УДК 636.03:575.852:578.834.1SARS-CoV-2
DOI: https://doi.org/10.31073/abg.62.16

COMPARATIVE ANALYSIS OF HUMAN AND LIVESTOCK ACE2 RECEPTORS FOR SARS-COV-2

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Angiotensin-converting enzyme 2 (ACE2) is a receptor for SARS-CoV-2 spike protein on the cell surface and plays a key role in the development of COVID-19. The high conservatism of ACE2 structure in different species and the large number of human contacts with livestock increase the risk of spreading SARS-CoV-2 among the ones if the virus will be able to penetrate and replicate in the cells of such animals successfully. The result of this course of events may be the emergence of the animal reservoirs of coronavirus disease.

To assess this possibility, a comparative analysis of the amino acid sequences of ACE2 receptors for SARS-CoV-2 in different species of livestock with human ACE2 was performed. High degrees of identity and similarity were found for ACE2 receptors of donkey, horse, rabbit, alpaca, lama, dromedary, pig, sheep, goat and cattle (taurine and zebu), lower – for poultry species (chicken, duck and turkey). The data obtained in this study are consistent with the results of previous experiments on the ability of SARS-CoV-2 to interact with ACE2 receptors of different animal species. Although there is evidence of pig, chicken and duck resistance to SARS-CoV-2 by intranasal inoculation, the risk of the virus adaptation to livestock infecting, given the mutational variability of the virus, remains high, which makes relevant the further studies of SARS-CoV-2 interactions with livestock.
Keywords: **coronavirus, SARS-CoV-2, ACE2, receptor, livestock, degree of identity, degree of similarity, phylogenetic analysis**

ПОРІВНЯЛЬНИЙ АНАЛІЗ ACE2 РЕЦЕПТОРІВ ЛЮДИНИ ТА СІЛЬСЬКОГОСПОДАРСЬКИХ ТВАРИН ДЛЯ SARS-COV-2

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Ангіотензин-перетворюючий фермент 2 (ACE2) є рецептором для спайк-білка SARS-CoV-2 на поверхні клітин і відіграє ключову роль у розвитку COVID-19. Висока консервативність структури ACE2 у різних біологічних видів та велика кількість контактів людини з сільськогосподарськими тваринами підвищує небезпеку поширення SARS-CoV-2 серед останніх у випадку, якщо даний вірус зможе успішно проникати та реплікуватися у клітинах таких

тварин. Результатом цього перебігу подій може бути виникнення тваринних резервуарів короновірусної інфекції.

Для оцінки такої можливості було здійснено порівняльний аналіз амінокислотних послідовностей ACE2 рецепторів для SARS-CoV-2 у різних видів сільськогосподарських тварин із ACE2 людини. Високі ступені ідентичності та подібності були виявлені для ACE2 рецепторів віслюка, коня, кролика, альпаки, лами, одногорбого верблюда, свині, вівці, кози та великої рогатої худоби (європейської та зебу), менші – для видів сільськогосподарської птиці (курки, качки та індички). Отримані в роботі дані узгоджуються з результатами проведених раніше експериментів щодо здатності SARS-CoV-2 взаємодіяти з ACE2 рецепторами різних видів тварин. Хоча існують підтвердження резистентності свиней, курок та качок до SARS-CoV-2 при інтраназальній інокуляції, ризик адаптації вірусу до зараження сільськогосподарських тварин, враховуючи мутаційну мінливість вірусу, залишається високим, що робить актуальним подальші дослідження взаємодій SARS-CoV-2 із клітинами сільськогосподарських тварин.

Ключові слова: **коронавірус, SARS-CoV-2, ACE2, рецептор, сільськогосподарські тварини, ступінь ідентичності, ступінь подібності, філогенетичний аналіз**

Introduction. Severe acute respiratory syndrome-related coronavirus-2 (SARS-CoV-2) is the pathogen that causes an infectious respiratory disease known as coronavirus disease 2019 (COVID-19) [1]. The first cases of COVID-19 were detected in December 2019 in Wuhan (Quebec, China) [2]. The disease has spread rapidly in China and other countries and reached pandemic proportions, as reported by the World Health Organization (WHO) in March 2021 [3].

SARS-CoV-2 belongs to the group of coronaviruses, which includes enveloped, nonsegmented, positive-sense single-stranded RNA viruses [4]. Coronaviruses (subfamily *Orthocoronavirinae*, family *Coronaviridae*) are divided into four genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus* [5]. Coronaviruses cause respiratory, intestinal and neurological diseases in animals, including birds (*Gallusformes*, *Passeriformes*) and mammals (bats, mice, rats, pigs, cows, sheep, goats, camels, lamas, alpacas, horses, dogs, cats, mink, ferrets, etc.) [5]. At the time of writing, there are seven known species of coronaviruses that cause human disease. Of these, four species: HCoV-229E, HCoV-NL63 (*Alphacoronavirus*) and HCoV-OC43, HCoV-HKU1 (*Betacoronavirus*) cause mild respiratory diseases [1, 4]. The other three species of *Betacoronavirus* genus: SARS-CoV (Severe acute respiratory syndrome-related coronavirus), MERS-CoV (Middle East respiratory syndrome coronavirus) and SARS-CoV-2 cause severe life-threatening respiratory diseases [1]. SARS-CoV and MERS-CoV are thought to have animal origin. Investigations indicated that SARS-CoV and MERS-CoV are transmitted from civet cats and dromedary camels, respectively [5]. The origin of SARS-CoV-2 continues to be investigated [6]. Phylogenetic analysis data show that SARS-CoV-2 is closer to bat coronaviruses detected in China in Zhoushan in 2018 (Bat-SL-CoVZC45 and Bat-SL-CoVZXC21, 88% identity) and in Yunnan in 2013 (Bat-CoV RaTG13, 96% identity) than to human coronaviruses SARS-CoV and MERS-CoV (79% and 50% identity, respectively) [7].

SARS-CoV-2 uses a trimeric spike glycoprotein to enter the host cell [8]. Each spike monomer contains 1273 amino acid residues and consists of two subunits: S1 (amino acid residues 14–685) and S2 (amino acid residues 686–1273), preceded by a short signal peptide (amino acid residues 1–13) [9, 10]. The S1 subunit is responsible for binding to the receptor on the surface of the host cell, and the S2 subunit ensures the fusion of the virus membrane with the cytoplasmic membrane [8].

It has been experimentally confirmed that SARS-CoV-2, as well as SARS-CoV, uses angiotensin-converting enzyme 2 (ACE2) as a receptor for cell entry [1, 11], which under physiological conditions participates in the functioning of the renin-angiotensin system, whose task is to maintain homeostasis of the cardiovascular system and the functioning of various organs, regulation of systolic pressure, osmotic and electrolyte balance [8]. ACE2 is a type I transmembrane protein consisting of an extracellular N-glycosylated N-terminal domain containing a carboxypeptidase site and a short

intracellular C-terminal cytoplasmic tail [2]. Crystallographic analysis data of the SARS-CoV-2 spike protein complex with human ACE2 indicate that the N-terminal peptidase domain is involved in the interaction with the virus [12]. In addition, a comparison of the complexes that form SARS-CoV and SARS-CoV-2 with human ACE2 shows that SARS-CoV-2 binds to ACE2 with higher affinity, and mutational adaptive changes in SARS-CoV-2 in comparison with SARS-CoV can cause high contagious capacity of SARS-CoV-2 and widespread of COVID-19 [8].

A significant role of ACE2 in the SARS-CoV-2 disease induction makes it relevant to study ACE2 for better understanding of the mechanisms of COVID-19 development, antiviral drug search, and prediction of possible routes of human-to-animal transmission and *vice versa*. The high conservatism of ACE2 in different species, as well as the prevalence of other coronavirus infections in animals, increases this potential.

A large number of human contacts with different species of livestock may increase the risk of SARS-CoV-2 spreading among the ones if the virus will be able to penetrate and replicate in the animal cells successfully. The result of this course of events may be the emergence of the animal reservoirs of the coronavirus disease, which, on the one hand, is dangerous to humans, and, on the other hand, can cause the economic damage to livestock industry. One approach for evaluation of this possibility is a comparative analysis of the amino acid sequences of ACE2 receptors for SARS-CoV-2 in different species of livestock with human ACE2. Obviously, the higher is the degree of their similarity, the more likely is the possibility of interpenetration of the coronavirus disease between humans and animals.

The ***aim*** of this study was to determine the similarity of the amino acid sequences of human ACE2 and different livestock species to assess the potential risks of SARS-CoV-2 transmission and the emergence of the animal reservoirs of COVID-19.

Materials and methods. The ACE2 amino acid sequences of human (*Homo sapiens*, GenBank: BAD99266.1) and 14 species were used for analysis: domestic pig (*Sus scrofa domesticus*, GenBank: ASK12083.1), taurine cattle (*Bos taurus*, isoform X2, NCBI RefSeq: XP_005228486.1), zebu cattle (*Bos inducus*, isoform X2, NCBI RefSeq: XP_019811720.1), sheep (*Ovis aries*, isoform X2, NCBI RefSeq: XP_011961657.1), goat (*Capra hircus*, isoform X1, NCBI RefSeq: XP_005701129.2), dromedary (*Camelus dromedarus*, NCBI RefSeq: XP_031301717.1), lama (*Lama glama,* GenBank: QWM88990.1), alpaca (*Vicugna pacos,* NCBI RefSeq: XP_006212709.1), horse (*Equus cabalus*, NCBI RefSeq: XP_001490241.1), donkey (*Equus asinus*, Ensembl ID: ENSEAST00005000893.1), rabbit (*Oryctolagus cuniculus*, NCBI RefSeq: XP_002719891.1), chicken (*Gallus gallus*, isoform X1, NCBI RefSeq: XP_040517014.1), wild turkey (*Meleagris gallopavo*, Uniport ID: G1N3R5), duck (*Anas platyrhynchos*, NCBI RefSeq: XP_012949915.3). If ACE2 in a particular species was represented by several isoforms, the isoform with the closest to human ACE2 amino acid composition was taken into account.

To assess the degree of identity and the degree of similarity of livestock ACE2 amino acid sequences with human ACE2, the pairwise alignment of the human ACE2 amino acid sequence with the corresponding sequences of animals was performed using the Needleman-Wunsch algorithm [13] and BLOSUM62 substitution matrix [14] in EMBOSS Neddle tool [15]. As "identical" were considered the same amino acids in two sequences (no substitutions), as "similar" were considered amino acid pairs with a score > 0 in BLOSUM62 substitution matrix.

In order to determine the conservatism of ACE2 receptors in different animal species and to conduct phylogenetic analysis, the multiple alignment was performed according to the ClustulW algorithm in MegaX software. The statistics obtained in MegaX on the content of individual amino acid residues in the ACE2 receptors were grouped using Venn diagram [18] according to physicochemical properties of amino acids [19]. The phylogenetic tree was built in MegaX using the Maximal Likelihood method and the JTT matrix-based model [20].

Data grouping, statistical processing and other necessary calculations were performed in Microsoft Office Excel software [21].

Results. Human ACE2 polypeptide chain consists of 805 amino acid residues. The same length

of the polypeptide chain have ACE2 of donkey, horse, dromedary, lama, alpaca, rabbit and pig. The results of the pairwise and multiple alignments showed that in the ACE2 sequences of these species there are no insertions or deletions of amino acids when compared with human ACE2. The ACE2 polypeptide chains of sheep, goat, cattle (taurine and zebu) consist of 804 amino acid residues and have a deletion at position 136. ACE2 receptors of poultry (chicken, duck, turkey) have both insertions and deletions when compared with human ACE2 and differ from human and mammalian ACE2 receptors more significantly. Despite the fact that duck ACE2 consists of 805 amino acid residues, as well as human ACE2, the same length of the polypeptide chain in this case is due to the presence of several insertions and deletions and does not indicate the greater similarity of duck ACE2 to human ACE2 than for other bird species.

Obtained through the pairwise alignment data on the degree of identity (% of identical amino acid residues in the sequences) and the degree of similarity (% of similar amino acid residues in the sequences) of the livestock ACE2 receptors with human ACE2 receptor were ranked in the descending order of alignment scores (Table 1). Donkey, horse and rabbit had the highest degrees of similarity with human ACE2. The ACE2 receptors of other mammals (dromedary, alpaca, lama, pig, sheep, goat, taurine and zebu cattle) were somewhat less similar. The lowest degree of similarity to human ACE2 was shown for the poultry ACE2 receptors.

Results of the determined ACE2 receptors' degrees of similarity were compared with experimental data obtained by C. Conceicao et al. [22] (Table 1). In that study authors used 2 related approaches to examine the capacity of SARS-CoV-2 to enter cells bearing different ACE2 proteins. The first approach, based on the pseudotyping of lentiviral particles with SARS-CoV-2 spike protein, mimicked particle entry. The second approach, based on a quantitative cell–cell fusion assay, assessed the capacity of spike protein to induce cell–cell fusion following receptor engagement [22]. The ability of SARS-CoV-2 to use human ACE2 receptor was taken as 100%, and for other animal species the value was taken relative to it.

1. Degrees of human and livestock ACE2 amino acid sequences' identity and similarity

Human ACE2 alignment with	Length (aa)	Identity (%)	Similarity (%)	Alignment score	ACE2 usage profile [22]	
					Pseudotype (%)	Cell-cell (%)
Human	805	100.0	100	4291.0	100	100
Donkey	805	87.0	93.4	3795.0	–	–
Horse	805	86.8	93.4	3791.0	102.1	96.8
Rabbit	805	85.2	92.8	3722.0	86.5	87.3
Dromedary	805	83.2	92.4	3666.0	–	–
Alpaca	805	83.4	91.9	3653.0	–	–
Lama	805	83.4	91.9	3652.0	–	–
Domestic pig	805	81.7	91.1	3587.0	78.0	103.9
Sheep	804	81.7	90.8	3579.0	117.8	126.7
Goat	804	81.6	90.6	3572.0	75.9	112.9
Taurine cattle	804	81.1	90.7	3568.0	91.9	139.4
Zebu cattle	804	81.1	90.4	3563.0	–	–
Chicken	808	65.6	79.3	2924.5	0.5	14.4
Duck	805	64.7	78.6	2847.0	–	–
Wild turkey	807	63.8	78.5	2827.0	0.5	15.6

Note: "–" experimental data is absent.

Results obtained in our study on the human and livestock ACE2 amino acid sequences' degrees of similarity are fully consistent with previously published experimental data on the ability of SARS-CoV-2 to use ACE2 receptors of different animal species. Pearson's correlation coefficient $r = 0.89$

between “receptor similarity” and “pseudotype entry assay”, and $r = 0.77$ between “receptor similarity” and “cell-cell fusion assay”. Thus, it can be argued that ACE2 receptors of horse, rabbit, pig, sheep, goat, cattle and other mammalian species have the significant similarity to human ACE2, as well as that ACE2 receptors of bird species have the insufficient similarity to ensure their effective interaction with SARS-CoV-2.

Data on the percentage of individual amino acids in human and livestock species ACE2 receptors were grouped according to the physicochemical properties of amino acids (Table 2). It was shown that there is a significant relative difference (RD) in the composition of charged amino acids. Thus, in ACE2 of all livestock species RD > 8% compared with human ACE2 for negative charged amino acids (Asp, Glu), and in sheep, goat, cattle (taurine and zebu) ACE2 receptors RD > 6% for positive charged amino acids (Lys, Arg, His). In addition, it is necessary to note significant differences compared with human ACE2 in the composition of aliphatic amino acids (Ile, Leu, Val) for some of studied species, as well as differences (both in the smaller and larger direction) in the composition of Proline (Pro).

The results of phylogenetic analysis, performed on the basis of the multiple alignment, were presented in the form of a phylogenetic tree (Fig. 1) and also demonstrated the greater similarity of human ACE2 receptors with mammalian receptors (rabbit, donkey, horse, dromedary, alpaca, lama, pig, taurine and zebu cattle, sheep, goat) than with bird receptors (chicken, duck, turkey). However, the smallest distances on the phylogenetic tree are between human ACE2 and ACE2 of donkey, horse and rabbit.

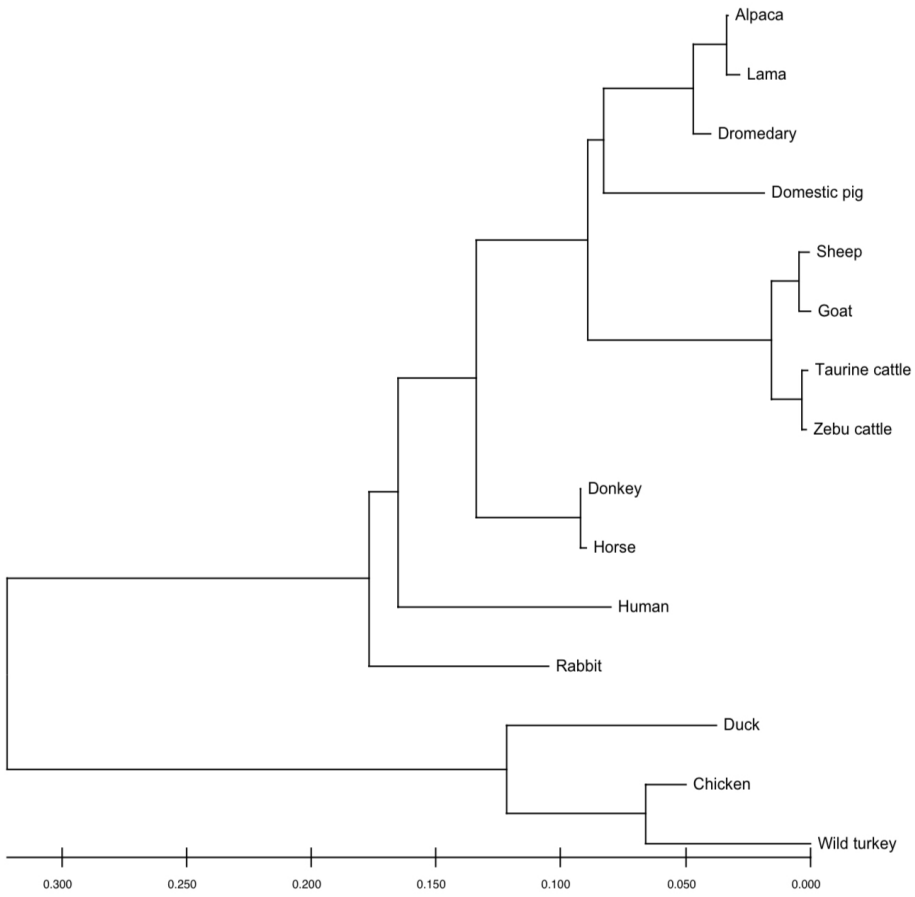

Fig. 1. **Phylogenetic analysis of human and livestock species ACE2 receptors**

2. Amino acid composition of human and livestock ACE2 receptors

	Hydrophobic (I, L, V, C, A, G, M, F, Y, W, H, K, T)		Charged				Polar (Y, W, H, K, R, E, Q, D, N, S, T)		Small (V, A, C, G, D, N, S, T, P)		Tiny (A, G, S)		Aliphatic (I, L, V,)		Aromatic (F, Y, W, H)		Proline (P)	
			Positive (H, K, R)		Negative (E, D)													
	Content (%)	RD	Content (%)	RD	Content (%)	RD	Content (%)	RD	Content (%)	RD	Content (%)	RD	Content (%)	RD	Content (%)	RD	Content (%)	RD
Human	61.12	–	11.68	–	12.30	–	53.91	–	47.08	–	20.00	–	20.62	–	13.79	–	4.60	–
Donkey	61.24	0.20%	11.80	1.06%	13.29	8.08%	54.29	0.69%	47.58	1.06%	21.37	6.83%	20.00	-3.01%	13.54	-1.80%	4.84	5.41%
Horse	61.12	-0.00%	11.80	1.06%	13.29	8.08%	54.41	0.92%	47.58	1.06%	21.49	7.45%	19.88	-3.61%	13.54	-1.80%	4.84	5.41%
Rabbit	60.25	-1.42%	11.30	-3.19%	13.66	11.11%	55.03	2.07%	46.58	-1.06%	21.86	9.32%	19.38	-6.02%	13.66	-0.90%	4.35	-5.41%
Dromedary	62.61	2.44%	11.68	0.00%	13.66	11.11%	54.41	0.92%	46.58	-1.06%	20.50	2.48%	20.25	-1.81%	14.29	3.60%	4.47	-2.70%
Alpaca	62.48	2.24%	12.05	3.19%	13.66	11.11%	54.41	0.92%	46.46	-1.32%	20.62	3.11%	20.12	-2.41%	14.16	2.70%	4.47	-2.70%
Lama	62.24	1.83%	12.05	3.19%	13.54	10.10%	54.41	0.92%	46.58	-1.06%	20.75	3.73%	20.12	-2.41%	14.04	1.80%	4.47	-2.70%
Domestic pig	61.37	0.41%	11.30	-3.19%	13.54	10.10%	54.04	0.23%	46.96	-0.26 %	21.61	8.07%	20.00	-3.01%	13.54	-1.80%	4.97	8.11%
Sheep	61.69	0.94%	12.56	7.58%	13.93	13.27%	55.97	3.82%	45.27	-3.84%	20.65	3.23%	19.53	-5.30%	14.30	3.73%	4.23	-7.99%
Goat	61.57	0.73%	12.44	6.52%	13.81	12.26%	55.85	3.58%	45.27	-3.84%	20.77	3.86%	19.53	-5.30%	14.30	3.73%	4.23	-7.99%
Taurine cattle	61.69	0.94%	12.44	6.52%	13.81	12.26%	55.85	3.58%	45.52	-3.31%	21.39	6.97%	19.65	-4.70%	14.43	4.63%	4.10	-10.70%
Zebu cattle	61.82	1.14%	12.44	6.52%	13.68	11.25%	55.72	3.35%	45.52	-3.31%	21.27	6.34%	19.78	-4.10%	14.43	4.63%	4.10	-10.70%
Chicken	62.38	2.06%	12.25	4.93%	13.37	8.69%	55.20	2.38%	47.77	1.47%	21.78	8.91%	18.94	-8.17%	14.23	3.22%	4.08	-11.14%
Duck	63.11	3.25%	11.68	0.00%	13.29	8.08%	54.66	1.38%	47.95	1.85%	21.61	8.07%	19.25	-6.63%	14.29	3.60%	4.35	-5.41%
Wild turkey	62.21	1.78%	12.14	4.00%	13.75	11.84%	55.02	2.05%	45.60	-3.14%	21.93	9.67%	19.95	-3.25%	14.25	3.35%	3.84	-16.42%

Note. Amino acids residues are denoted using the single-letter code.

RD – relative differences in amino acid composition of animal ACE2 receptors from human ACE2, %.

Conclusions. ACE2 is the SARS-CoV-2 receptor and plays a key role in the development of COVID-19. In this study were shown significant degrees of identity and similarity of ACE2 amino acid composition for human and various livestock species. Donkey and horse ACE2 are identical to human ACE2 by 87%, rabbit ACE2 – by 85.2%, alpaca, lama and dromedary – by about 83.4%, pig, sheep, goat – by about 81.7%, taurine and zebu cattle – by 81.1%. ACE2 of poultry species (chicken, duck, turkey) have less similarity to human ACE2 at about 65%.

The data obtained in this study are consistent with the results of previous experiments that detected the high ability of SARS-CoV-2 to interact with receptors on the cells of sheep, goat, cattle, pig, horse, rabbit, and low – with the receptors of bird species [22]. However, there is evidence of pig, chicken and duck resistance to SARS-CoV-2 by intranasal inoculation [23, 24]. Thus, pig is not susceptible to SARS-CoV-2 despite the high degree of similarity of its ACE2 to human ACE2, as well as the high ability of the virus to interact with pig ACE2. This fact makes it a priority to study in more detail both the virus-receptor interaction in pigs and other processes on which the development cycle of SARS-CoV-2 depends and which should occur for the development of infection in pigs. This may become the basis for identifying disease-blocking mechanisms even for the case when receptor interacts successfully.

Given the mutational variability of SARS-CoV-2 and the lack of large amount of experimental data on the possibility of disease in livestock, the risk of virus adaptation to livestock infecting remains high, so further studies of SARS-CoV-2 interactions with livestock remain relevant to prevent the new animal reservoirs of the virus.

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Одержано редколегією 20.09.2021 р.
Прийнято до друку 20.10.2021 р.