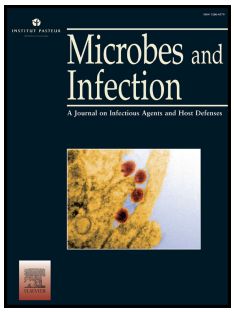

Molecular basis of COVID-19 relationships in different species: a one health perspective

Bruno Tilocca, Alessio Soggiu, Vincenzo Musella, Domenico Britti, Maurizio Sanguinetti, Andrea Urbani, Paola Roncada

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Molecular basis of COVID-19 relationships in different species: a one health perspective.

Bruno Tilocca[a], Alessio Soggiu[b], Vincenzo Musella[a], Domenico Britti[a], Maurizio Sanguinetti[c,d], Andrea Urbani[c,d], Paola Roncada[a,*]

[a] Department of Health Science, University "Magna Græcia" of Catanzaro, Viale Europa- 88100- Catanzaro, Italy

[b] Department of Veterinary Sciences, University of Milano, Via Celoria n10- 20133- Milano, Italy

[c] Institute of Microbiology, Catholic University of the Sacred Hearth, Largo S. Vito n1- 00168- Rome, Italy

[d] Dipartimento Scienze di Laboratorio e Infettivologiche, Fondazione Policlinico Universitario Gemelli IRCCS, Largo S. Vito n1- 00168- Rome, Italy

* Correspondence: Prof. Paola Roncada

Viale Europa 88100, Catanzaro, Italy

Tel. 09613694284

roncada@unicz.it

Abstract:

Outside the Hubei province, China, the mild form of infection and the progressive recover of the COVID-19 patients suggest the intervention of "unconventional" biological mechanisms worthy of attention. Based on the high-homology between the Spike protein epitopes of taxonomically-related coronaviruses, we hypothesized that past contact with infected dogs shield humans against the circulating SARS-CoV-2. Elseways, the recurrent virus exposure over a short time-lapse might result in the Antibody Dependent Enhancement, triggering the violent immune reaction responsible

for the severe clinical outcomes observed in the Hubei province. Nevertheless, further experimental studies are desired for a confidential evaluation of the postulated hypotheses.

Keywords: SARS-CoV-2; COVID-19; coronavirus; canine respiratory coronavirus; immunization; one health.

Since late December 2019, the world is facing a novel outbreak of an infectious disease, known as COVID-19, caused by an unidentified viral agent. Examination of patients cases reports clinical picture comparable to this observed in the SARS outbreak of 2003. This evidence is also supported by the early identification of the etiologic agent as a novel Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) and the molecular investigations describing a high percentage of sequence homology between SARS-CoV-2 and the previous SARS-causing virus (SARS-CoV) [1,2]. Coronaviruses are viruses with single-strand positive-sense RNA genome of approximately 30 kilobases [3]. They have been commonly found in a wide array of host spectrum including avian, camels, bats, masked palm civets, mice, dogs, and cats [2]. Since its early identification, in the very begin of 2020, several nucleotides-based surveys and sequence homology-based alignments have been carried out with the focus to elucidate the viral origin and delineate cell- and host-tropism of the viral agent on the attempt to guide better management and control of the virus diffusion [4]. Sequence similarity alignments have reported SARS-CoV-2 being highly similar to several wildlife animals such as bats, pangolins and masked palm civets [3]. Controversial results have been reported concerning the snake coronavirus [2,5]. Nevertheless, the sole investigation of the genetic sequences might result in a biased and/or incomplete depiction of the virus source, biology and its pathogenetic mechanism [4]. In the Hubei province, where the outbreak originated, severe cases or deaths attributed to SARS-CoV-2 infections are predominantly arising from patients suffering from one or more previous pathological conditions such as diabetes, cardiovascular and cerebrovascular

disease leading to sequelae, sometimes fatal, such as cellular immune deficiency, coagulation activation, myocardia injury, hepatic and kidney injury, and secondary bacterial infection [4,6].

Although featured by a higher fatality rate when compared with the virus of the seasonal influenza (3.4%, WHO Situation Report-37, 25/02/2020), outside the primordial outbreak site, much less warning clinical signs have been registered in the SARS CoV-2 infected patients manifesting a mild form of the disease that is not evolving into severe stages and can, in some cases, recover without medical intervention [4]. Altogether this suggests the important contribution of the biological mechanisms that cannot be explained by the sole investigation of the genetic sequences.

Protein repertoire of the coronaviruses consists of four main structural and approximately 16 major non-structural proteins [3,7]. Among the four structural proteins, the Spike protein (also known as S-protein) is involved in host tropism by means of recognition and attachment to the angiotensin-converting enzyme 2 (ACE2) receptor exposed in the outer layer of the host cell membrane [8]. A very recent amino acid sequence alignment of ACE2 from different animal species including humans, pets and the major domestic animals highlighted high protein sequence similarity between the tested animal species, suggesting a potential interaction of the viral particles with a wider array of host cells [8].

In accordance with the hypothesis of interspecies transmission of the beta coronaviruses [8], we performed sequence homology analysis of the aminoacidic sequence of the Spike protein from SARS CoV-2 against taxonomically related coronaviruses with tropism for other animal species. Although the low homology scored while comparing the whole protein sequence (Tab. 1), particular interest was dedicated to the canine respiratory coronavirus, the bovine coronavirus revealing and the human enteric coronavirus 36.93%, 38.42% and 37.68% sequence homology to SARS CoV-2 spike protein, respectively.

Table1

A previous study of Hua and colleagues on the amino acid sequence of SARS CoV spike protein identified six epitopes based on the hydrophilicity, surface probability, antigenic index, and secondary structure [9] (Tab.2).

Table2

The sequence homology analysis restricted to the epitope sequences of the SARS CoV-2 revealed instead high percentage homology towards taxonomically related coronaviruses. Of these, we retain it is worth of note the high similarity occurring among 4 epitope sequences of canine respiratory coronavirus (Canine respiratory CoV BJ 232) and the circulating SARS CoV-2 (Table 3).

Table3

Highlighting the similarity at the epitope level opens new avenues in understanding the biological mechanisms undertaken during viral infection. Before the current outbreak, several studies witness the common identification of coronavirus infection in dogs [10,11]. Previous studies performed by Szczepanski and colleagues report the possibility to grow both bovine and canine coronavirus on human cells lines such as the Human Rectal Tumor cells (HRT-18G) and Human Airway Epithelium (HAE) although featured by a strongly reduced replication efficiency [12,13]. In this view, a previous “contact” with the canine virus might provide at least a partial/basal immunization that shields humans against the circulating SARS CoV-2. This would partially explain the mild symptoms registered among patients outside the Hubei province and without previous co-morbidities. Accordingly, high similarity percentage have also been observed between SARS CoV-2 spike proteins epitopes and bovine coronavirus, which genome and proteins appear among those included in the patented vaccinal formulation released against coronavirus.

With regard to the most severe cases of infection registered both outside and within the Hubei province, it is most likely that these individuals have been primed by one or more coronavirus

exposure within a narrow time window, leading to the effects of Antibody-Dependent Enhancement (ADE) [4]. Such a hypothesis is in line with the previous observations dating back to the previous SARS outcome in 2003 when human coronaviruses, known to cause mild infections, were hypothesized of priming the high mortality scored in China. Here, anti-Spike protein antibodies were indicated as potentially involved in the enhancement mechanism [14–18]. In this view, it is plausible that Hubei province patients are featured by a more severe clinical picture, since more likely to be recurrently exposed to the coronavirus.

To summarize, animals have (had) a critical role in this outbreak onset and evolution. Acknowledged their pivotal role as virus reservoir, they might act in the first instance as "beneficial" source of immune-stimulating virus particles; thus, providing a shield against the circulating SARS CoV-2. However, the recurrent exposure within a narrow time-lapse might results in the detrimental triggering of violent immune responses and the evolution towards a more severe, or even fatal, clinical picture.

Above hypotheses are based on the assumption that sufficient viral titer of the SARS CoV-2 is transmitted from patient to patient [19]. The previous investigation on animal betacoronavirus such as the canine coronavirus have demonstrated a high transmission efficiency through direct contact between infected animals, acknowledged the high viral titers found in the aerosols of respiratory secretions [20]. Novel investigations to assess the transmission rate of the canine respiratory coronavirus to humans are needed to better suit the above hypotheses. Also, care should be taken while evaluating the data on the sole basis of sequence homology and further studies employing purified forms of the spike proteins and/or its epitopes are desired.

Conflict of interest: The authors declare no conflict of interest

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Table 2. Epitope details of the Spike glycoprotein of diverse coronaviruses.

Protein GI	Epitope AAs	Epitope sequence	Organism	NCBI TaxID
QHR63290			SARS coronavirus 2	2697049
	424-437	KLPDDFTGCVIAWN		
	447-458	NYNYLYRLFRK		
	560-571	LPFQQFGRDIAD		
	754-764	LQYGSFCTQLN		
	789-799	YKTPPIKDFGG		
	1139-1152	DPLQPELDSFKEEL		
QAY30020			Canine respiratory coronavirus	215681
	424-437	SGYTVAATFASLFP		
	447-458	FYLNVQYRINGI		
	560-571	QLSDSTLVKFSA		
	754-764	TYEYYVKWPWY		
	789-799	CGTSCFKKCGG		
	1139-1152	DPLQPELDSFKEEL		
AHA50776			Bovine coronavirus	11128
	424-437	ATSCQLYYNLPAAN		
	447-458	TWNRRFGFTEQS		
	560-571	EHCSGLAIKSDH		
	754-764	SGYCVDYSTKR		
	789-799	DSLEPVGGLYE		
	1139-1152	NGNHIISLVQNAPY		
ACJ35486			Human enteric coronavirus	166124
	424-437	ATSCQLYYNLPAAN		
	447-458	TWNRRFGFTEQS		
	560-571	EHCSGLAIKSDH		
	754-764	SGYCVDYSTKR		
	789-799	DSLEPVGGLYE		
	1139-1152	NGNHIISLVQNAPY		

Table3. Epitope sequence alignment. The table summarizes results from the alignment of SARS CoV-2 spike protein epitopes against the spike protein epitopes of other coronaviruses with known tropism for other animals than humans.

Epitope	Organism	% identity
	Bovine Coronavirus	80,00
424-437	Canine respiratory coronavirus	80,00
	Human enteric coronavirus	80,00
	Bovine Coronavirus	75,00
447-458	Canine respiratory coronavirus	-
	Human enteric coronavirus	75,00
	Bovine Coronavirus	83,33
754-764	Canine respiratory coronavirus	83,33
	Human enteric coronavirus	83,33
	Bovine Coronavirus	57,14
789-799	Canine respiratory coronavirus	57,14
	Human enteric coronavirus	57,14
	Bovine Coronavirus	70,00
1139-1152	Canine respiratory coronavirus	100,00
	Human enteric coronavirus	70,00

Table 1. Amino acid sequence alignment of SARS CoV-2 Spike protein (GI QHR63290) against betacoronavirus database. The table display alignment relative to taxonomically-related coronavirus having a tropism for other hosts than humans.

Protein Accession	**E-value**	**Organism**	**NCBITax ID**	**% identity**
AGO98871	1e-154	Bovine Coronavirus	11128	38.42%
QAY30020	3e-152	Canine respiratory coronavirus	215681	36.93%
ACJ35486	3e-150	Human enteric coronavirus	166124	37.68%
ACT10865	2e-105	Feline coronavirus	12663	32.26%
ABQ57216.1	4e-100	Bat coronavirus	693998	31.23%
AID16631	1e-145	Mouse coronavirus	1508222	36.56%
AID16649	8e-149	Rat coronavirus	1508223	37.60%