. J., Guérin J-L., de Vries R. P., Ducatez M. F., Verheije M. H., 2019. Guinea Fowl Coronavirus Diversity Has Phenotypic Consequences for Glycan and Tissue Binding. Journal of Virology, 93(10). Doi.org/10.1128/jvi.00067-19.

Chen N., Zhou M., Dong X., Qu J., Gong F., Han H., Qiu Y., Wang J., Liu Y., Wei Y., Xia J., Yu T., Zhang X., Zhang L., 2020. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 15; 395(10223): 507-513.

Chen Y. N., Loa C. C., Ababneh M. M. K., Wu C. C., Lin T. L., 2015. Genotyping of turkey coronavirus field isolates from various geographic locations in the Unites States based on the spike gene. Arch Virol, 160(11): 2719-26.

Clark K., Karsch-Mizrachi I., Lipman D. J., Ostell J., Sayers E. W., 2016. GenBank. Nucleic Acids Research, Volume 44, Issue D1, P: D67-D72.

Cowley J. A., Walker P. J., Flegel T. W., Lightner D. V., Tang-Nelson K. F. J., Bonami J. R., Snijder E. J., de Groot R. J., King A. M. Q., Adams M. J., Carstens E. B., Lefkowitz E. J., 2011. Virus Taxonomy, Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier, Part of book, Family Roniviridae, 829-834.

Derouiche L., Benzayed Y., Belmihoub M., Derouiche F., 2022. A study of genetic variants of SARS-CoV-2 using bioinformatics tools. Genet. Biodiv. J, 6 (1): 137-148.

Derouiche L., Latrach R., Seddaoui R., 2023. Studying mutations of SARS-Cov-2 different variants (Alpha, Beta, Delta, Gamma, Omicron). Genet. Biodiv. J, 2023; 7 (1): 75-87.

Dung V. N., Terada Y., Minami S., Yonemitsu K., Nagata N., Thanh L. E., Kuwata R., Shimoda H., Maeda K., 2017. Characterization of canine coronavirus spread among domestic dogs in Vietnam. J. Vet. Med. Sci. 79(2): 343-349.

Folegatti P. M., Bittaye M., Flaxman A., Dphil, Ramos L. F., Bellamy D., Kupke A., Mair C., Makinson R., Sheridan J., Rohde C., Halwe S., Jeong Y., Park Y.C., Kim J.K.,Song M., Boyd A.T. N., Silman D., Poulton I., Datoo M., Marshall J., Themistocleous., Lawrie A., Roberts R., Berrie E., Becker S., Lambe T., Hill A., Ewer K., Gilbert S., 2020. Safety and immunogenicity of a candidate Middle East respiratory syndrome coronavirus viral-vectored vaccine: a dose-escalation, open-label, non-randomised, uncontrolled, phase 1 trial. The lancet Infectious diseases. 20(7): 816-826.

Fung T.S., Liu D.X., 2019. Human Coronavirus: Host-Pathogen Interaction. Annual review of Microbiology, 73: 529-557. Doi.org/10.1146/annurev-micro-020518-115759.

Guan Y., Zheng B. J., He Y. Q., Liu X. L., Zhuang Z. X., Cheung C. L., Luo W. S., Li P. H., Zhangyj L. J., Guan Y. J., Butt K. M., Wong K. L., Chan K. W., Lim W., Shortridge K. F., Yuen K. Y., Peiris J. S. M., Poon L. L. M., 2003. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 302(5643): 276-8.

Guangwen L., Hu Y., Wang Q., Qi J., Gao F, Li Y., Zhang Y., Zhang W., Yuan Y., Bao J., Zhang B., Shi Y., Yan J., Gao G.F., 2013. Molecular basis of binding between novel human coronavirus MERS-CoV and its receptor CD26. Nature. 500: 227-231.

Kin N., Miszczak F., Diancourt L., Caro V., Moutou F., Vabret A., Gouilh M.R., 2016. Comparative molecular epidemiology of two closely related coronaviruses, bovine coronavirus (BCoV) and human coronavirus OC43 (HCoV-OC43), reveals a different evolutionary pattern. Infect Genet Evol. 40:186-191.

Korber B., Fischer W. M., Gnanakaran S., Yoon H., Theiler J., Abfalterer W., Hengartner N., Giorgi E., Bhattacharya T., Foley B., Hate K.M., Parker M. D., Perdrix D. G., Evans C. M., Homme libre T. M., de Silva T. I., 2020. Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. Cell. 182(4): 812-827.

Kuhls K., Mauricio I., 2019. Phylogenetic studies. Methods Mol. Biol. 1971: 9-68.

Li F., 2016. Structure, Function, and Evolution of Coronavirus Spike Proteins. Annual Review of Virology, 3: 237-261. DOI: 10.1146/annurev-virology-110615-042301.

Manuel B. F., Cardozo T., 2020. SARS-CoV-2 viral spike G614 mutation exhibits higher case fatality rate. Int. J. Clin. Pract. 74(8): e13525.

Nguyen V. D., Sueyoshi M., Norimine J., Hirai T., Myint O., Teh A. A. P., Izzati U. Z., Fuke N., Yamaguchi R., 2018. Molecular characterization of US-like and Asian non-S INDEL strains of porcine epidemic diarrhea virus (PEDV) that circulated in Japan during 2013-2016 and PEDVs collected from recurrent outbreaks. BMC veterinary search 14: 96.

Pérez C. R., Mendoza H. R., Elvira R. S., Velázquez R. S., Betancourt J. I., 2019. First report and phylogenetic analysis of porcine deltacoronavirus in Mexico. Transbound Emerg Dis. 66: 1436-1441.

Qingqing L., Zhang H., Li B., Qingwen D., Wang Y., Gao W., Guo D., Wei Z., Hu H., 2019. Susceptibility of Chickens to Porcine Deltacoronavirus Infection. Viruses. 11(6): 573.

Sayers E. W., Cavanaugh M., Clark K., Pruitt K. D., Schoch C. L., Sherry S. T., Karsch-Mizrachi I., 2020. GenBank. Nucleic Acids Research. 49(D1): D92-D96.

Siddell S., Coll M., 1983. Pathogénese des infections virales. Clinique vétérinaire de d'Amérique de Nord. 6 :1049-1079.

So R. T. A., Chu D. K. W., Miguel E., Perera R. A. P. M., Oladipo J. O., Fihri O. F., Aylet G., Ko R. L. W., Zhou Z., Cheng M. S., Kuranga S. A., Roger F. L., Chevalier V., Webby R. J., Woo P. C. Y., Poon L. L. M., Peiris M., 2019. Diversity of Dromedary Camel Coronavirus HKU23 in African Camels Revealed Multiple Recombination Events among Closely Related

Betacoronaviruses of the Subgenus Embecovirus. Journal virology, 93(23): e01236-19. DOI: 10.1128/JVI.01236-19.

Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S., 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Likelihood, Distance, and Parsimony methods. Molecular Biology and Evolution, 28 (10): 2731-2739. Doi.org/10.1093/molbev/msr121.

Than V. T., Choe S. E., Vu T. H., Tien D., Nguyen T. L., Bui T. T. N., Mai T. N., Cha R. M., Song D., An D. J., Van P. L., 2020. Genetic characterization of the spike gene of porcine epidemic diarrhea viruses (PEDVs) circulating in Vietnam from 2015 to 2016. Journal veterinary medicine and science, 6(3): 535-542. DOI: 10.1002/vms3.256.

Thompson J. D., Higgins D. G., Gibson T. J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22(22): 4673-80.

Woo P. C. W., Lau S. K. P., Lam C. C. F., Lau C. C. Y., Tsang A. K. L., Lau J. H., Baï R., Teng J. L. L., Tsang K. C. C., Wang M., Zheng B. J., Chan K. H., Yuen K. W., 2012. Discovery of Seven Novel Mammalian and Avian Coronaviruses in the Genus Deltacoronavirus Supports Bat Coronaviruses as the Gene Source of Alphacoronavirus and Betacoronavirus and Avian Coronaviruses as the Gene Source of Gammacoronavirus and Deltacoronavirus. Journal of virology, 86 (7): 3995-4008. Doi: 10.1128/JVI.06540-11.

Yuan P., Yang Z., Song H., Wang K., Yang Y., Xie L., Huang S., Liu J., Ran L., Song Z., 2018. Three Main Inducers of Alphacoronavirus Infection of Enterocytes: Sialic Acid, Proteases, and Low pH. Intervirology. 61(2): 53-63.

Zhang Z., Shen L., Gu X., 2016. Evolutionary Dynamics of MERS-CoV: Potential Recombination, Positive Selection and Transmission. Sci. Rep. 4(6): 25049.