DOI: 10.34101/ACTAAGRAR/1/15207

Assessing animal species at risk for SARS-CoV-2 transmission: Bioinformatic analysis based on Angiotensin-Converting-Enzyme (ACE2) homology in edible and other animals

Veronica Lissette Recalde-Soliz[1*] – Fabio Marcelo Idrovo-Espin[2]

[1]Doctoral School of Nutrition and Food Science, University of Debrecen, Debrecen 4032 Hungary
[2]Biochemistry Laboratory CL2-1/FCQ, Universidad Central del Ecuador, Quito 170521 Ecuador
*Correspondence: vlrecalde@mailbox.unideb.hu

SUMMARY

A novel coronavirus called SARS-CoV-2 was detected in December 2019, leading to the COVID-19 pandemic that began in Wuhan, China. This virus is classified as severe acute respiratory syndrome SARS-CoV-2 due to its significant similarities with the SARS-CoV virus. Initially, bats were recognized as the primary animal hosts, but later research indicated that other animals could also serve as reservoirs, posing health risks, particularly for those species consumed by humans. SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE2) as its cellular receptor, utilizing the receptor-binding domain (RBD) of its spike protein, much like SARS-CoV does.

The study aimed to identify animals, particularly edible animals, that may be susceptible to infection by SARS-CoV-2. This was achieved through bioinformatics techniques, including alignment analysis of genomic sequences from selected animals, identity percentages comparison, and phylogenetic analysis based on the interaction between ACE2 and the receptor-binding domain (RBD) of SARS-CoV-2. This analysis identified rabbits, donkeys, alpacas, horses, wild boars, field rats, and monkeys as potentially susceptible edible animals. Additionally, primates were highlighted due to their close genetic resemblance to humans. Overall, 22 animals worldwide were identified as susceptible, marking them as possible reservoirs and hosts for the virus, emphasizing the need for vigilance around animals that humans may contact or consume.

Keywords: *SARS-CoV-2; ACE2; edible animals; bioinformatics; reservoirs*

INTRODUCTION

The World Health Organization (WHO) explains that zoonosis is an infectious disease that is transmitted from an animal to a human. Given that animals are of great importance due to their role as food or simply as pets and as part of the natural environment, zoonoses represent a public health problem. Currently, COVID-19 caused by the new coronavirus SARS-CoV-2 has been classified as a global pandemic, and it is of fundamental importance to determine the animals that may become potential hosts of the virus to control the spread of the disease, especially those that are a food source for the public.

In December 2019, the first cases of SARS-CoV-2 emerged in Wuhan, located in Hubei province, China. On January 31, 2020, WHO formally classified the outbreak as a Public Health Emergency of International Concern, highlighting the global implications of the outbreak. It was classified as high risk on February 29, 2020, and identified as a pandemic on March 11, 2020 (Dhama et al., 2020). To date, the disease has impacted people globally, resulting in over 200 million infections and more than four million deaths, with United States reporting the highest confirmed cases (World Health Organization, 2021).

According to Chen, Guo, Pan, and Zhao (2020), structural analysis of the receptor-binding domain (RBD) of the spike glycoprotein (S), which is crucial for the binding of the coronavirus virion to host cells, has shown a 72% similarity between SARS-CoV and SARS-CoV-2. Molecular modelling further suggests that the RBD of 2019-nCoV interacts more effectively with angiotensin-converting enzyme 2 (ACE2). The ACE2 protein is widely found across the animal kingdom, with conserved primary structures in species ranging from amphibians and fish to birds, reptiles, and mammals. This structural analysis indicates that the ACE2 in these animals may bind to the RBD of 2019-nCoV, suggesting that they could serve as potential natural hosts for the virus, alongside bats. It has also been reported that 2019-nCoV spreads through saliva droplets from an infected individual, which can be inhaled by others. However, since ACE2 is primarily expressed in the intestines, testicles, and renal pathways, fecal-oral transmission and other routes may also represent potential sources of infection, posing a risk to global health (Chen et al., 2020).

Since vaccines have not yet been administered to animals, there is a risk of interspecies transmission, and it also poses a danger to those who have not received the vaccine. It is necessary to investigate other possible hosts that could harbor the SARS-CoV-2 virus. Adding to this, the original carrier of the virus has not yet been confirmed, as there is a controversy between the bat as the original animal and the pangolin. Although, according to Alvarado-Ortiz & Idrovo-Espín (2020), the pangolin represents the zoonotic agent for SARS-CoV-2. Eventually, this could prevent the consumption and trade of other animal species that could become infected and be hosts of the virus.

This bioinformatics research focuses on identifying the ACE2 protein presence in animals, particularly those that are intended for human consumption. Additionally, the study analyzes the binding of SARS-CoV-2 receptor binding domain (RBD) to this protein

DOI: 10.34101/ACTAAGRAR/1/15207

to assess whether these animals could potentially serve as reservoirs for SARS-CoV-2.

MATERIALS AND METHODS

In this research, Bioedit, Mega X, and Blast Parser software programs were used. In addition to genetic sequences of animals obtained from NCBI and Ensembl.

Search for animal genetic codings

A comprehensive selection of animal species was made based on their relevance for human consumption across various continents, as well as their cultural or ecological significance in the wild. The selection criteria were informed by an extensive review of the literature, which highlighted the importance of these species in different regions and ecosystems. The major taxonomic groups, including mammals, birds, reptiles, amphibians, and fish, all belong to the vertebrates category, as they possess a backbone or spine. Using a purposive sampling approach, representative species from these groups were prioritized, with a focus on those with potential relevance to SARS-CoV-2 transmission. Sequences for these species were then retrieved from publicly accessible databases, such as NCBI and Ensembl, with a focus on acquiring high-quality, complete coding sequences (CDS). These sequences were subjected to filtering to exclude incomplete or low-quality data.

Alignment of biological sequences

Using the Bioedit editor, an alignment of animal sequences with a base sequence of the ACE2 protein in SARS COV-2 was performed.

Blast local

Using the bioinformatics program Blast Parser, the E values were obtained, with a threshold of 10^-7.

Identity percentages

The Bioedit editor, based on sequence alignment, allowed for the determination of identity percentages with respect to humans and other animals. For this analysis, an identity percentage equal to or greater than 80% was used as the acceptance limit to predict which animals could be infected with SARS-COV-2 based on their ACE2 sequences.

Development of a phylogenetic tree

The Mega X tool allowed us to obtain a phylogenetic tree with animal sequences from the database, with a bootstrap of one thousand subsamples for its respective models.

RESULTS AND DISCUSSION

E values of animal coding sequences

A total of 42 animal genetic sequences coding for ACE2 were analyzed, including sequences from animals intended for human consumption and others, categorized by kingdom group: mammals, birds, reptiles, and amphibians. Fish were excluded as they did not meet the ACE2 criteria. From these sequences, E values were chosen with an already established limit of 10-^7. *Figure 1* presents the E values obtained after the analysis of pair alignment or pairing of coding sequences (cds) of animals based on the sequence of ACE2 [*Homo sapiens* (human)], designated as the query sequence along with the number of proteins that meet the selection parameters and their respective coding. Of the 42 animal cds, 28 obtained an E value equal to zero highlighted in red as indicated in *Figure 1*, assigned to 1 protein, except those of the yak and the tiger where zero values were obtained for 2 proteins, the wolf for 5 proteins, and the mouse for 3 proteins, the remaining 16 cds fall within the analysis limit of E number 10-^7.

Open reading frames for ACE2 protein

Open reading frames require start and stop codons to be translated by ribosomes, leading to protein synthesis. This mechanism allows for the evolutionary development of receptors, such as ACE2 proteins, which can potentially bind to the SARS-CoV-2 virus. By searching for genetic sequences with the desired E value, according to the established analysis limit (10-7) and translating the nucleotide sequences into protein sequences, different open reading frame options were obtained for each sequence.

Complex formation of the SARS-CoV-2 receptor-binding domain with the ACE2 receptor

The spike (S) glycoprotein enables the virus to bind to the host cell. Structurally, it is a trimer that splits into two subunits, S1 and S2, upon infection. The RBD is located in the S1 subunit and contains the receptor-binding motif (RBM), which specifically targets the ACE2 receptor on host cells. Meanwhile, the S2 subunit manages membrane fusion. To gain entry into the host cell, the virus binds to ACE2, a receptor primarily found in the heart, lungs, kidneys, and intestines (Rosu, 2021).

The RBD of the virus is structured with a β-sheet composed of five antiparallel strands, β1, β2, β3, β4, and β7. This β-sheet is interconnected by helices and loops, forming the core structure of the RBD. Between strands β4 and β7, there is an extended region that includes short strands (β5 and β6) and helices (α4 and α5), which together constitute the RBM, which holds the critical amino acid residues responsible for interacting with ACE2 for binding. The outer, concave surface of the RBM accommodates the N-terminal helix of ACE2, resulting in a notable contact area of 1,687 Å² at the SARS-CoV-2 RBD–ACE2 interface. This interface comprises 864 Å² from the RBD and 823 Å² from ACE2. A similar interaction is observed in SARS-CoV, where the buried contact area is 1,699 Å², made up of contributions from the SARS-CoV RBD (869 Å²) and ACE2 (830 Å²) (Lan et al., 2020). Within this interface, 17 amino acids from the SARS-CoV-2 RBD form interactions with 20 amino acids on ACE2, maintaining a distance cutoff of 4 Å. Analyzing the SARS-CoV RBD-ACE2 interface shows 16 amino

DOI: 10.34101/ACTAAGRAR/1/15207

acids on the SARS-CoV RBD in contact with 20 on ACE2. Of the 20 ACE2 amino acids interacting with both RBDs, 17 are common to both, with most located in the N-terminal helix (Lan et al., 2020).

Figure 1. **E-Value and protein analysis of animal species**

	No.	General Name	Proteins No.	Code	E
MAMMALS	1	Panda	1	PAN	0
	2	Yak	2	YAK. 1	0
				YAK. 2	0
	3	Wolf	5	LOB. 1	0
				LOB. 2	0
				LOB. 3	0
				LOB. 4	0
				LOB. 5	3E-18
	4	Chinchilla	1	CHI	0
	5	Kangaroo Rat	1	CAG	0
	6	Western Gorilla	1	GOR	0
	7	Ground Squirrel	1	ARD	0
	8	African Elephant	1	ELE	0
	9	Golden Hamster	1	HAM	0
	10	Siberian Musk Deer	1	CIE	0
	11	Mouse	3	RAT. 1	0
				RAT. 2	0
				RAT. 3	0
	12	Platypus	1	ORN	3E-95
	13	Lion	1	LEO	0
	14	Tiger	2	TIG. 1	0
				TIG. 2	0
	15	Koala	5	KOA. 1	2E-83
				KOA. 2	2E-83
				KOA. 3	2E-83
				KOA. 4	2E-83
				KOA. 5	2E-83
	16	Sumatran Orangutan	1	ORA	0
	17	Meerkat	1	SUR	0
	18	Black Bear	1	OSO	0
	19	Cow	1	VAC	2E-22
	20	Dromedary	1	DRO	2E-22
	21	Goat	3	CAB. 1	3E-18
				CAB. 2	4E-17
				CAB. 3	3E-18
	22	Guinea Pig	1	CNI	1E-22
	23	Rabbit	1	CON	4E-27
	24	Donkey	2	BUR. 1	2E-22
MAMMALS		Donkey		BUR.2	2E- 22
	25	Horse	1	CAB	3E- 22
	26	Cat	2	GAT.1	2E-22
				GAT.2	2E-22
	27	Sheep	1	OVE	1E-22
	28	Greater Horseshoe Bat	1	MUR.1	4E-23
	29	Wild Boar	1	JAB	6E-23
	30	Alpaca	1	ALP	6E-23
	31	Chacoan Peccary	1	PEC	0
BIRDS	32	Common Hawk	1	GAV	4E-44
	33	Burrowing Owl	4	TEL. 1	8E-30
				TEL. 2	8E-30
				TEL. 3	8E-30
				TEL. 4	8E-30
	34	Japanese Quail	1	COD	2E-24
	35	Turkey	2	PAV. 1	3E-40
				PAV. 2	3E-40
	36	Blue-Necked Ostrich	6	AVE. 1	1E-25
				AVE. 2	1E-25
				AVE. 3	1E-25
				AVE. 4	1E-25
				AVE. 5	1E-25
				AVE. 6	1E-25
	37	Rooster	1	GALL	9E-18
REPTILES	38	Gray Short-Tailed Opossum	1	COL	3E-15
	39	American Chameleon	1	CAM	8E-60
	40	Snapping Turtle	1	TORT	2E-40
	41	White Lizard	1	LAG	1E-47
AMPHIBIANS	42	African Clawed Frog	10	RAN. 1	1E-21
				RAN. 2	1E-21
				RAN. 3	1E-21
				RAN. 4	1E-21
				RAN. 5	1E-21
				RAN. 6	1E-21
				RAN. 7	1E-21
				RAN. 8	1E-21
				RAN. 9	1E-21
				RAN. 10	1E-21

Notes: Animals were classified by kingdom group (mammals in blue, birds in yellow, reptiles in light green, and amphibians in dark green). Next to each animal, the E-values are listed along with the number and names of the analyzed proteins. Low E-values suggest a non-random alignment, and an E-value of zero (highlighted in red) indicates a highly significant match, showing that multiple hits are unlikely to be by chance.

Analysis of animal ACE2 sequences and their possible potential to bind SARS-CoV-2

The starting point was a comparison of amino acid residues with animal sequences, selecting as the initial residue, amino acid L79 of ACE2 based on the binding amino acids at the SARS-CoV-2 RBD –ACE2 interface according to Lan et al. (2020), with the segment obtained in the study after trimming the alignment between the ACE2 sequences of the animal and the ACE 2 query sequence of Homo Sapiens. (*Table 1)* presents the original location of the amino acid residues and the ones after trimming.

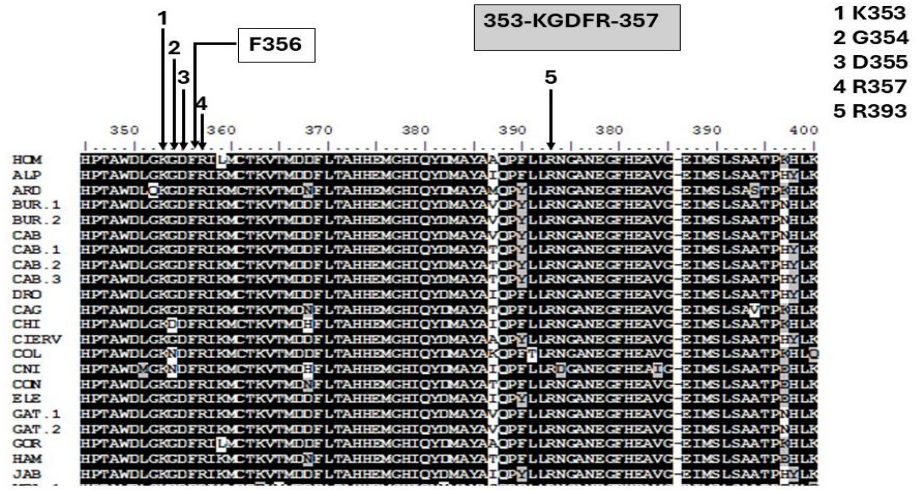
DOI: 10.34101/ACTAAGRAR/1/15207

Table 1. **Contact amino acid residues at the SARS-CoV-2 RBD-ACE2 interface**

Acid Residue without Trimming	Acid Residue with Trimming
L79	L19
M82	M22
Y83	Y23
N330	N306
K353	K329
G354	G330
D355	D331
R357	R333
R393	R373

Sequence alignment was carried out to identify amino acids that have binding capacity with SARS-CoV-2 RBD. Hayashi et al. (2020) found that multiple sequence alignments of ACE2 proteins across various species showed a high degree of identity, particularly conserving the amino acid sequence 353-KGDFR-357 in humans, dogs, cats, tigers, mink, and several other mammals. However, this sequence differed in snakes, which were analyzed separately. Consequently, the study concluded that ACE2 likely acts as a receptor for the SARS-CoV-2 spike glycoprotein in most mammals. According to Lan et al (2020), there are 20 amino acid residues of ACE2 that interact with the SARS-CoV-2 RBD, among which is the 353-KGDFR-357 series, except for residue F356. The block 345-400 as shown in *Figure 2* confirmed these studies and analyzed new animals, including mammals and one amphibian.

Figure 2. **Block Alignment (345-400) focused on common mammal's sequence**



Note: (a) The numbers correspond to specific residues indicated on the right side of the figure. The residue F356 is enclosed in a square to highlight its relevance, as it has been identified as a point of variation in previous studies. (b) The highlighted sequence 353-KGDFR-357 is noted as the key region of interest.

Identity percentages

The identity percentages of ACE2 sequences were analyzed, with an acceptance threshold set at 80% or higher. This threshold was used to predict which animals might be prone to SARS-CoV-2 infection based on the similarity of their ACE2 sequences. (*Table 2*) expresses the identity percentages of the selected animals from highest to lowest based on their closeness to 100%. In total, 22 animals were obtained that met this criterion, of which all are mammals. Within this group, primates stood out, with the highest value being the gorilla (99.1%), followed by the orangutan (97.7%), while the wild boar and bat presented the lowest values (80%). Some animals expressed more than one possibility of ACE 2 coding protein, which is part of a family of possible ACE2 genes and is the reason why 29 positions are observed. Those with this characteristic within (*Table 2)* were the donkey, tiger, cat, wolf and mouse, accompanied by their respective percentage of identity. The comparison made with the human ACE2 sequence revealed that animals belonging to the mammalian group have the highest values, compared to the subsequent groups of birds, reptiles and amphibians, which were discarded to be considered reservoirs of SARS-CoV-2.

Phylogenetic tree analysis

The importance of a phylogenetic tree lies in the topology, that is, how the branches are united or the branching pattern, which represents the evolutionary relationship between the different taxa (Mendoza-Revilla, 2012). In this study, it represents a parameter that strengthens the possibility of SARS-COV-2 infection, on an evolutionary basis due to the presence of ACE 2, the virus receptor. It is represented by the JTT matrix-based model and the Maximum Likelihood method, in *Figure 3.* The human immunodeficiency virus (HIV) was selected as an external group because it marks the beginning of the branching process, thereby indicating the most internal node shared by all, the root. An outgroup is a lineage that is closely related to, but not part of, the clade under investigation. (Mendoza-Revilla, 2012).

DOI: 10.34101/ACTAAGRAR/1/15207

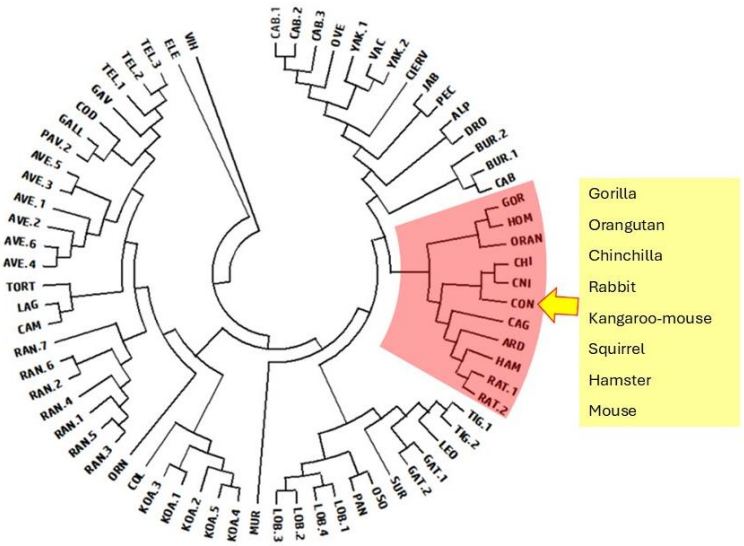
Table 2. **Identity percentages of animals with a predisposition to SARS-COV-2 infection**

N°	Animal	Protein Code	Identity Percentage
1	Western Gorilla	GOR	99.1%
2	Sumatran Orangutan	ORAN	97.7%
3	Donkey	BUR.1	86.6%
4	Horse	CAB	86.5%
5	Donkey	BUR.2	85.4%
6	Tiger	TIG.1	84.9%
7	Tiger	TIG.2	84.9%
8	Lion	LEO	84.6%
9	Squirrel	ARD	84.5%
10	Cat	GAT.2	84.5%
11	Cat	GAT.1	84 %
12	Rabbit	CON	83.8%
13	Wolf	LOB.4	83.7%
14	Wolf	LOB.1	83.4%
15	Wolf	LOB.3	83.4%
16	Chinchilla	CHI	83%
17	Wolf	LOB.2	83%
18	Black Bear	OSO	83%
19	Golden Hamster	HAM	82.7%
20	Panda	PAN	82.4%
21	Meerkat	SUR	82.2%
22	Alpaca	ALP	81.6%
23	Dromedary	DRO	81.4%
24	Kangaroo Rat	CAG	80.3%
25	Chacoan Peccary	PEC	80.1%
26	Mouse	RAT.1	80.1%
27	Mouse	RAT.2	80.1%
28	Wild Boar	JAB	80%
29	Greater Horseshoe Bat	MUR	80%

The more common ancestors two taxa share to the exclusion of others, the more closely related they are (Mendoza-Revilla, 2012). This is the case with primates and humans. It can be seen in *Figure 3* that they are directly related to their sister clades *Homo Sapiens* and the gorilla (*Gorilla gorilla)*, which is supported by their identity percentage of 99.1%. Likewise, the orangutan (*Pongo abelii*), which is closely related evolutionarily, has an identity percentage of 97.7%. Added to this is the great conservation of SARS-CoV-2 RBD-ACE2 binding amino acid residues, since in addition to belonging to the group of mammals, it conserves the largest number of amino acids under study.

Studies on SARS-CoV-2 infection in non-human primates, including *Rhesus* monkeys, long-tailed macaques (*Macaca fascicularis*), and vervet monkeys (*Chlorocebus pygerythrus*), have shown that some primate species can be infected by the virus and develop COVID-19-like symptoms similar to those seen in humans, including age-related effects. Acknowledging the potential risk that COVID-19 poses to non-human primates, the Great Apes section of the Primate Specialist Group within the IUCN (International Union for Conservation of Nature) issued guidelines advising researchers and caretakers on safety measures when interacting with great apes. Despite these precautions, the susceptibility of many primate taxa remains uncertain, suggesting that our closest living relatives may also be at risk of SARS-CoV-2 infection (Melin et al., 2020).

Figure 3. **Phylogeny of representative sequences of possible SARS-CoV-2 reservoirs from ACE2 sequences**



Notes: Phylogeny of representative sequences of possible SARS-CoV-2 reservoirs from ACE2 sequences. Evolutionary history was inferred using the Maximun Likelihood method and a matrix based on the JTT model (Jones et al. 1992). The analysis involved 71 amino a cid sequences with a total of 889 positions in the final data set. Evolutionary analysis was performed with MEGA X (Kumar et al. 2018).

The study highlights that rodents are a closely related group to the species being studied, with high identity percentages observed in squirrels (*Ictidomys tridecemlineatus*, 84.5%), rabbits (*Oryctolagus cuniculus*, 83.8%), chinchillas (*Chinchilla lanígera*, 83%), hamsters (*Mesocricetus auratus*, 82.7%),

DOI: 10.34101/ACTAAGRAR/1/15207

kangaroo rats (*Dipodomys ordii*, 80.3%), and mice (*Mus musculus*, 80.1%). Notably, Syrian hamsters (*Mesocricetus auratus*), could be consistently infected with SARS-CoV-2 and transmit the virus to other nearby animals, indicating that other rodent species might carry a similar risk (Imai et al., 2020).

Within the feline family, species such as tigers (*Panthera tigris*, 84.9%), lions (*Panthera leo*, 84.6%), and domestic cats (*Felis catus Linnaeus*, 84-84.5%) showed high identity percentages in ACE2, and studies have confirmed SARS-CoV-2 infections in tigers and lions, likely transmitted by human handlers. Domestic cats, particularly younger ones, are also susceptible to infection and can spread the virus to one another via airborne transmission (McAloose et al., 2020; Shi et al., 2020).

Other animals of interest include bears and wolves. American black bears (*Ursus americanus*, 83%) and pandas (*Ailuropoda melanoleuca*, 82.4%) show moderate identity percentages, while wolves (*Canis lupus*, 83–83.7%) share a close evolutionary relationship with domestic dogs. Research indicates that dogs are less susceptible to getting infected with SARS-CoV-2, therefore they do not spread the virus to other animals (Shi et al., 2020; Zhai et al., 2020).

The only marsupial analyzed, the meerkat *(Suricata suricatta*), showed a compatibility percentage of 82.2%. Although Luan et al. (2020a) indicated that the meerkat had a low probability of binding to the virus's S protein, their study focused on only five ACE2 amino acids. The sequence from the meerkat included two of those five residues. By expanding the range of interacting amino acids to nine in this research, it is suggested that the meerkat could be considered a potential host for the virus.

Camelids, represented by alpacas (*Vicugna pacos*, 81.6%) and dromedaries (*Camelus dromedarius*, 81.4%), are also evolutionarily close to the species of interest. Research indicates that SARS-CoV-2 can interact with the ACE2 receptors of various animals, including alpacas, suggesting potential susceptibility (Wu et al., 2020).

The phylogenetic analysis further placed donkeys (*Equus asinus asinus*, 86.6-85.4%) and horses (*Equus caballus*, 86.5%) as closely related, exhibiting high identity percentages, which may correlate with an increased likelihood of SARS-CoV-2 infection. Wu et al. (2020) suggested that within the *Perissodactyla* family, horses may interact with ACE2, while Damas et al. (2020) predicted, through in silico analysis, that donkeys could be potential hosts of SARS-CoV-2.

Wild boars (*Sus scrofa*, 80%) and Chacoan peccaries (*Catagonus wagneri*, 80.1%) cluster together in the phylogenetic tree, suggesting comparable infection risks based on their genetic similarity.

Finally, the Greater Horseshoe Bat (*Rhinolophus ferrumequinum*, 80%), analyzed from the family *Chiroptera*, confirmed the findings of Buonocore et al. (2020). Through *in silico* molecular docking of ACE2 from *Chiroptera* species with the SARS-CoV-2 spike (S) protein, the study identified *Rhinolophus ferrumequinum* as a bat species in Italy that may serve as a potential primary reservoir for the virus.

To summarize, nearly all mammalian species identified as vulnerable to SARS-CoV-2 infection, such as ferrets, exhibit mutations in several amino acids of their ACE2 proteins. This suggests that these species, especially those in frequent contact with humans, are prone to infection and may contribute to the emergence of new animal reservoirs. Species like gorillas, orangutans, chinchillas, rabbits, kangaroo rats, squirrels, hamsters, and rats carry a greater risk due to their evolutionary closeness to humans, as shown in *Figure 3*. In contrast, pigs (*Sus scrofa domesticus*) are not susceptible to SARS-CoV-2, despite their ACE2 proteins being able to serve as receptors for the virus (Zhai et al., 2020).

Other susceptibility studies based on statistical models, artificial intelligence, in vitro cell culture infections and in silico 3D structure models (ACE2-SARS-CoV-2 spike protein interactions) have systematically included non-human primates (especially *catarrhines*), bats (particularly *rhinolophids*), rodents, viverrids, and pholidates as potential hosts of SARS-CoV-2 (Melin et al., 2020 cited in Chaves et al., 2021).

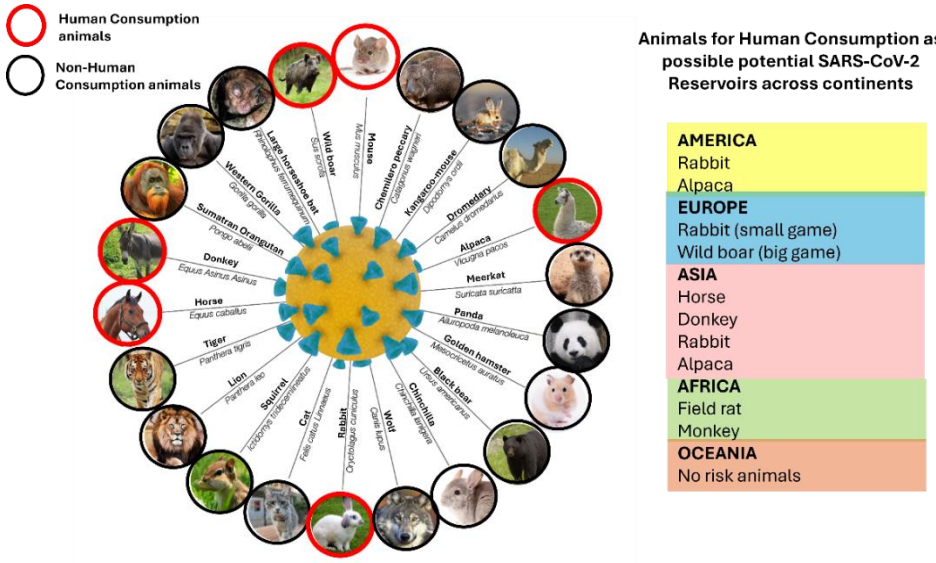
Investigations into the susceptibility of pigs, chickens, and ducks (*Anas platyrhynchos domesticus*) to SARS-CoV-2 using the viral RNA detection strategy by analyzing swabs collected from these animals that had been inoculated with the virus, showed that they were seronegative for SARS-CoV-2 when tested by ELISA. This demonstrated that pigs, chickens, and ducks are not susceptible to SARS-CoV-2 (Shi et al., 2020). This in turn, focusing on the results from chickens, extends to embryonated chicken eggs, which are a classic substrate for the isolation and propagation of a plethora of zoonotic viruses, posing no risk (Schlottau et al., 2020).

Regarding the reptile group, a study demonstrated that ACE2 in turtles (*Testudines*) and snakes (*Serpentes*) lost the ability to bind with the SARS-CoV-2 spike (S) protein, leading to their exclusion as potential hosts for the virus, along with birds. Additionally, since all known coronavirus hosts are homeothermic animals, reptiles were deemed unlikely to be infected by SARS-CoV-2. This conclusion is also applicable to amphibians (Luan et al., 2020b). An amino acid comparison analysis of the SARS-CoV-2-RBD and ACE2 junction further revealed low conservation of amino acid residues, reinforcing that these animal groups are not considered potential hosts for the virus.

According to this study, the animals that can contract the SARS-CoV-2 virus and potentially become hosts were 22. It is recommended to create an official list of animals that are possible reservoirs of the SARS-CoV-2 virus, including the Western gorilla, Sumatran orangutan, donkey, horse, tiger, lion, squirrel, cat, rabbit, wolf, chinchilla, American black bear, golden hamster, panda, meerkat, alpaca, dromedary, kangaroo mouse, Chacoan peccary, mouse, wild boar, and Greater horseshoe bat. These findings were based on sequence alignment, identity percentages, and phylogenetic analysis and it can be seen in *Figure 4*.

DOI: 10.34101/ACTAAGRAR/1/15207

Figure 4. **Global Assessment of possible potential SARS-CoV-2 reservoirs based on ACE2 similarity in human consumption animals and others**



Note: (a) The red circles represent animals intended for human consumption, while the black circles denote other animals. Both categories were identified as possible potential reservoirs for SARS-CoV-2. (b) The right side of the figure categorizes the animals for human consumption by continent.

Possible potential hosts of SARS-CoV-2 in human-consumed animals: A continental analysis

In most American countries, rabbits represent the primary animal species at risk of SARS-CoV-2 infection among those intended for human consumption. According to the Manual of Procedures for the Regulation and Control of Meat Products and By-products (Ministerio de Agricultura y Ganadería & Agrocalidad, 2020), the species permitted for slaughter and consumption include guinea pigs, rabbits, chicken, hens, turkeys, ducks, bovines, pigs, and sheep. Although this manual was created in Ecuador, it encompasses species consumed across the American continent, noting that rabbits and guinea pigs are more commonly consumed in Latin America, in contrast to the United States, where they are primarily regarded as pets. The manuals and regulations of other American countries are largely similar, with these exceptions being notable.

Animal products consumed in Europe include beef, pork, poultry, sheep, goats, fish, game meat, milk, dairy products, eggs and honey (European Commission, n.d-a). Game meat refers to terrestrial mammals and birds not considered domestic animals, excluding cattle, domestic pigs, sheep, goats, and various birds (European Commission, n.d-b). It is classified into large games, which include deer and wild boar, and small games, which include partridge, rabbit, and hare. Although the variety of animals may differ in other European countries, these are the most representative (Asociación Interprofesional de la Carne de Caza, n.d). According to the study, the animals in Europe that can be infected with SARS-CoV-2 and could function as possible hosts were rabbits (small game) and wild boar (large game).

In the context of the COVID-19 pandemic, which originated in China, the Ministry of Agriculture and Rural Affairs (MARA) issued a comprehensive list of 33 species of livestock and poultry approved for human consumption and various commercial applications. This initiative seeks to curtail illegal wildlife trade and the consumption of wild animals, thereby promoting public health and safety (MARA, 2020). This National Catalog of Genetic Resources is divided into three parts: the first includes 17 types of traditional livestock and birds such as goats, cows, pigs and chickens; the second, 16 types of livestock and special birds such as reindeer and ostriches; and the third lists species that can be raised but not consumed, such as mink and raccoons. These animals are common in the diet and husbandry in China and most Asian countries, except in India, where cows are considered sacred (Longe, 2018). Among the species mentioned, the horse and donkey stand out for representing a risk of SARS-CoV-2 infection.

The diet across the African continent primarily consists of grains, but it also includes a diverse range of protein sources, such as various meats from cattle, sheep, chickens, and goats. Typically, beef is reserved for holidays and special occasions. Fish is commonly eaten, particularly in coastal regions, while pork is mainly found in areas with non-Muslim populations due to Islamic influences. In these regions, the meat from wild animals, including field rats, antelope, and monkeys, is widely consumed. Additionally, giant snails are eaten in various parts of West Africa (Longe, 2018). This study identifies monkeys and field rats, both categorized as wild animals, as being at risk of hosting the SARS-CoV-2 virus. Although the specific species consumed are not detailed, monkeys, being part

DOI: 10.34101/ACTAAGRAR/1/15207

of the primate family, and rats, related to the mouse, could serve as potential reservoirs for the virus.

The Australian red meat sector involves the production and processing of sheep, cattle, goats, and buffalo aimed at both domestic consumption and international exports. Among these, cattle and sheep are the primary sectors. Kangaroo meat is also completely sustainably produced (Australian Government, n.d). Australia's products are representative of the other countries that comprise the continent of Oceania. According to this research, no animal for human consumption on this continent can be the host of the SARS-CoV-2 virus.

Insights into wildlife health and public safety implications

The findings regarding COVID-19 in animals have had significant implications for wildlife management, food safety policies, and zoonotic disease surveillance. As evidence of animal-to-human, human-to-animal and animal-to-animal transmission emerged (Centers for Disease Control and Prevention [CDC], 2022), it highlighted the need for enhanced monitoring of wildlife and livestock populations for emerging zoonotic diseases. In response, wildlife management strategies have been adapted to include more rigorous surveillance programs aimed at detecting novel viruses in animal populations.

For instance, the Collaborative Partnership on Sustainable Wildlife Management (CPW) with support from the Food and Agriculture Organization (FAO) published a report called Wildlife Management Conservation Risks: The COVID-19 Challenge: Zoonotic Diseases and Wildlife, which outlines four key principles to reduce zoonotic disease risks. These include preventing disease emergence by protecting habitats, regulating wildlife trade, and improving biosecurity; promoting the sustainable use of wildlife to support conservation and local livelihoods; adopting a One Health approach that integrates human, animal, and ecosystem health; and strengthening policies, legal frameworks, and global cooperation for better surveillance and regulation. These principles aim to mitigate future pandemics through sustainable wildlife management and coordinated action (FAO, 2020).

In the context of human-wildlife interaction, policies have increasingly focused on managing this relationship to safeguard public health, conserve biodiversity, and maintain ecological balance. These policies address critical issues such as wildlife trade, habitat protection, and the prevention of zoonotic disease transmission. Recognizing the significant risks posed by human-wildlife interactions, the World Health Organization (WHO), the World Organization for Animal Health (WOAH), and the United Nations Environment Programme (UNEP) have recommended suspending the trade of live wild mammals, known to carry a high risk of undetected infections, and closing high-risk market sections to mitigate the likelihood of outbreaks such as COVID-19. While traditional food markets, where live animals are handled and slaughtered, remain essential for millions of people, these measures aim to protect both workers and consumers from potential future health threats (European Parliamentary Research Service [EPRS], 2020).

The wildlife trade, though highly profitable, remains difficult to quantify and is regulated under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). The European Union (EU) and its Member States are signatories to CITES, which employs a permit system to ensure that trade in listed species is sustainable, legal, and traceable. However, illegal wildlife trade continues to pose a significant challenge. In response, the EU launched an action plan to combat wildlife trafficking in 2016, initially set to run until 2020, which is currently under evaluation. The European Parliament has expressed support for extending and strengthening these measures to enhance enforcement and address ongoing challenges in wildlife trafficking (European Parliamentary Research Service [EPRS], 2020).

Additionally, food safety policies have been reviewed and strengthened, to prevent potential cross-species transmission. The U.S. Department of Agriculture (USDA, 2021) allocated $300 million from the American Rescue Plan Act to enhance surveillance for SARS-CoV-2 and other zoonotic diseases in animals. Led by the Animal and Plant Health Inspection Service (APHIS), this initiative aimed to build an early warning system to detect potential threats and enable public health partners to take swift action in preventing future pandemics and aiming to enhance biosecurity measures in live animal markets and farms. Furthermore, the World Wildlife Fund (WWF, 2020) focused on addressing high-risk wildlife trade and markets by prioritizing the closure of dangerous markets, particularly in urban areas, which sell wildlife for human consumption. Its goal was to crack down on the illegal wildlife trade that bypasses health and veterinary checks, including the online sale of illegally sourced animals and products, as well as combating misinformation that fuels anti-wildlife sentiment linked to COVID-19.

The European Food Safety Authority (EFSA, 2020) recognized that SARS-CoV-2 can infect several animal species, such as American mink, raccoon dogs, cats, ferrets, hamsters, and white-tailed deer, with mink being the most likely to spread the virus on farms. In the EU, mink farm outbreaks decreased from 44 in 2021 to 6 in 2022. While companion animals like cats and ferrets can be infected, it was established that they do not significantly impact human transmission. However, it recommended disposing of human waste and avoiding contact with sick or dead wildlife, but did not recommend wildlife monitoring, except for testing hunter-harvested animals that show symptoms or are found dead. For example, bats, being natural hosts for many coronaviruses, should be monitored.

Regarding international collaboration, Gallo-Cajiao et al. (2023) mentioned there was an inadequate global governance of the wildlife trade as a major risk factor for zoonotic disease spillover and future pandemics. They mentioned that existing regulations, such as

DOI: 10.34101/ACTAAGRAR/1/15207

CITES, primarily focused on conservation and trade legality but failed to address public health risks, allowing high-risk wildlife trade to persist due to weak enforcement. In addition, they recognized poor collaboration between health, environmental, and trade sectors, resulting in fragmented efforts to prevent zoonotic spillover. Concerning wildlife trade monitoring, they noted there was no global system that can detect early warning signs of disease outbreaks. To address governance gaps, they proposed strengthening international regulations by integrating public health into agreements like CITES and improving enforcement. They also call for intersectoral collaboration between health, environmental, and trade organizations to develop cohesive wildlife trade strategies, and the implementation of risk-based policies for high-risk species and trade practices, along with global surveillance systems.

However, amid ongoing global efforts to combat zoonotic diseases, the latest initiative focusing on Southeast Asia is the United Nations Office on Drugs and Crime's (UNODC, 2021) SAFE (Strengthening Anti-Microbial Resistance and Food Safety) project. Launched in response to the persistent risks posed by the illegal wildlife trade, this initiative is the most recent step in addressing the region's vulnerabilities. In July 2024, experts from the Association of Southeast Asian Nations (ASEAN) countries, the European Union, and the One Health community convened to develop strategies for safe and sustainable wildlife trade. Their discussions underscored the need for stronger inter-agency coordination and the crucial role of criminal justice in preventing future pandemics. Assessments of over 150 human-wildlife interaction sites identified key legal and operational gaps, shaping what is now the region's most up-to-date strategy for mitigating zoonotic disease risks.

CONCLUSIONS

In this study, we identified 22 animal species as vulnerable to SARS-CoV-2 infection based on sequence alignment analysis, percentage identity comparisons, and phylogenetic analysis. These methods highlighted the presence of ACE2 and its interaction with the viral RBD, indicating that homologous ACE2 sequences in these animals can bind to the SARS-CoV-2 spike glycoprotein and potentially facilitate infection. While bioinformatics offers strong predictive power, the study acknowledges that experimental validation, such as in vitro binding assays, is necessary to confirm these findings.

The animals that exposed the risk of infection to SARS-CoV-2 for human consumption and could represent a possible reservoir of the virus, were the rabbit of national importance and from some countries in America and Europe. Donkey, alpaca and horse which are consumed in Asian countries. Game animals like wild boar, along with the rabbit on the European continent. Species of monkeys and field rats stood out in Africa. Other animals analyzed within this study that could also contract the SARS-CoV-2 virus and become possible potential hosts of the virus were: the Western gorilla, Sumatran orangutan, donkey, horse, tiger, lion, squirrel, cat, rabbit, wolf, chinchilla, American black bear, golden hamster, panda, meerkat, alpaca, dromedary, kangaroo mouse, Chacoan peccary, mouse, wild boar and Greater horseshoe bat.

Mammals obtained the highest degree of similarity both in genomic sequences and in percentages of identity, unlike other groups in the animal kingdom. And since they represent the largest number of hosts of the virus and exhibit the characteristics of homeothermic animals, they ruled out reptiles and amphibians as possible hosts of SARS-CoV-2.

The comparative analysis of the ACE2 protein sequences of animal species and humans indicated that primates were the most susceptible to being infected by the SARS-CoV-2 virus, due to their great evolutionary similarity with humans and its conservation of amino acid residues that interfere with SARS-CoV-2 RBD-ACE-2 binding.

The highest percentages of identity, to determine the similarity of ACE2 genomic sequences, were obtained by primates, specifically the gorilla and the orangutan, and, therefore, they represent the riskiest animals to become reservoirs of the virus.

The bird group exhibited low conservation of amino acids in the SARS-CoV-2 RBD-ACE2 interaction and low identity percentages. Furthermore, considering the evolutionary distance from humans, this group of potential hosts was excluded, as they are not considered a risk for virus transmission, despite the fact that many birds are consumed by humans. This conclusion also extends to reptiles and amphibians, as all known coronavirus hosts are homeothermic animals, a fact confirmed by this study.

Future research should focus on identifying and monitoring potential animal reservoirs of SARS-CoV-2 to better understand interspecies transmission dynamics. This includes conducting comprehensive surveillance studies on at-risk species, assessing their susceptibility to the virus, and evaluating the potential for spillover events. Additionally, research should explore the effectiveness of vaccination programs for animals, particularly those in close contact with humans or at risk of extinction, to mitigate viral transmission and protect biodiversity.

The ongoing research into COVID-19 in animals has highlighted critical challenges and opportunities in wildlife management, food safety policies, and zoonotic disease surveillance. The latest efforts, such as the UNODC's SAFE project and regional strategies developed by ASEAN countries, EU and One Health community emphasize the growing need for global collaboration and stronger enforcement in wildlife trade and disease monitoring. Despite progress, gaps in governance and weak enforcement persist, underscoring the importance of a unified, risk-based approach to monitoring high-risk species and trade practices. Continued investment in surveillance and international cooperation is essential to prevent future pandemics and protect both wildlife and public health.

DOI: 10.34101/ACTAAGRAR/1/15207

REFERENCES

Alvarado-Ortiz, J.; Meneses Olmedo, L.; Idrovo-Espín, F. (2020): Origen probable y transmisión entre especies del SARS-CoV-2. *infoANALÍT, 8*(1). https://doi.org/10.26807/ia.v8i1.142

Asociación Interprofesional de la Carne de Caza. (n.d.). Disfruta de la carne más noble. Carne de caza silvestre de España. Available online: https://www.carnesilvestreuropea.com/carne-silvestre-europea.html (September 13, 2021)

Australian Government. Department of Agriculture, Water and the Environment. Red meat - Department of Agriculture. Red Meat. (n.d.). Available online: https://www.agriculture.gov.au/ag-farm-food/meat-wool-dairy/red-meat-livestock (September 13, 2021)

Buonocore, M. et al. (2020): New putative animal reservoirs of SARS-COV-2 in Italian fauna: A bioinformatic approach, *Heliyon*, *6*(11). doi:10.1016/j.heliyon.2020.e05430.

Centers for Disease Control and Prevention. (2022, February 10). COVID-19 and animals. Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/daily-life-coping/animals.html

Chaves, A.; Montecino-Latorre, D.; Alcázar, P.; Suzán, G. (2021): Wildlife rehabilitation centers as a potential source of transmission of SARS-CoV-2 into native wildlife of Latin America. *Biotropica*, *53*(4). 987–993. https://doi.org/10.1111/btp.12965

Chen, Y.; Guo, Y.; Pan, Y.; Zhao, Z.J. (2020): Structure analysis of the receptor binding of 2019-nCoV. *Biochem. Biophys. Res. Commun*, *525(1)*, 135–140. https://doi.org/10.1016/j.bbrc.2020.02.071

Damas, J. et al. (2020): Broad host range of SARS-COV-2 predicted by comparative and structural analysis of ACE2 in vertebrates. *PNAS*, *117*(36). 22311–22322. doi:10.1073/pnas.2010146117.

Dhama, K.; Patel, S.K.; Sharun, K.; Pathak, M.; Tiwari, R.; Yatoo, M.I.; Malik, Y.S.; Sah, R.; Rabaan, A.A.; Panwar, P.K.; Singh, K.P.; Michalak, I.; Chaicumpa, W.; Martinez-Pulgarin, D.F.; Bonilla-Aldana, D.K.; Rodriguez-Morales, A.J. (2020): SARS-CoV-2 jumping the species barrier: Zoonotic lessons from SARS, MERS and recent advances to combat this pandemic virus. *Travel Med. Infect. Dis*, *37*, 101830. https://doi.org/10.1016/j.tmaid.2020.101830

European Comission (n.d-a). Animales y productos animales. Available online: https://ec.europa.eu/info/food-farming-fisheries/animals-and-animal-products_es (September 13, 2021)

European Comission (n.d-b). Game meat. Food Safety. Available online: https://ec.europa.eu/food/animals/animals-products-trade-imports/game-meat_en (September 13, 2021)

European Food Safety Authority (2020): Scientific opinion on the animal health and welfare aspects of the trade in live wild animals for human consumption. *EFSA Journal*, *18*(11), 7822. https://www.efsa.europa.eu/en/efsajournal/pub/7822

European Parliamentary Research Service (2020): The COVID-19 pandemic and its impact on the EU economy (EPRS Briefing No. 649409). European Parliament. https://www.europarl.europa.eu/RegData/etudes/BRIE/2020/649409/EPRS_BRI%282020%29649409_EN.pdf

Food and Agriculture Organization of the United Nations (2020): The COVID-19 challenge: Zoonotic diseases and wildlife (Joint CPW Statement). https://openknowledge.fao.org/server/api/core/bitstreams/3cc5ad00-91c6-420f-8d39-04abf532a342/content (April 13, 2025)

Gallo-Cajiao, E. et al. (2023):'Global governance for pandemic prevention and the Wildlife Trade', *The Lancet Planetary Health*, *7*(4). doi:10.1016/s2542-5196(23)00029-3

Hayashi, T.; Abiko, K.; Mandai, M.; Yaegashi, N.; Konishi, I. (2020): Highly conserved binding region of ACE2 as a receptor for SARS-CoV-2 between humans and mammals. *Vet. Q., 40(1)*, 243–249. https://doi.org/10.1080/01652176.2020.1823522

Imai, M.; Iwatsuki-Horimoto, K.; Hatta, M.; Loeber, S.; Halfmann, P.J.; Nakajima, N.; Watanabe, T.; Ujie, M.; Takahashi, K.; Ito, M.; Yamada, S.; Fan, S.; Chiba, S.; Kuroda, M.; Guan, L.; Takada, K.; Armbrust, T.; Balogh, A.; Furusawa, Y.; . . . Kawaoka, Y. (2020): Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development. *Proc. Natl. Acad. Sci*., 202009799. https://doi.org/10.1073/pnas.2009799117

Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. (2018): MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol. Biol*, *35*, 1547–1549.

Lan, J.; Ge, J.; Yu, J.; Shan, S.; Zhou, H.; Fan, S.; Zhang, Q.; Shi, X.; Wang, Q.; Zhang, L.; Wang, X. (2020): Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature*, *581*(7807), 215–220. https://doi.org/10.1038/s41586-020-2180-5

Longe, J.L. (2018): *The Gale Encyclopedia of Environmental Health*. 1st ed. Gale a Cengage Company.

Luan, J.; Jin, X.; Lu, Y.; Zhang, L. (2020a): SARS-CoV-2 spike protein favors ACE2 from Bovidae and Cricetidae. *J. Med. Virol., 92*(9), 1649–1656. https://doi.org/10.1002/jmv.25817

Luan, J.; Lu, Y.; Jin, X.; Zhang, L. (2020b): Spike protein recognition of mammalian ACE2 predicts the host range and an optimized ACE2 for SARS-CoV-2 infection. *Biochem. Biophys. Res. Commun*, *526*(1), 165–169. https://doi.org/10.1016/j.bbrc.2020.03.047

McAloose, D.; Laverack, M.; Wang, L.; Killian, M.L.; Caserta, L.C.; Yuan, F.; Mitchell, P.K.; Queen, K.; Mauldin, M.R.; Cronk, B.D.; Bartlett, S.L.; Sykes, J.M.; Zec, S.; Stokol, T.; Ingerman, K.; Delaney, M.A.; Fredrickson, R.; Ivančić, M.; Jenkins-Moore, M., . . . Diel, D.G. (2020): From People to Panthera: Natural SARS-CoV-2 Infection in Tigers and Lions at the Bronx Zoo. *mBio*, *11*(5). https://doi.org/10.1128/mbio.02220-20

Melin, A.D.; Janiak, M.C.; Marrone, F.; Arora, P.S.; Higham, J.P. (2020): Comparative ACE2 variation and primate COVID-19 risk. *Commun. Biol., 3*(1). https://doi.org/10.1038/s42003-020-01370-w

Mendoza-Revilla, J. (2012): Aportes de la filogenética a la investigación médica. *Rev. Méd. Herediana*, *23*(2), 119. https://doi.org/10.20453/rmh.v23i2.1042

Ministerio de Agricultura y Ganadería & Agrocalidad (2020): *Manual de procedimientos para la regulación y control de origen de productos y subproductos cárnicos en estado primario destinados a consumo humano (N.o 1)*. Inocuidad de Alimentos.

MARA. Ministry of Agriculture and Rural Affairs of the People's Republic of China (2020): '农 业农村部关于《国家畜禽遗传资源目录》公开征求意见的通知'. Available at: http://www.moa.gov.cn/hd/zqyj/202004/t20200408_6341067.htm (September 13, 2021)

Rosu, I. (2021): Resumen de los avances de la vacuna frente a SARS-CoV-2. *Farmacéuticos Comunitarios*, *13*(1), 60–64. https://doi.org/10.33620/FC.2173-9218.(2021/Vol13).001.07

DOI: 10.34101/ACTAAGRAR/1/15207

Schlottau, K.; Rissmann, M.; Graaf, A.; Schön, J.; Sehl, J.; Wylezich, C.; Höper, D.; Mettenleiter, T.C.; Balkema-Buschmann, A.; Harder, T.; Grund, C.; Hoffmann, D.; Breithaupt, A.; Beer, M. (2020): SARS-CoV-2 in fruit bats, ferrets, pigs, and chickens: an experimental transmission study. *The Lancet Microbe*, *1*(5), e218–e225. https://doi.org/10.1016/s2666-5247(20)30089-6

Shi, J.; Wen, Z.; Zhong, G.; Yang, H.; Wang, C.; Huang, B.; Liu, R.; He, X.; Shuai, L.; Sun, Z.; Zhao, Y.; Liu, P.; Liang, L.; Cui, P.; Wang, J.; Zhang, X.; Guan, Y.; Tan, W.; Wu, G.; . . . Bu, Z. (2020): Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS–coronavirus 2. *Science*, *368*(6494), 1016–1020. https://doi.org/10.1126/science.abb7015

United Nations Office on Drugs and Crime (2021, July 20). Doing nothing is not an option. United Nations Office on Drugs and Crime. https://www.unodc.org/unodc/en/environment-climate/webstories/safe-doing-nothing-is-not-an-option.html

U.S. Department of Agriculture (2021, August 24). USDA announces proposed framework for advancing surveillance of SARS-CoV-2 and other emerging zoonotic diseases. U.S. Department of Agriculture. https://www.usda.gov/about-usda/news/press-releases/2021/08/24/usda-announces-proposed-framework-advancing-surveillance-sars-cov-2-and-other-emerging-zoonotic

World Health Organization (2021): Coronavirus disease (COVID-19). World Health Organization. https://www.who.int/emergencies/diseases/novel-coronavirus-2019 (September 7, 2021)

World Wildlife Fund (2020): COVID-19 and wildlife trade: Perspectives and proposed actions. World Wildlife Fund. https://www.worldwildlife.org/pages/covid-19-and-wildlife-trade-perspectives-and-proposed-actions

Wu, L.; Chen, Q.; Liu, K.; Wang, J.; Han, P.; Zhang, Y.; Hu, Y.; Meng, Y.; Pan, X.; Qiao, C.; Tian, S.; Du, P.; Song, H.; Shi, W.; Qi, J.; Wang, H.W.; Yan, J.; Gao, G.F.; Wang, Q. (2020): Broad host range of SARS-CoV-2 and the molecular basis for SARS-CoV-2 binding to cat ACE2 *Cell Discov*, *6*(1). https://doi.org/10.1038/s41421-020-00210-9

Zhai, X.; Sun, J.; Yan, Z.; Zhang, J.; Zhao, J.; Zhao, Z.; Gao, Q.; He, W.T.; Veit, M.; Su, S. (2020): Comparison of Severe Acute Respiratory Syndrome Coronavirus 2 Spike Protein Binding to ACE2 Receptors from Human, Pets, Farm Animals, and Putative Intermediate Hosts. *Virol. J.*, *94*(15). https://doi.org/10.1128/jvi.00831-20