Predicting susceptibility for SARS-CoV-2 infection in domestic and wildlife animals using ACE2 protein sequence homology

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Abstract
The article is presenting a bioinformatics based method predicting susceptibility for SARS-CoV-2 infection in domestic and wildlife animals. Recently, there were reports of cats and ferrets, dogs, minks, golden hamster, rhesus monkeys, tigers, and lions testing for SARS-CoV-2 RNA which indicated for the possible interspecies viral transmission. Our method successfully predicted the susceptibility of these animals for contracting SARS-CoV-2 infection. This method can be used as a screening tool for guiding viral RNA testing for domestic and wildlife animals at risk of getting COVID-19. We provide a list of the animals at risk of developing COVID-19 based on the susceptibility score.

KEYWORDS
ACE2, COVID-19, pets, SARS-CoV-2, susceptibility, wildlife

1 | INTRODUCTION
The ongoing epidemic of COVID-19 is a global concern for human health, but its impact on domestic and wildlife animals is largely un-evaluated. The epidemic was believed to have started from the wet wildlife market in Wuhan in the People’s Republic of China, but until now, no study has located the actual source of the virus. Genomic analyses suggest that bat is the most likely origin of this virus (Zhou et al., 2020). The virus has been detected from the pangolins, but there is no concrete evidence that this animal can act as an intermediate host (Lam et al., 2020). Currently, very limited data is available that evaluates the susceptibility of the domestic and wildlife stocks for SARS-CoV-2 infection using laboratory testing. There have been reports for positive tests for SARS-CoV-2 RNA in cats and ferrets, dogs, minks, golden hamster, rhesus monkey, tigers, and lions (Imai et al., 2020; Kim et al., 2020; Shi et al., 2020; United States Department of Agriculture [USDA], 2020; Yu et al., 2020). Cats do show disease symptoms and transmit the infection to other animals of their species (Shi et al., 2020). In all the cases of pet or zoo animals testing positive, the source of infection was either confirmed or suspected to be a human (American Veterinary Medical Association, 2020).

SARS-CoV-2 infection in humans depends on the binding of its spike protein to a human cell receptor angiotensin-converting enzyme 2 (ACE2) which is expressed across the animal species as a cell surface receptor (Bibiana et al., 2020). Evolutionary conservation of protein sequence of ACE2 across vertebrate species, and more specifically, in hominids and primates, remains strong plausibility (Braun et al., 2020). Recent studies have decoded the receptor-binding domain (RBD) of SARS-CoV-2 spike protein and demonstrated its structural binding with the N-terminal peptidase domain of human ACE2 (hACE2). Receptor-binding motif (RBM) on SARS-CoV-2 RBD makes direct contacts with amino acid residues at hACE2 (Lan et al., 2020; Wan et al., 2020). We assumed that a homology to hACE2, more particularly to viral RBD-binding interface, of ACE2 protein sequences of animal species could be used in predicting their susceptibility for contracting SARS-CoV-2 infection.
2 | MATERIALS AND METHODS

The complete protein sequence of hACE2 (Q9BYF1.2) was retrieved from the open-access protein sequence data base Uniprot.org. Crystal structures of hACE2 and SARS-CoV-2 RBD were created based on X-RAY diffraction data retrieved from RCSB protein data bank, PBD1D-6M0J (https://www.rcsb.org/structure/6m0j) using software: PyMOL built from Schrodinger, Inc. Amino acid residues at hACE2/RBD interface, and conserved RBD-binding hotspots at hACE2, were annotated in consultation with published literature (Lan et al., 2020; Othman et al., 2020; Wan et al., 2020).

Further, the NCBI protein blast tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp) was used to analyze open-access protein sequence data of ACE2 for the species in animal kingdom available at NCBI databases. To minimize the prediction error, a two-step homology search was performed. First, we performed a comparative analysis of the variability of hACE2 with that of wildlife and domestic animal species in complete protein sequences. The accession numbers of the species which showed significant homology (E value ≤ 0) for the complete sequence were selected for further analysis for the second step. In the second step, we narrowed down our homology search to a subrange of 36–53 amino acid residues of hACE2 (AEDLFYQSSLAWNYNTN), which contain conserved hotspots for binding of SARS-CoV-2 RBD (Othman et al., 2020).

A fixed query cover of 100% was applied for the sequence homology match in the second analysis. The final ACE2 sequence homology scores along with the statistical significance value (E value ≤ 1E-08) were used to determine the susceptibility ranking of the animal for contracting SARS-CoV-2 infection. A higher sequence homology score with E-value closure to zero signified higher susceptibility ranking for the species. Details of NCBI protein blast and statistical methods predicting significance can be consulted at https://www.ncbi.nlm.nih.gov/books/NBK20261/.

3 | RESULTS

Figure 1 presents crystal structures of hACE2 and SARS-CoV-2 RBD (1a), and shows annotations for conserved RBD-binding hotspots (1b) and other interacting amino acids (1c) and at the ACE2/RBD interface. Significant alignments of the species-specific ACE2 orthologous to hACE2 (Accession: Q9BYF1.2) for the complete sequence (Table S1) and a partial sequence at the RBD interface (a subrange of 36–53 amino acid residues) (Figure 1b and Table 1) were noted across the animal kingdom. In the search for complete sequence homology as well as a subrange of 36–53 amino acid residues at the RBD interface, hominids and other primates showed the highest homology (up to 100%) with hACE2, followed by...
| Description                                      | Order/Family      | Query cover (%) | E value  | Percentage identity | Accession no.      | Susceptibility ranking |
|--------------------------------------------------|-------------------|-----------------|----------|---------------------|--------------------|------------------------|
| ACE2 ([Gorilla gorilla][Gorilla])                 | Primate/Hominidae | 100             | 2E−16    | 100                 | XP_018874749.1     | 1                      |
| ACE2 isoform X1 ([Pan troglodytes][Chimpanzee])  | Primate/Hominidae | 100             | 2E−16    | 100                 | XP_016798468.1     | 2                      |
| ACE2 isoform X1 ([Pan paniscus][Bonobo])         | Primate/Hominidae | 100             | 2E−16    | 100                 | XP_024096013.1     | 4                      |
| ACE2 isoform X1 ([Pongoabelii][Sumatran orangutan]) | Primate/Hominidae | 100             | 2E−16    | 100                 | XP_008972428.1     | 3                      |
| ACE2 ([Hylobatesmoloch][Silvery gibbon])         | Primate/Hylobatidae | 100             | 2E−16    | 100                 | XP_032612508.1     | 5                      |
| ACE2 ([Nomascus leucogenys][Northern white-cheeked gibbon]) | Primate/Hylobatidae | 100             | 2E−16    | 100                 | XP_003261132.2     | 6                      |
| ACE2 ([Pilocolobus tephrosceles][Ugandan red colobus]) | Primate/Cercopithecidae | 100             | 2E−16    | 100                 | XP_025227847.1     | 7                      |
| ACE2 ([Rhinopithecus roxellana][Golden snub-nosed monkey]) | Primate/Cercopithecidae | 100             | 2E−16    | 100                 | XP_010364367.2     | 8                      |
| ACE2 ([Theropithecus gelada][Gelada])            | Primate/Cercopithecidae | 100             | 2E−16    | 100                 | XP_021788732.1     | 9                      |
| ACE2 ([Papioanubis][Olive baboon])               | Primate/Hominidae | 100             | 2E−16    | 100                 | XP_011733505.1     | 10                     |
| ACE2 ([Macaca nemestrina][Southern pig-tailed macaque]) | Primate/Cercopithecidae | 100             | 2E−16    | 100                 | XP_011733505.1     | 11                     |
| ACE2 ([Macaca mulatta][Rhesus macaque])          | Primate/Cercopithecidae | 100             | 2E−16    | 100                 | XP_005593094.1     | 12                     |
| PREDICTED: ACE2 ([Macaca fascicularis][Crab-eating macaque]) | Primate/Cercopithecidae | 100             | 2E−16    | 100                 | XP_005593094.1     | 13                     |
| PREDICTED: ACE2 ([Cercocebus atys][Sooty mangabey]) | Primate/Cercopithecidae | 100             | 2E−16    | 100                 | XP_018911198.1     | 14                     |
| PREDICTED: ACE2 ([Mandrillus sphinx][Mandrill])  | Primate/Cercopithecidae | 100             | 2E−16    | 100                 | XP_018859231.1     | 15                     |
| PREDICTED: ACE2 ([Chlorocebus sabaeg][Green monkey]) | Primate/Cercopithecidae | 100             | 2E−16    | 100                 | XP_007989304.1     | 16                     |
| ACE2 ([Ursusarctos horribilis][Grizzly bear])    | Carnivora/Ursidae | 100             | 2E−15    | 94.44               | XP_026338651.1     | 17                     |
| PREDICTED: ACE2 ([Alluropa melanoleuca][Giant panda]) | Carnivora/Ursidae | 100             | 2E−15    | 94.44               | XP_002930657.1     | 18                     |
| PREDICTED: ACE2 ([Ursus maritimus][Polar bear])  | Carnivora/Ursidae | 100             | 2E−15    | 94.44               | XP_008694637.1     | 19                     |
| PREDICTED: ACE2 isoform X1 ([Chinchilla lanigera][Long-tailed chinchilla]) | Rodentia/Chinchillidae | 100            | 3E−14    | 94.44               | XP_013362428.1     | 20                     |
| ACE2 ([Heterocephalus glaber][Naked mole-rat])   | Rodentia/Heterocephalidae | 100            | 3E−14    | 94.44               | XP_004866157.1     | 21                     |
| ACE2 ([Cameleusferus][Wild Bactrian camel])       | Artiodactyla/Camelidae | 100            | 3E−14    | 94.44               | XP_006194263.1     | 22                     |
| PREDICTED: ACE2 ([Jaculus jaculus][Lesser Egyptian jerboa]) | Rodentia/Dipodidae | 100            | 3E−14    | 94.44               | XP_004671523.1     | 23                     |
| PREDICTED: ACE2 isoform X1 X1 [Dipodomys ordii][Ord’s kangaroo rat]) | Rodentia/Heteromyidae | 100            | 3E−14    | 94.44               | XP_018887572.1     | 24                     |
| ACE2 ([Physeter catodon][Sperm whale])            | Artiodactyla/Physeteridae | 100            | 3E−14    | 94.44               | XP_023971279.1     | 25                     |
| ACE2 ([Lipotes vexillifer][baiji])                | Artiodactyla/Lipotidae | 100            | 3E−14    | 94.44               | XP_007466389.1     | 26                     |
| ACE2 ([Octodon degus][Common degu])               | Rodentia/Octodontidae | 100            | 3E−13    | 88.89               | XP_023575315.1     | 27                     |

(Continues)
| Description                                      | Order/Family               | Query cover (%) | E value | Percentage identity | Accession no. | Susceptibility ranking |
|--------------------------------------------------|----------------------------|-----------------|---------|----------------------|---------------|------------------------|
| ACE2 (Eumetopias jubatus [Steller sea lion])     | Carnivora/Otaridae        | 100             | 3E-13   | 88.89                | XP_027970822.1 | 28                     |
| PREDICTED: ACE2 (Fukomys damarensis [Damara land Mole-rat]) | Rodentia/Bathyergidae     | 100             | 3E-13   | 88.89                | XP_010643477.1 | 29                     |
| ACE2 (Puma concolor [Cougar])                    | Carnivora/Felidae         | 100             | 3E-13   | 88.89                | XP_025790417.1 | 30                     |
| PREDICTED: ACE2 isoform X1 (Pantherapardus [Leopard]) | Carnivora/Felidae         | 100             | 3E-13   | 88.89                | XP_019273508.1 | 31                     |
| ACE2 isoform X1 (Acinonyx jubatus [Cheetah])     | Carnivora/Felidae         | 100             | 3E-13   | 88.89                | XP_026910297.1 | 32                     |
| ACE2 precursor (Felis catus [Cat])               | Carnivora/Felidae         | 100             | 3E-13   | 88.89                | XP_001034545.1 | 33                     |
| ACE2 (Lynx pardinus [Iberian lynx])              | Carnivora/Felidae         | 100             | 3E-13   | 88.89                | XP_017505746.1 | 34                     |
| ACE2 (Lynx canadensis [Canada lynx])             | Carnivora/Felidae         | 100             | 3E-13   | 88.89                | XP_030160839.1 | 35                     |
| ACE2 (Mesocricetus auratus [Golden hamster])    | Rodentia/Cricetidae       | 100             | 3E-13   | 88.89                | XP_005074266.1 | 36                     |
| ACE2 (Cricetulus griseus [Chinese hamster])     | Rodentia/Cricetidae       | 100             | 3E-13   | 88.89                | XP_003503283.1 | 37                     |
| ACE2 (Manis javanica [Sunda pangolin])          | Pholidota/Manidae         | 100             | 3E-13   | 88.89                | XP_017505746.1 | 38                     |
| ACE2 (Peromyscus bairdii [North American deer mouse]) | Rodentia/Cricetidae       | 100             | 3E-13   | 88.89                | XP_006973269.1 | 39                     |
| ACE2 (Microtus ochrogaster [Prairie vole])      | Rodentia/Cricetidae       | 100             | 3E-3    | 88.89                | XP_005358818.1 | 40                     |
| PREDICTED: ACE2 (Panthera tigris (Tiger))       | Carnivora/Felidae         | 100             | 3E-13   | 88.89                | XP_007090142.1 | 41                     |
| PREDICTED: ACE2 (Panthera leo [lion])           | Carnivora/Felidae         | 100             | 3E-13   | 88.89                | Query_32935   | 42                     |
| ACE2 (Balaenaoptera acutorostrata [Minke whale]) | Cetartiodactyla/           | 100             | 1E-12   | 88.89                | XP_028020351.1 | 43                     |
| PREDICTED: ACE2 (Saimiri boliviensi [Black-capped squirrel monkey]) | Primate/Cebidae           | 100             | 1E-12   | 83.33                | XP_010334925.1 | 44                     |
| ACE2 (Sapajus apella [Tufted capuchin])        | Primate/Cebidae           | 100             | 1E-12   | 83.33                | XP_032141854.1 | 45                     |
| PREDICTED: ACE2 (Cebus capucinus [Panamanian White-faced Capuchin]) | Primate/Cebidae           | 100             | 1E-12   | 83.33                | XP_017367865.1 | 46                     |
| ACE2 (Aotus nancymaae [Nancy Ma’s night monkey]) | Primate/Aotidae           | 100             | 1E-12   | 83.33                | XP_012290105.1 | 47                     |
| ACE2 (Callithrix jacchus [Common marmoset])     | Primate/Callitrichidae    | 100             | 1E-12   | 83.33                | XP_008987241.1 | 48                     |
| ACE2 (Carlito syrichta [Philippine tarsier])   | Primate/Tarsiidae         | 100             | 1E-12   | 83.33                | XP_008062810.1 | 49                     |
| PREDICTED: ACE2 (Propliopithecus coquereli [Coquerel’s sifaka]) | Primate/Indriidae         | 100             | 2E-12   | 83.33                | XP_012494185.1 | 50                     |
| PREDICTED: ACE2 (Oryctolagus cuniculus [European rabbit]) | Lagomorpha/Leporidae     | 100             | 2E-12   | 83.33                | XP_002719891.1 | 51                     |
| ACE2 (Mustela aurina [Stoat])                   | Carnivora/Mustelidae      | 100             | 2E-12   | 83.33                | XP_032187677.1 | 52                     |
| ACE2 (Mustela putorius furo [European domestic ferret]) | Carnivora/Mustelidae      | 100             | 2E-12   | 83.33                | Q2WG88        | 53                     |
### TABLE 1  (Continued)

| Description                          | Order/Family          | Query cover (%) | E value | Percentage identity | Accession no. | Susceptibility ranking |
|--------------------------------------|-----------------------|-----------------|---------|---------------------|---------------|------------------------|
| PREDICTED: ACE2 (Ceratotherium simum [White rhinoceros]) | Perissodactyla/Rhinocerotidae | 100             | 4E-12   | 83.33               | XP_004435206.1 | 54                     |
| ACE2 (Vicugnapacos [Alpaca])          | Cetartiodactyla/Camelidae | 100             | 6E-12   | 88.89               | XP_006212709.1 | 55                     |
| ACE2 Isoform XI (Canis lupus familiaris [Dog])b | Carnivora/Canidae     | 100             | 6E-12   | 83.33               | XP_005641049.1 | 56                     |
| PREDICTED: ACE2 (Marmota marmota [Alpine marmot]) | Rodentia/Sciuridae   | 100             | 2E-11   | 88.89               | XP_015343540.1 | 57                     |
| ACE2 (Marmota flaviventris [Yellow-bellied marmot]) | Rodentia/Sciuridae   | 100             | 2E-11   | 88.89               | XP_027802308.1 | 58                     |
| PREDICTED: ACE2 (Ochotona princeps [American pika]) | Lagomorpha/Ochotonidae | 100             | 2E-11   | 77.78               | XP_004597549.2 | 59                     |
| ACE2 (Paguma larvata [Masked palm civet]) | Carnivora/Viverridae  | 100             | 6E-11   | 77.78               | Q56NL1.1      | 60                     |
| ACE2 (Ictidomys tridecemlineatus [Thirteen-lined ground squirrel]) | Rodentia/Sciuridae | 100             | 1E-10   | 83.33               | XP_005316051.3 | 61                     |
| ACE2 isoform X1 (Myotis lucifugus [Little brown bat]) | Chiroptera/Vespertilionidae | 100             | 2E-10   | 77.78               | XP_023609437.1 | 62                     |
| PREDICTED: ACE2 (Equus przewalskii [Przewalski's horse]) | Perissodactyla/Equidae | 100             | 3E-10   | 77.78               | XP_008542995.1 | 63                     |
| ACE2 (Equus caballus [Horse])         | Perissodactyla/Equidae | 100             | 3E-10   | 77.78               | XP_001490241.1 | 64                     |
| ACE2 enzyme (Crocuta crocuta [Spotted hyena]) | Carnivora/Hyaenidae  | 100             | 6E-09   | 72.22               | KAF0878287.1  | 65                     |
| ACE2 isoform X1 (Urocitellus parryii [Arctic ground squirrel]) | Rodentia/Sciuridae | 100             | 1E-08   | 77.78               | XP_026252505.1 | 66                     |

Note: hACE2 Accession no.: Q9BYF1.2; query subrange: 36–53aa; subject subrange: 36–53aa.

Species/Family compared: Primates (taxid: 9443), cat family (taxid: 9681), Equus (taxid: 9789), ferret (taxid: 9669), dog, coyote, wolf, fox (taxid: 9608), elephants (taxid: 9779), oxen, cattle (taxid: 9903), water buffalo (taxid: 89462), goats (taxid: 9925), pigs (taxid: 9821), deer (taxid: 9850), lion (taxid: 9689), Ursidae (taxid: 9632), Camelidae (taxid: 9635), carnivores (taxid: 33554), Mammalia (taxid: 40674), Rodents and rabbits (taxid: 314147), house mouse (taxid: 10090), birds (taxid: 8782), fishes (taxid: 7898), snakes (taxid: 8570), mouse (taxid: 10088), bats (taxid: 9397).

*SARS-CoV-2 RNA testing positive (Imai et al., 2020; Kim et al., 2020; Shi et al., 2020; United States Department of Agriculture [USDA], 2020; Yu et al., 2020).*

*ACE2 protein sequence for Panthera leo (Lion) is not available in NCBI data base. A predicted ACE2 sequence has been referred from Alexander et al. (2020).*
carnivores (up to 94.4%), rodents (up to 94.4%), and artiodactyles (even-toed ungulates) (up to 94.4%). Oxen, water buffaloes, goats, pigs, birds, fishes, and snakes showed no significant match in either of the searches. Sequence homology scores and susceptibility ranking (for contracting SARS-CoV-2 infection) for the animal species have been provided in the columns for “Percentage Identity” and “Susceptibility Ranking” in Table 1. The animals which have been described positive for the SARS-CoV-2 RNA in the recent literature have been marked (Table 1, footnote b). ACE2 sequence alignment data for the complete and partial sequences have been provided as Supporting Information, Files 2 and 3.

4 | DISCUSSION

Zoonotic spillover of human infectious diseases is not uncommon. Recent reports of SARS-CoV-2 infection and COVID-19 symptoms in selected wildlife and domestic animals warranted for caution that the epidemic can have possible spread in animal settings like zoos and wildlife sanctuaries, including a threat of viral transmission from domestic animals (CDC, 2020; Tiwari et al., 2020; Yoo & Yoo, 2020). The species-specific protein sequence homology to hACE2 may be a valuable tool for predicting susceptibility for contracting SARS-CoV-2 infection. It can be used as a screening tool for guiding viral RNA-based laboratory testing to contain COVID-19 spread in wildlife and domestic animals during an epidemic outbreak.

Currently, polymerase chain reaction (PCR)-based laboratory testing for SARS-CoV-2 RNA is a standard practice for detecting viral infection and development of COVID-19 in humans. However, viral infectivity studies involving animals, especially wildlife, are very limited for now. We used open-access bioinformatics tools to predict susceptibility for SARS-CoV-2 infection in domestic and wildlife animals. For this purpose, we performed a comparative analysis of the variability of complete protein sequence and SARS-CoV-2 RBD-binding hotspots of hACE2 with that of the animal species. On the basis of the sequence homology, we predicted the highest susceptibility for hominids and other primates, followed by carnivores, rodents, and artiodactyles (ungulates), however, sequence match varied widely among the species in any order of the animal kingdom. A similar pattern of susceptibility prediction was recently reported by Damas et al., (2020) who compared ACE2 sequence homology across species, however, their species list varied from us, which can be explained by differences of data sources. Damas et al., (2020) also noted conservation of ACE2 protein sequence among evolutionarily linked species; this has been reflected in our study also, as we find the highest sequence homology to hACE2 in the members of order hominids and primate who are closest to humans, and further across mammals, and other categories of vertebrates. A lesser sequence homology was found with the species which are remotely related to humans.

Our susceptibility predictions for cats, dogs, golden hamster, rhesus monkey, tigers, and lions get confirmation from the recent reports in literature which had performed PCR-based laboratory testing for SARS-CoV-2 RNA in the selective animals (Table 1, footnote b) (Imai et al., 2020; Kim et al., 2020; Shi et al., 2020; United States Department of Agriculture USDA, 2020; Yu et al., 2020). However, a viral RNA-based susceptibility information is currently highly limited, especially for the animals inhabiting in natural settings.

For certain species, such as American mink and ferret, which was recently reported to get SARS-CoV-2 infection (Shi et al., 2020; USDA, 2020); although we found their significant homology to hACE2 (Table S1), we couldn’t get homology score for SARS-CoV-2 RBD-binding hotspots in absence of complete protein sequence of ACE2 for these species. However, we found significant homology for SARS-CoV-2 RBD-binding hotspots for the species which are closely related to them, such as, European mink and stoat for which the complete protein sequences for ACE2 were available (Table 1).

Our two-step comparison for sequence homology (for complete sequence and SARS-CoV-2 RBD-binding hotspots, respectively) has reduced the chances of false inclusions and provides a susceptibility rank which may help to identify the most vulnerable animal species for contracting SARS-CoV-2 infection. Many of the wild animals noted in the list, such as giant panda, white rhinoceros, are endangered, which raises immediate concern to protect them from the reach of the COVID-19 pandemic. Laboratory testing of viral RNA for wildlife animals in zoos and sanctuaries is limited by many practical factors, in that sense software-based tool to predict COVID-19 susceptibility in the animals presents a very easy and cheap option for COVID-19 prevention and containment in these settings. However, there remains a need to further confirm their susceptibility through laboratory testing of viral RNA.

4.1 | Limitations

In the present study, we considered only viral host cell entry receptor ACE2 homology for predicting the susceptibility, however, SARS-CoV-2 host cell entry and replication, in addition, may also depend on many other factors like tissue proteases TMPRSS2 or CTSL, and ADAM-17 (Zhang et al., 2020), thus, a hACE2 homology may not be sufficient, and a viral infectivity testing of the listed animals will be necessary to confirm their susceptibility for contracting SARS-CoV-2 infection. Also, some animals may not develop clinical symptoms or transmit the virus even after contracting SARS-CoV-2 infection, hence their identification through viral RNA testing accompanied by clinical observation will be necessary to understand their susceptibility risk.

Additionally, our susceptibility prediction list (Table 1) has only provided an assessment for the animal species for which ACE2 protein sequence (more particularly from amino acid residues 36–53) is available in the NCBI data base (except Panthera leo, for which ACE2 sequence has been referred from Alexander et al., 2020). However, this tool can be suitably used to screen remaining animals if their ACE2 protein sequence is made available.
CONFLICT OF INTERESTS
The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT
Sequence alignment data generated in this study have been provided as Supporting Information Files 2 and 3. ACE2 protein sequences used for this study can be retrieved from NCBI protein (https://www.ncbi.nlm.nih.gov/protein) and Uniprot.org (https://www.uniprot.org/) databases using species-specific accession numbers provided in Tables 1 and S1.

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REFERENCES
American Veterinary Medical Association. (2020). SARS-CoV-2 in animals. AVMA. https://www.avma.org/resources-tools/animal-health-and-welfare/covid-19-sars-cov-2-animals-including-pets

Alexander, M. R., Schoeder, C. T., Brown, J. A., Smart, C. D., Moth, C., Wikswo, J. P., Capra, J. A., Meiler, J., Chen, W., & Madhur, M. S. (2020). Which animals are at risk? Predicting species susceptibility to Covid-19. bioRxiv: the preprint server for biology. https://doi.org/10.1101/2020.07.19.94563

Braun, M., Sharon, E., Unterman, I., Miller, M., Shtern, A. M., Benenson, S., Vainstein, A., & Tabach, Y. (2020). ACE2 co-evolutionary pattern suggests targets for pharmaceutical intervention in the COVID-19 pandemic. iScience, 23(8), 101384. https://doi.org/10.1016/j.isci.2020.101384

Bibiana, S. O. F., Vargas-Pinilla, P., Amorim, C., Sortica, V. A., & Bortolini, M. C. (2020). ACE2 diversity in placental mammals reveals the evolutionary strategy of SARS-CoV-2. Genetics and Molecular Biology, 43(2), e20200104. https://doi.org/10.1590/1678-4685-gmb-2020-0104

CDC. (2020). COVID-19 and animals. https://www.cdc.gov/coronavirus/2019-ncov/daily-life-coping/animals.html

Dam, J., Hughes, G. M., Keough, K. C., Painter, C. A., Persky, N. S., Corbo, M., Miller, M., Koepfli, K. P., Pfenning, A. R., Zhao, H., Genereux, D. P., Swofford, R., Pollard, K. S., Ryder, O. A., Nweeia, M. T., Lindblad-Toh, K., Teeling, E. C., Karlsson, E. K., & Lewin, H. A. (2020). Broad host range of SARS-CoV-2 predicted by comparative and structural analysis of ACE2 in vertebrates. Proceedings of the National Academy of Sciences of the United States of America, 117(36), 22311–22322. https://doi.org/10.1073/pnas.201046117

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., ... Kawaoa, Y. (2020). Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development. Proceedings of the National Academy of Sciences of the United States of America, 117(28), 16587–16595. https://doi.org/10.1073/pnas.2009799117

Kim, Y. I., Kim, S. G., Kim, S. M., Kim, E. H., Park, S. J., Yu, K. M., Chang, J. H., Kim, E. J., Lee, S., Casel, M. A. B., Um, J., Song, M. S., Jeong, H. W., Lai, V. D., Kim, Y., Chin, B. S., Park, J. S., Chung, K. H., Foo, S. S., ... Choi, Y. K. (2020). Infection and Rapid Transmission of SARS-CoV-2 in Ferrets. Cell Host and Microbe, 27(5), 704–709. https://doi.org/10.1016/j.chom.2020.03.023

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

Lam, T. T. Y., Shum, M. H. H., Zhu, H. C., Tong, Y. G., Ni, X. B., Liao, Y. S., & Guan, Y. (2020). Identifying SARS-CoV-2 related coronaviruses in Malayan pangolins. Nature, 1–4. https://doi.org/10.1038/s41586-020-2169-0

Othman, B., Boulama, Z., Brandenburg, J. T., Da Rocha, J., Hamdi, Y., Ghedira, K., Srairi-Abid, N., & Hazehurst, S. (2020). Interaction of the spike protein RBD from SARS-CoV-2 with ACE2: similarity with SARS-CoV, hot-spot analysis and effect of the receptor polymorphism. Biochemical and Biophysical Research Communications, 527(3), 702–708.

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 (New York, N.Y.), 368(6494), 1016–1020. https://doi.org/10.1126/science.abb7015

Tiwari, R., Dhama, K., Sharun, K., Iqbal Yatoo, M., Malik, Y. S., Singh, R., Michalak, I., Sah, R., Bonilla-Aldana, D. K., & Rodriguez-Moreales, A. J. (2020). COVID-19: animals, veterinary and zoonotic links. Veterinary Quarterly, 40(1), 169–182. https://doi.org/10.1080/01652176.2020.1766725

United States Department of Agriculture (USDA). (2020, September). Confirmed cases of SARS-CoV-2 in Animals in the United States. USDA. https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/sa_one_health/sars-cov-2-animals-us

Wan, Y., Shang, J., Graham, R., Baric, R. S., & Li, F. (2020). Receptor recognition by the novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS coronavirus. Journal of Virology, 94(7), e00127–e00220. https://doi.org/10.1128/jvi.00127-20

Yoo, H. S., & Yoo, D. (2020). COVID-19 and veterinarians for one health, zoonotic- and reverse-zoonotic transmissions. Journal of Veterinary Science, 21(3), e51. https://doi.org/10.4142/jvs.2020.21.e51

Yu, P., Qi, F., Xu, Y., Li, F., Liu, P., Liu, J., Bao, L., Deng, W., Gao, H., Xiang, Z., Xiao, C., Lv, Q., Gong, S., Liu, J., Song, Z., Qu, Y., Xue, J., Wei, Q., Liu, M., ... Qin, C. (2020). Age-related rhesus macaque models of COVID-19. Animal Models and Experimental Medicine, 3(1), 93–97. https://doi.org/10.1002/am2.12108

Zhou, P., Yang, X. L., Wang, X. G., Hu, B., Zhang, L., Zhang, W., Si, H. R., Zhu, Y., Li, B., Huang, C. L., Chen, H. D., Chen, J., Luo, Y., Guo, H., Jiang, R. D., Liu, M. Q., Chen, Y., Shen, X. R., Wang, X., ... Shi, Z. L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature, 579(7798), 270–273. https://doi.org/10.1038/s41586-020-2122-7

Zhang, H., Rostami, M. R., Leopold, P. L., Mezey, J. G., O’Beirne, S. L., Strulovici-Barel, Y., & Crystal, R. G. (2020). Expression of the SARS-CoV-2 ACE2 receptor in the human airway epithelium. American Journal of Respiratory and Critical Care Medicine, 202(2), 219–229. https://doi.org/10.1164/rccm.202003-0541OC

SUPPORTING INFORMATION
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