

ACEII gene analysis exposes SARS-CoV-2 as a potential threat to agricultural and national security.

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Abstract

Coronavirus is now a significant human pathogen with the emergence of SARS-CoV-2. Until now there has been no data to support a threat to agricultural industries. Using a comparative genomic protein analysis, this study examined the angiotensin-converting enzyme II (ACEII) gene of 17 animal species with an emphasis on agriculture. To determine viral vulnerability the 20 known SARS-CoV-2 receptor-binding domain (RBD)/ACEII receptor interaction sites were compared to determine their potential susceptibility to the SARS-CoV-2 virus. With the known bat host's (XP_032963186) number of binding sites as a threshold, we note that ALL animal species examined in this study contained significant numbers (≥ 10) of SARS-CoV-2 binding sites and could be at risk for SARS-CoV-2 infection. The data from this study suggest SARS-CoV-2 imposes a grave threat to the safety and security of the agricultural industry. Urgent studies are needed to determine if infected animals can transmit SARS-CoV-2 before and/or after processing.

Introduction

Until recently it was unknown whether animals could become infected by SARS-CoV-2. Despite the widespread suspicion that SARS-CoV-2 originated from a bat (RaTG13|MN996532.1), it remains unclear whether other animal species may be viable primary or

secondary hosts. Preliminary data (Chen et al. 2020) suggest various animal groups contain SARS-CoV-2 interaction sites between the SARS-CoV-2 spike protein receptor-binding domain (RBD) and the angiotensin-converting enzyme II (ACEII) gene. This RBD has 16 amino acid residues capable of interacting with 20 ACEII amino acid sites (Li et al. 2005, Chen et al. 2020).

Since the onset of the SARS-CoV-2 pandemic, we have identified and verified that tigers (Gollakner and Capua 2020, Mahdy 2020) and lions serve as SARS-CoV-2 hosts. To determine if SARS-CoV-2 poses a potential threat to agricultural and national security this study examines the SARS-CoV-2 spike protein RBD sites that are capable of interacting with the ACEII gene of 17 animals identified as having agricultural significance.

Materials and Methods

Human and animal ACEII gene sequences (Table 1) were queried on the NCBI gene database (Anjay 2012) and compiled to fasta format using Notepad++ (Ho 2020). The resulting fasta file of ACEII protein sequences was aligned using Muscle (Edgar 2004a, 2004b) with the default algorithm and parameters within UGENE v34 (Okonechnikov et al. 2012). A second alignment was performed using the Cobalt tool within the NCBI site (Papadopoulos and Agarwala 2007). The second alignment was necessary as UGENE lacks a nexus file format option when exporting alignments.

Using UGENE a distance matrix was generated using the following parameters: Distance algorithm = Similarity, Profile mode = Percentage, Save profile to file= checked, File = Comma-separated (.CSV). The human reference sequence must be in the first position when generating a distance matrix. The distance matrix generation process was repeated after removing all amino acid residues other than the 20 known ACEII interactive sites.

Using ACEII gene alignment, all sequences were examined for SARS-CoV-2/ACEII gene interaction sites using the 20 known human sites as the reference (Li et al. 2005, Chen et al. 2020). Amino acid changes were annotated and represented graphically using both MSWord and SnagIt Editor (Bragg 2002). The number of matching amino acid residue sites were denoted into a spreadsheet and exported as for visualization.

Using human (NP_001358344) reference sequence, amino acids that differ from human SARS-CoV-2 interaction sites were queried manually and recorded in table format. The similarity value obtained from the lowest scoring known host was used as a threshold value to determine the possibility of COVID-19 infection.

Results

Amino acid alignment of the ACEII binding domain (Appendix 1) reveals a highly homologous binding domain between species. Most SARS-CoV-2/ACE II interactive sites in this binding domain lie within the α -helices and β -sheets of the ACEII complex structure.

The similarity distance matrix demonstrates that the human ACEII gene shares $\geq 78\%$ whole gene identity to all species in this study except the 4 birds; emu 65%, turkey 55%, chicken 66%, and pheasant 66% (Appendix 2a-c). When only the 20 ACEII interaction sites are analyzed, all species except the 4 birds; emu 53%, turkey 58%, chicken 58%, and pheasant 58%, in addition to bat (55%) demonstrate $\geq 75\%$ similarity (Appendix 3a-c).

Our study noted the following AA residue differences in the hydrophobic pockets created by F28, L79, Y83, and L97: Birds p.L79N, Pig p.L79I, and all others except donkey and horse p.L79M; birds and bat p.Y83F; and birds p.L97I in birds (Appendix 1). F28 was conserved in all species.

The known host species with the least number of SARS-CoV-2/ACEII complex structure interaction sites were identified as being horseshoe bat (XP_032963186) with 10 interaction sites. The known host tiger, as well as cattle, sheep, goat, bison, and deer, contained the most SARS-CoV-2 interaction sites outside of humans (Table 2).

Table 2. SARS-CoV-2/ACEII amino acid (AA) residue interaction sites. Known SARS-CoV-2 hosts are denoted with a black background.

Accession number Common name	Amino acid interactions
XP_005228485 Cattle	17
XP_027389727 Hybrid cattle X1	17
XP_027389729 Hybrid Cattle X2	17
NP_001116542 Pig	15
XP_011961657 Domestic sheep	17
NP_001277036 Domestic goat	17
XP_025976569 Emu	10
XP_001490241 Horse	15
XP_014713133 Donkey	15
XP_006212709 Alpaca	15
XP_416822 Chicken	10
XP_019467554 Turkey	10
XP_010833001 American bison	17

XP_020768965 Texas whitetail deer	17
XP_031451919 Common pheasant	10
NP_001358344 Human	20
XP_007090142 Tiger	17
XP_032963186 Horseshoe bat	10

Species with the greatest number of SARS-CoV-2 interaction sites contained 17; including cattle, sheep, goat, bison, deer, and tiger. Donkey, horse, and alpaca have 15 binding sites.

Discussion

Structural analysis

It has been previously described that the unique SARS-CoV-2 F486 site is capable of binding deep in the ACEII hydrophobic pockets created by F28, L79, Y83, and L97 (Chen et al. 2020). The interspecies AA residue differences of those sites, noted in this study, conserve the hydrophobic pockets in the ACEII complex structure by use of other hydrophobic residue substitutions. Of note is the p.Y83F substitution in birds and bats which creates π -stacking of aromatic residues (McGaughey et al. 1998), an effect that can alter drug effectiveness/design (Graci and Cameron 2008). It also creates a disulfide bond with F486 increasing virus binding affinity ($k=6$)(Chen et al. 2007). This improved binding affinity may alter our AA interaction threshold estimate, 10 in known host bat, for establishing infection in a host. It is conceivable that these species with p.Y83F would not require as many sites to cause infection since they could theoretically acquire similar virus-host bonding strengths with less interacting residues.

Other than birds, all animals in this study contained at least 15 SARS-CoV-2/ACEII complex structure interaction sites. This number of interaction sites is well above our threshold value established by the know bat host at 10. This may or may not be cause for concern but it certainly leads to a high index suspicion of their SARS-CoV-2 host viability.

Historical coronavirus evidence in agricultural species

It is important to understand that ALL species in this study have been previously identified as a host for a coronavirus; cattle (Weiyang and Chengping 1990), chicken, pheasant (Culver et al. 2008), turkey (Guy et al. 1997), pig (Woo et al. 2017), sheep (Tråvén et al. 1999), goat, bison (Weiyang and Chengping 1990), emu (Kummrow 2015), donkey, horse, (Nagano 1982, OUE et al. 2013, Fielding et al. 2015), alpaca (Jin et al. 2007, Crossley et al. 2012), bison (Amer 2019, Harms et al. 2019), and deer (Amer 2019). While not SARS-CoV-2, they were of

the same closely related viral coronavirus genus. The possibility to host SARS-CoV-2 should not be disregarded until experimentally proven otherwise.

It is still not known what the pathogenic effect, if any, will be in SARS-CoV-2 infected animals. Looking at historical data from bovine coronavirus (BCoV) infections (Boileau and Kapil 2010), it is conceivable that SARS-CoV-2 could have serious implications in the morbidity and mortality of cattle < 2years old.

SARS-CoV-2 agricultural and national security risk

SARS-CoV-2 has demonstrated surface survivability of up to 9 days (Kampf et al. 2020). Coronavirus in excreted body fluids can survive >7 days (Lai et al. 2005). Our understanding is that survivability in biological tissues has yet to be determined but from excreted fluids, we can infer survivability of the virus is also >7 days. Within biological tissues the virus' survivability could increase beyond 20 days when refrigerated (Guionie et al. 2013), a vital process to avert food spoilage. A commonly used method to increase food preservation is gamma irradiation (Diehl 2002, Piri et al. 2011). This process may not inactivate enough of the virus to prevent viral transmission as demonstrated by gamma irradiation studies with the Ebola virus (Hume et al. 2016). In that study, it was determined that "no dosage could be considered to inactivate 100% of a sample" (Hume et al. 2016). Principally, viral transmission was possible after gamma irradiation.

Infected animals and humans may also contaminate a water supply if in direct contact. Studies have shown that coronavirus inactivation in water can take over 500 days at 4°C or 10 days at 23°C (Gundy et al. 2009). This study also indicates the possibility of human to animal spread through an open, contaminated water source such as a water trough. Due to the extreme importance of this viral capacity, the water study should be repeated with SARS-CoV-2 for comparative results.

Until now there has been no data to support a threat to the global food supply. However, existing control measures are not adequate to mitigate SARS-CoV-2 propagation. Food poverty, hunger, and food inequality lead to instability at the local, national, and international levels (Pinstrup-Andersen 2003). These factors highlight the importance of agricultural industries to the security of a nation (Tweeten 1999, Falcon and Naylor 2005, Etim et al. 2017). One needs only to look at recent events in Venezuela for an example of this concept. Preparing and mitigating any potential impact of SARS-CoV-2 on animals is paramount to agricultural and national

security (Batie and Healy 1980, Winters 1990, HORN and BREEZE 1999, Casagrande 2000). Agricultural industries should begin preparation for a worst-case-scenario before they are forced to respond to one.

Mitigation and control

Coronavirus is now a significant human pathogen with the emergence of SARS-CoV-2. This pathogen has caused severe illness and death in humans and until recently no evidence existed to suggest the agricultural industry was in jeopardy until the recent confirmation of SARS-CoV-2 infections in several Bronx Zoo tigers and lions. To date, it is unknown if the tigers and lions contracted the disease through human interaction, another intermediate vector, or a contaminated food source. If by an alternative vector or food/water-borne mode of spread, it poses significant challenges to the control of agricultural disease propagation.

Currently, social isolation, respiratory barrier masks, and personal sanitation measures are the only available forms of SARS-CoV-2 mitigation. This is not a possibility for agricultural industry animals where animals are typically kept in large numbers and close proximity to each other. As a result, the risk of one animal infecting another would appear to be extremely high. Even with free-range animals, their natural herd tendencies sustain a viral transmission risk. While numerous studies have shown that viruses can spread quickly with a high degree of population penetrance, no studies are found to document coronavirus transmission to humans or other animals through food/water consumption or handling during processing. The absence of this data does not eliminate this prospect. The rate of raw meat processing plant worker infections is alarming and should raise concern as to the root source of spread since this phenomena is not observed in other industries of similar scale.

While the ACEII gene is highly conserved between species, this study was limited by using the human ACEII gene sequence as a reference. Actual SARS-CoV-2/ACEII animal complex structure interactions could differ in animals. Another limitation of this study was that SARS-CoV-2 survivability in tissues and water must be inferred from other human coronaviruses. SARS-CoV-2 survivability in these media may differ under identical environmental conditions. Lack of animal testing precludes a definitive viral susceptibility analysis. Our work establishes the framework for a SARS-CoV-2 infection risk amongst animals. Future studies should test living and processed animal samples to determine primary and secondary host viability.

Summary

Using a comparative genomic protein analysis this study found that all animal species in this study contained significant numbers of SARS-CoV-2/ACEII complex structure interaction sites and could be considered at risk for SARS-CoV-2 infection. Data from this study suggest SARS-CoV-2 imposes a grave threat to agricultural and national security. Urgent studies are needed to determine if infected animals can transmit SARS-CoV-2 before and/or after processing.

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Table 1. Accession list

Accession number common name [scientific name]
NP_001358344 Human [<i>Homo sapiens</i>]
XP_005228485 Cattle [<i>Bos taurus</i>]
XP_027389727 Hybrid cattleX1 [<i>Bos indicus</i> x <i>Bos taurus</i>]
XP_027389729 Hybrid cattleX2 [<i>Bos indicus</i> x <i>Bos taurus</i>]
NP_001116542 Pig [<i>Sus scrofa</i>]
XP_011961657 Domestic sheep [<i>Ovis aries</i>]
NP_001277036 Domestic goat [<i>Capra hircus</i>]
XP_025976569 Emu [<i>Dromaius novaehollandiae</i>]
XP_001490241 Horse [<i>Equus caballus</i>]
XP_014713133 Donkey [<i>Equus asinus</i>]
XP_006212709 Alpaca [<i>Vicugna pacos</i>]
XP_416822 Chicken [<i>Gallus gallus</i>]
XP_019467554 Turkey [<i>Meleagris gallopavo</i>]
XP_010833001 American bison [<i>Bison bison bison</i>]
XP_020768965 Texas whitetail deer [<i>Odocoileus virginianus texanus</i>]
XP_031451919 Common pheasant [<i>Phasianus colchicus</i>]
XP_007090142 Tiger [<i>Panthera tigris altaica</i>]
XP_032963186.1 Horseshoe bat [<i>Rhinolophus ferrumequinum</i>]

Appendices

Appendix 1. ACEII protein sequence alignment (1a, 1b, 1c, and 1d). Only regions containing SARS-CoV-2 interaction sites are shown. Bold white letters with black background indicate the similarity of SARS-CoV-2 interaction sites to the human ACEII reference sequence. Clear boxes denote alpha-helices, shaded boxes denote β -sheets. Conserved interactive amino acid residues are seen at F28, E37, L45, N331, K354, D356, and R358.

1a.

REFERENCE	20	---Q--TP--K--H--ED--YQ--L-----	70
Cattle	20	VTEERAKHTLEKFNFEATQLSVSSASWNYNTNITDENVQKMEARAKH	70
Hybrid cattle X1	20	VTEERAKHTLEKFNFEATQLSVSSASWNYNTNITDENVQKMEARAKH	70
Hybrid cattle X2	20	VTEERAKHTLEKFNFEATQLSVSSASWNYNTNITDENVQKMEARAKH	70
Pig	20	VTEELAKHTLEKFNFEATQLSVSSASWNTINTNITDENIQKMDARAKH	70
Domestic sheep	20	VTEERAKHTLEKFNFEATQLSVSSASWNYNTNITDENVQKMEARAKH	70
Domestic goat	20	VTEERAKHTLEKFNFEATQLSVSSASWNYNTNITDENVQKMEARAKH	70
Emu	20	VTE---AAGITLTFENIKAGELISVSSASWNYNTNITTEETSMKMEADAKH	70
Horse	20	VTEELAKHTLEKFNFEATQLSVSSASWNYNTNITDENVQKMEAGARH	70
Donkey	20	VTEELAKHTLEKFNFEATQLSVSSASWNYNTNITDENVQKMEAGARH	70
Alpaca	20	VTEELAKHTLEKFNFEATQLSVSSASWNYNTNITDENVQKMDARAKH	70
Chicken	20	VTE---EAGITLAEFNVRAAGISVSSASWNYNTNITTEETARKMSAGAKH	70
Turkey	20	VTE---EAGITLAEFNVRAAGISVSSASWNYNTNITTEETARKMSAGAKH	70
American bison	20	VTEERAKHTLEKFNFEATQLSVSSASWNYNTNITDENVQKMEARAKH	70
Texas whitetail deer	20	VTEERAKHTLEKFNFEATQLSVSSASWNYNTNITDENVQKMEARAKH	70
Common pheasant	20	VTE---EAGITLAEFNVRAAGISVSSASWNYNTNITTEETARKMSAGTKH	70
Human	20	VTEERAKHTLEKFNFEATQLSVSSASWNYNTNITDENVQKMEAGAKH	70
Tiger	20	VTEERAKHTLEKFNFEATQLSVSSASWNYNTNITDENVQKMEAGAKH	70
Horseshoe bat	20	VTEELAKHTLEKFNFEATQLSVSSASWNYNTNITDENVQKMEAGAKH	70

1c.

REFERENCE	303	-----Q-----EN-----	353
Cattle	303	WDAERTFKEAEKFFVSLPMMTGFNFNSMLITLPGDGRKVVCH	PTAWDLG 353
Hybrid cattle X1	303	WDAERTFKEAEKFFVSLPMMTGFNFNSMLITLPGDGRKVVCH	PTAWDLG 353
Hybrid cattle X2	303	WDAERTFKEAEKFFVSLPMMTGFNFNSMLITLPGDGRKVVCH	PTAWDLG 353
Pig	303	WDARTFKEAEKFFVSLPMMTGFNFNSMLITLPGDGRKVVCH	PTAWDLG 353
Domestic sheep	303	WDAERTFKEAEKFFVSLPMMTGFNFNSMLITLPGDGRKVVCH	PTAWDLG 353
Domestic goat	303	WDAERTFKEAEKFFVSLPMMTGFNFNSMLITLPGDGRKVVCH	PTAWDLG 353
Emu	303	WDALKLFFKTAFAFFASVGLVNMTEGFNFNSMLITLPTDRKVVCH	PTAWDMG 353
Horse	303	WDARRFKEAEKFFVSLPMMTGFNFNSMLITLPGDGRKVVCH	PTAWDLG 353
Donkey	303	WDARRFKEAEKFFVSLPMMTGFNFNSMLITLPGDGRKVVCH	PTAWDLG 353
Alpaca	303	WDARRFKEAEKFFVSLPMMTGFNFNSMLITLPGDGRKVVCH	PTAWDLG 353
Chicken	303	WDARKLFFKTAFAFFASVGLVNMTEGFNFNSMLITLPTDRKVVCH	PTAWDMG 353
Turkey	303	WDALKLFFKTAFAFFASVGLVNMTEGFNFNSMLITLPTDRKVVCH	PTAWDMG 353
American bison	303	WDAERTFKEAEKFFVSLPMMTGFNFNSMLITLPGDGRKVVCH	PTAWDLG 353
Texas whitetail deer	303	WDAERTFKEAEKFFVSLPMMTGFNFNSMLITLPGDGRKVVCH	PTAWDLG 353
Common pheasant	303	WDARRFKEAEKFFVSLPMMTGFNFNSMLITLPGDGRKVVCH	PTAWDLG 353
Human	303	WDARRFKEAEKFFVSLPMMTGFNFNSMLITLPGDGRKVVCH	PTAWDLG 353
Tiger	303	WDARRFKEAEKFFVSLPMMTGFNFNSMLITLPGDGRKVVCH	PTAWDLG 353
Horseshoe bat	303	WDARRFKEAEKFFVSLPMMTGFNFNSMLITLPGDGRKVVCH	PTAWDLG 353

1b.

REFERENCE	71	-----L-----NY-----	120
Cattle	71	SAFYEEQSRMAKTPSLEETQNLTLKRLQKALQHSQTSALSAEKSRLNTITL	120
Hybrid cattle X1	71	SAFYEEQSRMAKTPSLEETQNLTLKRLQKALQHSQTSALSAEKSRLNTITL	120
Hybrid cattle X2	71	SAFYEEQSRMAKTPSLEETQNLTLKRLQKALQHSQTSALSAEKSRLNTITL	120
Pig	71	SAFYEEQSRMAKTPSLEETQNLTLKRLQKALQHSQTSALSAEKSRLNTITL	120
Domestic sheep	71	SAFYEEQSRMAKTPSLEETQNLTLKRLQKALQHSQTSALSAEKSRLNTITL	120
Domestic goat	71	SAFYEEQSRMAKTPSLEETQNLTLKRLQKALQHSQTSALSAEKSRLNTITL	120
Emu	71	SAFYDEASRNASNPPLANTTDLRLQLQTSQDQSGSSVLSSEKYTRLSTVL	120
Horse	71	SAFYEEQSRMAKTPSLEETQNLTLKRLQKALQHSQTSALSAEKSRLNTITL	120
Donkey	71	SAFYEEQSRMAKTPSLEETQNLTLKRLQKALQHSQTSALSAEKSRLNTITL	120
Alpaca	71	SAFYEEQSRMAKTPSLEETQNLTLKRLQKALQHSQTSALSAEKSRLNTITL	120
Chicken	71	SAFYEEQSRMAKTPSLEETQNLTLKRLQKALQHSQTSALSAEKSRLNTITL	120
Turkey	71	SAFYEEQSRMAKTPSLEETQNLTLKRLQKALQHSQTSALSAEKSRLNTITL	120
American bison	71	SAFYEEQSRMAKTPSLEETQNLTLKRLQKALQHSQTSALSAEKSRLNTITL	120
Texas whitetail deer	71	SAFYEEQSRMAKTPSLEETQNLTLKRLQKALQHSQTSALSAEKSRLNTITL	120
Common pheasant	71	SAFYEEQSRMAKTPSLEETQNLTLKRLQKALQHSQTSALSAEKSRLNTITL	120
Human	71	SAFLKEQSTLAEVPLAEIHNITVKRQLQALQDQSGSSVLSSEDKSRLNTITL	120
Tiger	71	SAFYEEQSRMAKTPSLEETQNLTLKRLQKALQHSQTSALSAEKSRLNTITL	120
Horseshoe bat	71	SDFYKQSKLAKNFSLLEEIHNTVKRQLQALQDQSGSSVLSSEDKSRLNTITL	120

1d.

REFERENCE	354	KGD-R-----	415
Cattle	354	KGDVREKRIKVTIPDDFLTAHHEMGIHQYDMMVAQPYLLLRINGANEGFHEAVGEIMSLSAAT	415
Hybrid cattle X1	354	KGDVREKRIKVTIPDDFLTAHHEMGIHQYDMMVAQPYLLLRINGANEGFHEAVGEIMSLSAAT	415
Hybrid cattle X2	354	KGDVREKRIKVTIPDDFLTAHHEMGIHQYDMMVAQPYLLLRINGANEGFHEAVGEIMSLSAAT	415
Pig	354	KGDVREKRIKVTIPDDFLTAHHEMGIHQYDMMVAQPYLLLRINGANEGFHEAVGEIMSLSAAT	415
Domestic sheep	354	KGDVREKRIKVTIPDDFLTAHHEMGIHQYDMMVAQPYLLLRINGANEGFHEAVGEIMSLSAAT	415
Domestic goat	354	KGDVREKRIKVTIPDDFLTAHHEMGIHQYDMMVAQPYLLLRINGANEGFHEAVGEIMSLSAAT	415
Emu	354	KGDVREKRIKVTIPDDFLTAHHEMGIHQYDMMVAQPYLLLRINGANEGFHEAVGEIMSLSAAT	415
Horse	354	KGDVREKRIKVTIPDDFLTAHHEMGIHQYDMMVAQPYLLLRINGANEGFHEAVGEIMSLSAAT	415
Donkey	354	KGDVREKRIKVTIPDDFLTAHHEMGIHQYDMMVAQPYLLLRINGANEGFHEAVGEIMSLSAAT	415
Alpaca	354	KGDVREKRIKVTIPDDFLTAHHEMGIHQYDMMVAQPYLLLRINGANEGFHEAVGEIMSLSAAT	415
Chicken	354	KGDVREKRIKVTIPDDFLTAHHEMGIHQYDMMVAQPYLLLRINGANEGFHEAVGEIMSLSAAT	415
Turkey	354	KGDVREKRIKVTIPDDFLTAHHEMGIHQYDMMVAQPYLLLRINGANEGFHEAVGEIMSLSAAT	415
American bison	354	KGDVREKRIKVTIPDDFLTAHHEMGIHQYDMMVAQPYLLLRINGANEGFHEAVGEIMSLSAAT	415
Texas whitetail deer	354	KGDVREKRIKVTIPDDFLTAHHEMGIHQYDMMVAQPYLLLRINGANEGFHEAVGEIMSLSAAT	415
Common pheasant	354	KGDVREKRIKVTIPDDFLTAHHEMGIHQYDMMVAQPYLLLRINGANEGFHEAVGEIMSLSAAT	415
Human	354	KGDVREKRIKVTIPDDFLTAHHEMGIHQYDMMVAQPYLLLRINGANEGFHEAVGEIMSLSAAT	415
Tiger	354	KGDVREKRIKVTIPDDFLTAHHEMGIHQYDMMVAQPYLLLRINGANEGFHEAVGEIMSLSAAT	415
Horseshoe bat	354	KGDVREKRIKVTIPDDFLTAHHEMGIHQYDMMVAQPYLLLRINGANEGFHEAVGEIMSLSAAT	415

Appendix 2. Distance matrix table comparing ACEII sequences (2a: humans, cattle, pigs, and sheep; 2b: goat, emu, horse, donkey, alpaca, and chicken; 2c: turkey, bison, deer, pheasant, tiger, and bat).

2a.							
	Human	Cattle	Hybrid cattle X1	Hybrid cattle X2	Pig	Sheep	
NP_001358344 Human [Homo sapiens]	100%	78%	78%	81%	81%	82%	
XP_005228485 Cattle [Bos taurus]	78%	100%	99%	95%	84%	92%	
XP_027389727 Hybrid cattle X1 [Bos indicus x Bos taurus]	78%	99%	100%	95%	84%	93%	
XP_027389729 Hybrid cattle X2 [Bos indicus x Bos taurus]	81%	96%	96%	100%	88%	97%	
NP_001116542 Pig [Sus scrofa]	81%	84%	84%	88%	100%	87%	
XP_011961657 Domestic sheep [Ovis aries]	82%	93%	93%	97%	88%	100%	
NP_001277036 Domestic goat [Capra hircus]	82%	94%	94%	98%	88%	99%	
XP_025976569 Emu [Dromaius novaehollandiae]	65%	65%	65%	66%	66%	66%	
XP_001490241 Horse [Equus caballus]	87%	82%	82%	85%	87%	85%	
XP_014713133 Donkey [Equus asinus]	86%	81%	81%	84%	86%	84%	
XP_006212709 Alpaca [Vicugna pacos]	83%	84%	84%	88%	90%	88%	
XP_416822 Chicken [Gallus gallus]	66%	64%	64%	65%	66%	66%	
XP_019467554 Turkey [Meleagris gallopavo]	55%	55%	55%	55%	55%	56%	
XP_010833001 American bison [Bison bison bison]	81%	100%	100%	100%	89%	98%	
XP_020768965 Texas whitetail deer [Odocoileus virginianus texanus]	78%	92%	92%	92%	85%	92%	
XP_031451919 Common pheasant [Phasianus colchicus]	66%	65%	65%	66%	66%	66%	
XP_007090142 Tiger [Panthera tigris altaica]	86%	80%	80%	83%	83%	83%	
XP_032963186.1 Horseshoe bat [Rhinolophus ferrumequinum]	81%	77%	77%	80%	80%	80%	

2b.							
	Goat	Emu	Horse	Donkey	Alpaca	Chicken	
NP_001358344 Human [Homo sapiens]	82%	65%	87%	83%	83%	66%	
XP_005228485 Cattle [Bos taurus]	93%	64%	82%	78%	84%	64%	
XP_027389727 Hybrid cattle X1 [Bos indicus x Bos taurus]	93%	64%	82%	78%	84%	64%	
XP_027389729 Hybrid cattle X2 [Bos indicus x Bos taurus]	98%	66%	86%	82%	88%	66%	
NP_001116542 Pig [Sus scrofa]	88%	66%	87%	83%	90%	66%	
XP_011961657 Domestic sheep [Ovis aries]	99%	66%	86%	82%	88%	66%	
NP_001277036 Domestic goat [Capra hircus]	100%	66%	85%	82%	88%	66%	
XP_025976569 Emu [Dromaius novaehollandiae]	66%	100%	67%	65%	66%	84%	
XP_001490241 Horse [Equus caballus]	85%	67%	100%	96%	88%	67%	
XP_014713133 Donkey [Equus asinus]	84%	66%	98%	100%	87%	66%	
XP_006212709 Alpaca [Vicugna pacos]	88%	66%	88%	84%	100%	67%	
XP_416822 Chicken [Gallus gallus]	66%	83%	67%	64%	67%	100%	
XP_019467554 Turkey [Meleagris gallopavo]	56%	65%	56%	54%	55%	72%	
XP_010833001 American bison [Bison bison bison]	98%	67%	86%	79%	89%	68%	
XP_020768965 Texas whitetail deer [Odocoileus virginianus texanus]	92%	66%	82%	78%	85%	67%	
XP_031451919 Common pheasant [Phasianus colchicus]	66%	84%	67%	64%	67%	96%	
XP_007090142 Tiger [Panthera tigris altaica]	83%	66%	88%	85%	85%	68%	
XP_032963186.1 Horseshoe bat [Rhinolophus ferrumequinum]	80%	64%	86%	82%	82%	65%	

2c.							
	Turkey	Bison	Deer	Pheasant	Tiger	Horseshoe bat	
NP_001358344 Human [Homo sapiens]	59%	43%	66%	66%	85%	81%	
XP_005228485 Cattle [Bos taurus]	59%	53%	77%	65%	78%	76%	
XP_027389727 Hybrid cattle X1 [Bos indicus x Bos taurus]	59%	53%	77%	64%	78%	76%	
XP_027389729 Hybrid cattle X2 [Bos indicus x Bos taurus]	59%	54%	78%	66%	82%	80%	
NP_001116542 Pig [Sus scrofa]	59%	48%	72%	66%	83%	80%	
XP_011961657 Domestic sheep [Ovis aries]	60%	52%	78%	67%	82%	80%	
NP_001277036 Domestic goat [Capra hircus]	59%	53%	78%	67%	82%	80%	
XP_025976569 Emu [Dromaius novaehollandiae]	69%	36%	55%	84%	66%	65%	
XP_001490241 Horse [Equus caballus]	60%	46%	69%	67%	87%	86%	
XP_014713133 Donkey [Equus asinus]	59%	43%	68%	67%	86%	84%	
XP_006212709 Alpaca [Vicugna pacos]	59%	47%	72%	67%	84%	82%	
XP_416822 Chicken [Gallus gallus]	77%	36%	56%	96%	67%	65%	
XP_019467554 Turkey [Meleagris gallopavo]	100%	34%	49%	74%	56%	54%	
XP_010833001 American bison [Bison bison bison]	67%	100%	97%	67%	81%	80%	
XP_020768965 Texas whitetail deer [Odocoileus virginianus texanus]	62%	62%	100%	67%	79%	76%	
XP_031451919 Common pheasant [Phasianus colchicus]	79%	36%	56%	100%	67%	65%	
XP_007090142 Tiger [Panthera tigris altaica]	61%	44%	67%	68%	100%	83%	
XP_032963186.1 Horseshoe bat [Rhinolophus ferrumequinum]	58%	43%	64%	66%	82%	100%	

Appendix 3. Distance matrix table using only the 20 known SARS-CoV-2/ACEII interaction sites (3a: humans, cattle, hybrid cattle, pigs, and sheep; 3b: goat, emu, horse, donkey, alpaca, and chicken; 3c: turkey, bison, deer, pheasant, tiger, and bat).

3a.	Human	Cattle	Hybrid cattle		Pig	Sheep
			X1	X2		
NP_001358344 Human [Homo sapiens]	100%	85%	85%	85%	85%	85%
XP_005228485 Cattle [Bos taurus]	85%	100%	100%	100%	100%	100%
XP_027389727 Hybrid cattle X1 [Bos indicus x Bos taurus]	85%	100%	100%	100%	100%	100%
XP_027389729 Hybrid cattle X2 [Bos indicus x Bos taurus]	85%	100%	100%	100%	100%	100%
NP_001116542 Pig [Sus scrofa]	80%	85%	85%	85%	85%	85%
XP_011961657 Domestic sheep [Ovis aries]	85%	100%	100%	100%	100%	100%
NP_001277036 Domestic goat [Capra hircus]	85%	95%	95%	95%	95%	95%
XP_025976569 Emu [Dromaius novaehollandiae]	53%	53%	53%	53%	53%	53%
XP_001490241 Horse [Equus caballus]	80%	75%	75%	75%	75%	75%
XP_014713133 Donkey [Equus asinus]	80%	75%	75%	75%	75%	75%
XP_006212709 Alpaca [Vicugna pacos]	75%	80%	80%	80%	80%	80%
XP_416822 Chicken [Gallus gallus]	58%	58%	58%	58%	58%	58%
XP_019467554 Turkey [Meleagris gallopavo]	58%	58%	58%	58%	58%	58%
XP_010833001 American bison [Bison bison bison]	85%	100%	100%	100%	100%	100%
XP_020768965 Texas whitetail deer [Odocoileus virginianus texanus]	85%	100%	100%	100%	100%	100%
XP_031451919 Common pheasant [Phasianus colchicus]	58%	58%	58%	58%	58%	58%
XP_007090142 Tiger [Panthera tigris altaica]	85%	80%	80%	80%	80%	80%
XP_032963186.1 Horseshoe bat [Rhinolophus ferrumequinum]	55%	50%	50%	50%	50%	50%

3b.	Goat	Emu	Horse	Donkey	Alpaca	Chicken
	NP_001358344 Human [Homo sapiens]	85%	85%	80%	80%	80%
XP_005228485 Cattle [Bos taurus]	95%	100%	85%	75%	75%	80%
XP_027389727 Hybrid cattle X1 [Bos indicus x Bos taurus]	95%	100%	85%	75%	75%	80%
XP_027389729 Hybrid cattle X2 [Bos indicus x Bos taurus]	95%	100%	85%	75%	75%	80%
NP_001116542 Pig [Sus scrofa]	90%	85%	100%	80%	80%	80%
XP_011961657 Domestic sheep [Ovis aries]	95%	100%	85%	75%	75%	80%
NP_001277036 Domestic goat [Capra hircus]	100%	95%	90%	75%	75%	75%
XP_025976569 Emu [Dromaius novaehollandiae]	58%	53%	58%	42%	42%	58%
XP_001490241 Horse [Equus caballus]	75%	75%	80%	100%	100%	70%
XP_014713133 Donkey [Equus asinus]	75%	75%	80%	100%	100%	70%
XP_006212709 Alpaca [Vicugna pacos]	75%	80%	80%	70%	70%	100%
XP_416822 Chicken [Gallus gallus]	58%	58%	58%	47%	47%	63%
XP_019467554 Turkey [Meleagris gallopavo]	58%	58%	58%	47%	47%	63%
XP_010833001 American bison [Bison bison bison]	95%	100%	85%	75%	75%	80%
XP_020768965 Texas whitetail deer [Odocoileus virginianus texanus]	95%	100%	85%	75%	75%	80%
XP_031451919 Common pheasant [Phasianus colchicus]	58%	58%	58%	47%	47%	63%
XP_007090142 Tiger [Panthera tigris altaica]	80%	80%	85%	95%	95%	75%
XP_032963186.1 Horseshoe bat [Rhinolophus ferrumequinum]	55%	50%	60%	65%	65%	55%

3c.	Turkey	Bison	Deer	Pheasant	Tiger	Horseshoe bat
	NP_001358344 Human [Homo sapiens]	55%	55%	55%	50%	85%
XP_005228485 Cattle [Bos taurus]	55%	55%	55%	50%	80%	50%
XP_027389727 Hybrid cattle X1 [Bos indicus x Bos taurus]	55%	55%	55%	50%	80%	50%
XP_027389729 Hybrid cattle X2 [Bos indicus x Bos taurus]	55%	55%	55%	50%	80%	50%
NP_001116542 Pig [Sus scrofa]	55%	55%	55%	55%	85%	60%
XP_011961657 Domestic sheep [Ovis aries]	55%	55%	55%	50%	80%	50%
NP_001277036 Domestic goat [Capra hircus]	55%	55%	55%	55%	80%	55%
XP_025976569 Emu [Dromaius novaehollandiae]	79%	74%	74%	100%	47%	63%
XP_001490241 Horse [Equus caballus]	45%	45%	45%	40%	95%	65%
XP_014713133 Donkey [Equus asinus]	45%	45%	45%	40%	95%	65%
XP_006212709 Alpaca [Vicugna pacos]	60%	60%	60%	55%	75%	55%
XP_416822 Chicken [Gallus gallus]	100%	95%	95%	79%	53%	53%
XP_019467554 Turkey [Meleagris gallopavo]	95%	100%	100%	74%	53%	47%
XP_010833001 American bison [Bison bison bison]	55%	55%	55%	50%	80%	50%
XP_020768965 Texas whitetail deer [Odocoileus virginianus texanus]	55%	55%	55%	50%	80%	50%
XP_031451919 Common pheasant [Phasianus colchicus]	95%	100%	100%	74%	53%	47%
XP_007090142 Tiger [Panthera tigris altaica]	50%	50%	50%	45%	100%	60%
XP_032963186.1 Horseshoe bat [Rhinolophus ferrumequinum]	50%	45%	45%	60%	60%	100%