Molecular phylogeny of coronaviruses and host receptors among domestic and close-contact animals reveals subgenome-level conservation, crossover, and divergence

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Abstract

Background: Coronaviruses have the potential to cross species barriers. To learn the molecular intersections among the most common coronaviruses of domestic and close-contact animals, we analyzed representative coronavirus genera infecting mouse, rat, rabbit, dog, cat, cattle, white-tailed deer, swine, ferret, mink, alpaca, Rhinolophus bat, dolphin, whale, chicken, duck and turkey hosts; reference or complete genome sequences were available for most of these coronavirus genera. Protein sequence alignments and phylogenetic trees were built for the spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins. The host receptors and enzymes aminopeptidase N (APN), angiotensin converting enzyme 2 (ACE2), sialic acid synthase (SAS), transmembrane serine protease 2 (TMPRSS2), dipeptidyl peptidase 4 (DPP4), cathepsin L (and its analogs) and furin were also compared.

Results: Overall, the S, E, M, and N proteins segregated according to their viral genera (α, β, or γ), but the S proteins of alphacoronaviruses lacked conservation of phylogeny. Interestingly, the unique polybasic furin cleavage motif found in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) but not in severe acute respiratory syndrome coronavirus (SARS-CoV) or Middle East respiratory syndrome coronavirus (MERS-CoV) exists in several β-coronaviruses and a few α- or γ-coronaviruses. Receptors and enzymes retained host species-dependent relationships with one another. Among the hosts, critical ACE2 residues essential for SARS-CoV-2 spike protein binding were most conserved in white-tailed deer and cattle.

Conclusion: The polybasic furin cleavage motif found in several β- and other coronaviruses of animals points to the existence of an intermediate host for SARS-CoV-2, and it also offers a counternarrative to the theory of a laboratory-engineered virus. Generally, the S proteins of coronaviruses show crossovers of phylogenies indicative of recombination events. Additionally, the consistency in the segregation of viral proteins of the MERS-like coronavirus (NC_034440.1) from pipistrelle bat supports its classification as a β-coronavirus. Finally, similarities in host enzymes...
Background
The ongoing pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has led to unprecedented interest in the study of coronaviruses, although the first coronavirus was identified in the 1930s [1]. Given the most recent outbreaks by SARS-CoV-2, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), the potential for zoonoses and reverse zoonoses, in conjunction with viral, host and environmental factors, have elevated the need to identify the origin, transmission, pathogenesis and control of and novel therapeutic and preventive strategies against these viruses.

The first two-thirds of the coronavirus genome encodes proteins needed for replication, and the remaining one-third encodes accessory and structural proteins, which include hemagglutinin esterase (HE) (present in only Group 2 coronaviruses), spike (S), envelope (E), membrane (M) and nucleocapsid (N) [2, 3]. Previously grouped based on serology, coronaviruses are now categorized into α (alpha), β (beta), γ (gamma) and δ (delta) groups using genetics. Coronaviruses affect many host species ranging from mammals to birds [3, 4].

Cross-species coronavirus infections have been reported among humans and animals. The SARS-CoV epidemic in 2002, for example, appears to have jumped from bats to infect palm civets, then to raccoon dogs, Chinese ferret badgers and finally into the human population [5]. In domestic animals, the most likely recent interspecies transmission leading to an outbreak may be that of canine respiratory coronavirus discovered in 2003 [6]. In Africa, a recent study by Burimuah et al. also showed that the close association of ruminants can lead to a spillover of coronaviruses from cattle to other small ruminants [7]. A high degree of sequence identity among some canine, bovine and human β-coronaviruses [2, 5, 8] strongly suggests that coronaviruses in close-contact environments could recombine or jump the species barrier to initiate emerging infections with sustained transmissions in new hosts. In the last two decades, SARS-CoV-2 has been the third zoonotic coronavirus to originate from animals and cause a pandemic in humans. Although the intermediate host for SARS-CoV-2 has not yet been identified [9, 10], recent comparative molecular analysis indicates that the pangolin and bats harbor the closest relative viruses [11–14].

With the current public availability of whole-genome sequences and additional tools for the analysis of both proteome and genome data, it is now possible to examine viruses and other microbes in great detail even before experimental validations. In this study, we analyzed the phylogenetic relationships among selected coronaviruses that infect domestic and close-contact animals to sketch the subgenomic relationships among the viruses. The molecular phylogeny of coronavirus proteins (spike, envelope, membrane and nucleocapsid), putative host-cell receptors and key functional domains of host enzymes were compared.

Results
Viruses of the same genera may form variable clades at subgenomic levels
Overall, viral S, E, M, and N proteins clustered together according to the viral genera groups (Fig. 1 A-D), although intragroup discordances were evident. For alpaca respiratory α-coronavirus, the S protein shared the same origin as that of the porcine epidemic diarrhea (PED) virus, but distinct from its clade, the E protein shared a distant origin with β-coronaviruses (Fig. 1B). Among β-coronaviruses, proteins from canine respiratory, bovine, rabbit, and white-tailed deer coronaviruses were closely related. The E, M, and N proteins from transmissible gastroenteritis (TGE) virus, canine coronavirus (also referred to as canine enteric coronavirus) and PED virus showed close phylogeny with each other, while the S protein showed discordance. Feline coronavirus (strain UU11) and feline infectious peritonitis (FIP) virus E, M, and N proteins were closely related (Fig. 1 B-D). However, the phylogeny of the S protein from FIP virus was distant from that of feline coronavirus (strain UU11) (Fig. 1 A). To test for possible past recombination events that may have led to the crossover phylogeny between viruses of unrelated host species, we performed a SimPlot analysis of the entire genomes of canine coronavirus, PED virus, TGE virus and alpaca respiratory coronavirus, holding the canine coronavirus sequence as a query against the other three. The results showed that both TGE- and PED-coronaviruses maintained 70-98% similarity with the canine coronavirus along the genome region (approximately 20,000 bases) proximal to the segment coding for the S protein. However, in the S gene segment, the PED virus showed no similarity to either...
canine coronavirus or TGE virus. TGE virus regained 90-96% similarity to canine coronavirus after a drop of the curve in the proximal region of the S gene (Fig. 1E). The alpaca respiratory coronavirus shared very limited similarity with the canine coronavirus between the proximal 10,000 and 20,000 bases but was similar to the PED virus between the 23,000 and 25,000 base marks corresponding to the spike gene segment. This suggests that the S gene segments of some coronaviruses may originate from other viruses via recombination, leading to divergent subgenome phylogeny among proteins of the same virus.

**The polybasic furin cleavage motif in SARS-CoV-2 is present in several β-coronaviruses**

In SARS-CoV-2, the S protein contains a polybasic furin cleavage site with the motif RRAR (where R and A are arginine and alanine residues, respectively). This motif is present in the murine β-coronavirus but absent in SARS-CoV, while MERS-CoV has an RSVR sequence at approximately the same location. This cleavage site, located at the junction of the S1/S2 subdomains, specifically has an arginine residue at the second position (RRAR), which is essential for efficient cleavage of the S protein [15]. We show that this furin cleavage motif RRXR (where X is arginine) is present in SARS-CoV-2, as well as in several other β-coronaviruses. This motif may play a crucial role in the infection and spread of these viruses, as it allows for efficient cleavage of the S protein and viral entry into host cells.
an alanine residue or another amino acid) is present in several β-coronaviruses and the avian infectious bronchitis virus (a γ-coronavirus) as shown in Fig. 2. Notably, a variation in the sequence is also evident in other S proteins, such as RVGR in a β-coronavirus of bat, RSRR in an α-coronavirus of cat, and RKRR in a γ-coronavirus of turkey. Therefore, this motif or its mutant variants exist in natural coronaviruses belonging to different groups.

The TMPRSS2 cleavage site on the viral S protein and the catalytic residues of the host TMPRSS2 enzyme are conserved

The TMPRSS2 cleavage site of the SARS-CoV-2 S protein is well conserved among all viruses studied, although the residues flanking the cleavage site vary widely (Fig. 3A). TMPRSS2 is expressed in tissues of the aerodigestive tract of humans [16], and it is important for initiating SARS-CoV-2 infection by processing the S protein to release fusion peptides for membrane fusion [17]. On the host TMPRSS2 enzyme, we found both the substrate binding site and the triad of catalytic residues (histidine (H) 296, aspartic acid (D) 345 and serine (S) 441) located in the active site to be well conserved among all host species as shown in Fig. 3B and C, respectively.

Comparison of host ACE2, TMPRSS2, APN SAS, DPP4, cathepsin L and furin proteins

Phylogenetic analysis of host enzymes and receptors was performed. TMPRSS2, SAS, APN, DPP4, cathepsin L (and its analogs) and furin showed high similarity among mammals, with distinct separation from those of birds. An overall species-based segregation pattern was observed for the various host enzymes and receptors, except for the ACE2 enzyme, where bat, rabbit and beluga whale ACE2 proteins were distantly related to proteins of other mammals and birds (Fig. 4A-G). Unlike the phylogenies of host species cathepsin and furin, which generally followed species relationship patterns, the phylogeny of DPP4 was rather peculiar in that proteins of cattle and white-tailed deer or beluga whale and bottlenose dolphin were very distantly related. On the other hand, cathepsins of dog and cattle were in the same clade (Fig. 4 E-G). Upon analyzing both the required and critical residues of ACE2 needed for SARS-CoV-2 S protein binding, the cattle and white-tailed deer proteins shared the most similarity scores with humans, followed closely by the dolphin and pig proteins and then by the cat, alpaca and dog proteins (Fig. 4H).

Discussion

We compared the protein-level phylogenetic relationships among common coronaviruses of domestic and close-contact animals and key infection-associated proteins in the hosts. In this report, we highlight that the polybasic furin cleavage site found in SARS-CoV-2, but not in SARS-CoV or MERS-CoV [15], exists in several β-coronaviruses included in this report, although the

Fig. 2 Polybasic furin cleavage motif of SARS-CoV-2 is present in other coronaviruses. The protein sequence of the furin cleavage site within the S protein, with polybasic amino acid residues conserved among some coronaviruses, is shown. The residues constituting the RRXR motif (where R is an arginine residue and X is another amino acid) are marked with rectangles. The exact RRAR configuration of this motif in SARS-CoV-2 is present in the murine coronavirus but absent in others, including the bat virus and MERS-CoV. The RRXR motif is reversed in others such as the rabbit HKU14 coronavirus and turkey coronavirus. Amino acids are listed in single letter code
configuration varied in some of those. For example, in
the murine coronavirus (accession no. ACN89705.1),
the exact RRAR motif is observed, but in others, it is
either reversed or palindromic (Fig. 2). The polybasic
furin cleavage site in the SARS-CoV-2S protein is con-
sidered unique to the novel virus that causes COVID-19
[18]. This has led to speculations about the possibility of
a laboratory-engineered virus. However, the presence
of the same and similar motifs in other β-coronaviruses
and even other coronaviruses eliminates the theory of an
artificially engineered virus. Although the current search
for the intermediate host of SARS-CoV-2 suggests several
potential candidates [19], possible recombination events
of viruses in the same or different host species, which
can give rise to a hybrid or a variant species, should not
be ruled out. RpYN09 bat coronavirus, the most recently
found closest relative of SARS-CoV-2, lacks the RRAR
motif [14], suggesting that the parent virus containing
this motif has yet to be discovered.

Although the identities of all host receptors for coro-
naviruses are not fully known, ACE2 is documented as
a receptor mediating both infection and transmission
of SARS-CoV-2 [20, 21]. ACE2 is a key regulator of the
angiotensin system and is well expressed in the vascular
endothelium, smooth muscle cells of the intestines, kid-
neys and heart muscle cells [22–24]. During infection,
twenty key amino acid residues in the ACE2 enzyme
interact with the S protein of SARS-CoV-2 [25]. Five of
these residues, lysine (K), glutamic acid (E), aspartic acid
(D), methionine (M) and lysine (K), at positions 31, 35,
38, 82 and 353, respectively, are critical for S protein
binding [26]. Sequence alignment analysis showed that
cattle and white-tailed deer share the most similarity
scores to humans for both required and critical amino
acid residues. Interestingly, a recent USDA/APHIS study
showed that approximately 40% of the wild white-tailed
deer population in four states in the USA tested positive
for anti-SARS-CoV-2 immunoglobulins (https://www.
aphis.usda.gov/animal_health/one_health/downloads/
qa-covid-white-tailed-deer-study.pdf). This finding indi-
cates the possibility that SARS-CoV-2 may establish and
spread in hosts with high affinity receptors. However,
the similarity of a single receptor may not explain or predict
cross-species infections. For example, while the bovine-
canine cross-species jump of a β-coronavirus is obvi-
ous from the phylogeny, the comparison of their ACE2
does not indicate a unique relationship of this receptor
between cow and dog, although their cathepsins belong
to the same clade. Therefore, ACE2 may not necessar-
ily be a receptor for bovine-canine interspecies viral
infection. Similarly, ACE2 of bottlenose dolphin and
pig shared more common residues with those of human
ACE2 than other hosts. Specifically, the Rhinolophus
bat reference sequence has one of the least phylogenetic
and key amino acid commonalities with human ACE2
(Fig. 4H). This brings into question the relevance of the
ACE2 receptor in this bat species.

The TMPRSS2 cleavage site in SARS-CoV-2 was con-
served among all viruses we included (Fig. 3A). In the host
species, both the substrate binding and active sites of the
enzyme were well conserved. Notably, the triad of catalytic
residues histidine (H) 296, aspartic acid (D) 345 and serine
(S) 441 [27], which interact with the SARS-CoV-2S pro-
tein, are conserved among all species included in this study
(Fig. 3B). The overall phylogenetic segregation pattern of
host enzymes and receptors was species-related except for
the ACE2 enzyme, where bat, rabbit and whale proteins
were distantly related to those of other mammals.

Among the viruses we analyzed, consistent pat-
terns with little variation were observed among
γ-coronaviruses. The canine respiratory, rabbit, bovine
respiratory, and white-tailed deer coronaviruses also
consistently clustered together. However, phylogenetic
crossovers were evident among canine coronavirus, TGE
virus, PED virus, feline coronavirus, and FIP virus. The S
protein of the feline coronavirus included in this report
was distant from that of the FIP virus, displaying dis-
cordant relationships that indicate past recombination
events [28]. An interesting phylogenetic common origin
was also noted for the S proteins of PED virus and the
distantly related alpaca respiratory coronavirus. These
crossovers are most likely from past recombination and
mutation events. Coronaviruses are noted for their high
frequency of recombination [29], and the patterns of
recombination among coronaviruses of domestic ani-

![Fig. 3](see figure on next page.)

**Fig. 3** S protein cleavage site and catalytic residues of the TMPRSS2 enzyme are conserved. A S protein sequence alignment at the site where human TMPRSS2 cleaves the SARS-CoV-2 spike protein. The TMPRSS2 enzyme cleaves between the arginine (R) and serine (S) residues. Only the FIP virus has a glycine (G) in place of the arginine (R) residue. Aligned protein sequences of the substrate binding site (B) and active site (C) of the TMPRSS2 enzyme of various host species are shown. Arrows in panel C point to the triad of catalytic residues histidine (H) 296, aspartic acid (D) 345 and serine (S) 441 that are essential for binding to the SARS-CoV-2 S protein. All conserved residues are shaded blue, and sequence consensus conservation is shown as colored bars (red, tall bars mean more conserved). The threshold for showing a consensus is set at > 70 for A and > 50% for B and C. The letter X denotes no consensus, and amino acids are listed in single letter codes.
Fig. 3 (See legend on previous page.)
of cross-species infections and to design effective disease control strategies.

Finally, we also noted that the MERS-like bat coronavirus (accession number YP_009361857.1; https://www.ncbi.nlm.nih.gov/protein/1189488876) is annotated as unclassified in the National Center for Biotechnology Information (NCBI) database. Considering the phylogenetic clustering of the S, M, E, and N proteins, they most likely belong to the β-coronavirus group.

Limitations of our study include the unknown nature of the complete receptor repertoire for coronaviruses among domestic and close-contact animals. Although we based our comparative phylogeny primarily on the reported or putative human receptor or coronavirus infection-associated proteins, coronaviruses may not use the same receptors in different species. It is possible that a virus crossing to another species will adapt to using different receptors or associated proteins.

Conclusion
Recombination or a few critical mutations in viral receptor binding domains or receptor protein key residues may contribute to cross-species infections [30]. We highlighted key molecular relationships that exist among common coronaviruses and their host receptors. Notably, the existence of similar polybasic residues within the S proteins of several coronaviruses...
suggests that the RRXR sequence motif is not unique to SARS-CoV-2. Unlike genome-level phylogenies, sub-genomic-level comparisons provide stronger insights into functional ontogeny and the identification of consequential recombination events. However, not all factors that determine species-specific or cross-species infections are currently known. Therefore, additional studies of host determinants for virus attachment and productive infectivity are urgently needed to understand the role different hosts play in coronavirus infections, maintenance, and host adaptations. Such studies will be critical to identify molecules that can be targeted for the prevention and control of diseases caused by these viruses, especially in hosts of economic or public health importance.

Methods

All protein sequences included in our analysis were collected from the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/nuccore/), stored in FASTA formats and analyzed. In total, 32 coronaviruses, comprising eight α-coronaviruses, 19 β-coronaviruses and five γ-coronaviruses, were included in our comparative study as shown in Table 1. For each of the viruses, we assembled protein sequence data on the spike (S), membrane (M), envelope (E) and nucleocapsid (N) structural proteins. Furthermore, protein sequence data on key receptors and enzymes reported to be involved in viral infection, namely, angiotensin converting enzyme 2 (ACE2), transmembrane serine protease 2 (TMPRSS2), aminopeptidase N (APN), sialic acid synthase (SAS),

| Virus Group               | Name of Virus                              | Accession ID | Host Species        |
|--------------------------|--------------------------------------------|--------------|---------------------|
| Alphacoronavirus (α)     | Feline Infectious Peritonitis (FIP)        | NC_002306.3  | Cat                 |
|                          | Feline Coronavirus (strain UU11)           | FJ938052.1   | Cat                 |
|                          | Canine Coronavirus                         | KC175340.1   | Dog                 |
|                          | Porcine Epidemic Diarrhea (PED) virus      | NC_028806.1  | Pig                 |
|                          | Transmissible Gastroenteritis (TGE) virus  | NC_038861.1  | Pig                 |
|                          | Ferret Coronavirus                         | NC_030292.1  | Ferret              |
|                          | Mink Coronavirus                           | NC_023760.1  | Mink                |
|                          | Alpaca Respiratory Coronavirus             | JW41000.1    | Alpaca              |
| Betacoronavirus (β)      | Canine Respiratory Coronavirus             | KV432213.1   | Dog                 |
|                          | Bovine Coronavirus                         | NC_003045.1  | Cattle              |
|                          | White-tailed Deer Coronavirus              | FJ425187.1   | Whitetail Deer      |
|                          | Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) | NC_045512.2  | Human              |
|                          | Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) | YP_009825051.1 | Human |
|                          | Middle East Respiratory Syndrome Coronavirus MERS-CoV | YP_009047204.1 | Human         |
|                          | MERS-like Coronavirus (PREDICT/PDF-2180)   | NC_034440.1  | Pipistrelle Bat     |
|                          | Mouse Coronavirus                          | FJ647220.1   | Mouse               |
|                          | Rat Coronavirus                            | KF294371.1   | Rat                 |
|                          | Rabbit Coronavirus                         | JN874562.1   | Rabbit              |
|                          | SARS-CoV-2                                 | QLC48407.1   | Tiger               |
|                          | Murine Coronavirus                          | ACN89705.1   | Mouse               |
|                          | Sable Antelope Coronavirus                 | ABP38306.1   | Sable Antelope      |
|                          | Giraffe Coronavirus beta                   | ABP38334.1   | Giraffe             |
|                          | Human Coronavirus OC43 beta                | AMK59677.1   | Human               |
|                          | Human Coronavirus OC43                     | AWW13551.1   | Chimpanzee          |
|                          | Camel Coronavirus HKU23 beta               | ALAS0080.1   | Camel               |
|                          | Buffalo Coronavirus                        | ANJ04717.1   | Buffalo             |
|                          | Equine Coronavirus                         | BAS18866.1   | Horse               |
| Gammacoronavirus (γ)     | Avian Infectious Bronchitis                | NC_001451.1  | Chicken             |
|                          | Turkey Coronavirus                         | NC_010800.1  | Turkey              |
|                          | Duck Coronavirus                           | NC_048214.1  | Duck                |
|                          | Bottlenose Dolphin Coronavirus             | MN690608.1   | Bottlenose Dolphin  |
|                          | Beluga Whale Coronavirus                   | NC_010646.1  | Beluga Whale        |
dipeptidyl peptidase 4 (DPP4), cathepsin L and furin, of seventeen hosts were also assembled, and their details are shown in Table 2. The FASTA format of all sequences was organized in Text Editor for analyses. Using MEGA X software version 11, sequence alignments and phylogenetic trees were developed. Alignments were generated using the Muscle Tool with neighbor-joining as the cluster method, and the degree of consensus among conserved residues was visualized using Snap Gene software. The SimPlot program [31] was used to graphically depict similarities among selected sequences. Phylogenetic trees were constructed using the neighbor-joining method with a Poisson model of substitution at 1000 bootstrap replications, and all the results were validated with the maximum likelihood method. All other default settings of the bioinformatics programs used were applied.

### Abbreviations

- α: Alpha
- β: Beta
- γ: Gamma
- S: Spike
- E: Envelope
- M: Membrane
- N: Nucleocapsid
- APN: Aminopeptidase-N
- ACE2: Angiotensin Converting Enzyme 2
- SAS: Sialic Acid Synthase
- TMPRSS2: Transmembrane Serine Protease 2
- SARS-CoV-2: Acute Respiratory Syndrome Coronavirus-2
- SARS-CoV: Acute Respiratory Syndrome Coronavirus
- MERS-CoV: Middle East Respiratory Syndrome Coronavirus
- TGE: Transmissible gastroenteritis
- PED: Porcine Epidemic Diarrhea
- FIP: Feline Infectious Peritonitis
- NCBI: National Center for Biotechnology Information

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### Authors’ information (optional)

None Declared.

### Authors’ contributions

TS and PM: conceptualization, investigation, methodology, review and editing, data curation, validation, supervision. KB: data curation, comparative analysis, methodology, writing manuscript draft, review and editing. SS and CW: data curation and organization, methodology, comparative analysis. GR, WA and RF: scientific and conceptual input, review and editing. The author(s) read and approved the final manuscript.

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| Host                | Host Enzyme/Receptor Accession Number (Source: NCBI) |
|---------------------|------------------------------------------------------|
|                     | ACE2 | TMPRSS2 | Aminopeptidase N (APN) | Sialic Acid Synthase (SAS) | Dipeptidyl Peptidase 4 (DPP4) | Furin | Cathepsin L |
| Human               | NP_001373189.1 | NP_001369649.1 | NP_001368853.1 | NP_061819.2 | NP_001926.2 | NP_001369548.1 | EAW62736.1 |
| Bat                 | XP_032945086.1 | XP_032944708.1 | XP_032956109.1 | XP_032979625.1 | XP_011356841.1 | XP_011374459.1 | XP_011383262.1 |
| Mouse               | NP_001123985.1 | NP_056590.2 | NP_032512.2 | NP_444409.1 | NP_034204.1 | NP_001074923.1 | EDL1624.1 |
| Rat                 | NP_001012006.1 | NP_569108.2 | NP_011274.1 | NP_001100125.2 | NP_036921.2 | NP_011374599.1 | BPM14518.1 |
| Rabbit              | NP_001164540.1 | NP_001373057.1 | NP_001075795.1 | XP_002707999.1 | NP_008256890.1 | XP_002721548.2 | NP_008255787.1 |
| Ferret              | NP_001297119.1 | NP_001373056.1 | NP_012917463.1 | NP_004747730.1 | NP_012907449.1 | NP_004763758.1 | XP_012903814.1 |
| Cat                 | NP_001034545.1 | NP_023094479.1 | NP_001009252.2 | NP_023098256.1 | NP_001009838.1 | NP_023110662.2 | NP_011286595.1 |
| Dog                 | NP_001158732.1 | NP_038299491.1 | NP_001139506.1 | NP_0538746.2 | NP_038302823.1 | NP_038517318.1 | CAC08809.1 |
| Pig                 | NP_001116654.1 | NP_001373060.1 | NP_999442.1 | NP_001172086.1 | NP_999422.1 | NP_020954578.1 | CAC44793.1 |
| Alpaca              | XP_006212709.1 | NP_031540200.1 | NP_006198515.1 | NP_006204124.1 | NP_006196279.1 | NP_002602997.1 | XP_006209297.1 |
| Cattle              | NP_001019673.2 | NP_001075054.1 | NP_001068612.1 | NP_001039947.1 | NP_776646.1 | NP_745661.1 | Procathepsin L |
| White-tailed Deer   | XP_020768965.1 | XP_020763597.1 | XP_020765004.1 | XP_020761234.1 | XP_020737504.1 | XP_020761451.1 | XP_020765041.1 |
| Bottlenose Dolphin | XP_019781177.2 | XP_033712275.1 | XP_033708027.1 | NP_004322851.1 | NP_033715971.1 | NP_033708070.1 | NP_004320974.1 |
| Beluga whale        | XP_022444817.1 | XP_022408670.1 | XP_022419728.1 | XP_022455827.1 | XP_022425590.1 | XP_022419719.1 | XP_030615313.1 |
| Duck                | XP_012949915.3 | XP_027304175.2 | XP_027322378.2 | XP_027302136.1 | XP_035187422.1 | XP_038040754.1 | Procathepsin L |
| Chicken             | XP_4168222 | XP_015156666.1 | AC9Z5799.1 | NP_001007976.1 | NP_001026426.2 | NP_001338040.1 | Procathepsin L |
| Turkey              | XP_019467554.1 | XP_010722229.1 | XP_010715973.1 | XP_010724239.2 | NOT FOUND | XP_010715981.1 | XP_010723392.1 | Procathepsin L |

**Table 2** Host receptor and enzyme sequences included in this study
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