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Research Article

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. However, 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 of animals. The 20 known SARS-CoV-2 ribosomal binding domain (RBD)-ACEII gene interaction sites were compared to the 17 animal species to determine their potential susceptibility to the SARS-CoV-2 virus. Using 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 should be considered at serious risk for SARS-CoV-2 infection. The data from this study suggests 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 after processing.

1. 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 [1], suggest that various animal groups contain SARS-CoV-2 interaction sites between the SARS-CoV-2 spike protein ribosomal 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 [1, 2].

Since the onset of the SARS-CoV-2 pandemic, we have identified and verified that tigers [3, 4] and lions serve as SARS-CoV-2 hosts. To determine if SARS-CoV-2 poses a potential threat to agricultural security, this study examines the SARS-CoV-2 spike protein RBD sites capable of interacting with the ACEII gene of 17 animals identified as having agricultural significance.

2. Materials and Methods

Human and animal ACEII gene sequences (Table 1) were queried on the NCBI gene database [5]. Sequences were then sequestered into a Notepad++ [6] text file and saved using the fasta format suffix. The resulting fasta file of ACEII protein sequences was then imported into UGENE v34 [7] and aligned using Muscle [8, 9] default algorithm. A second alignment was performed using the Cobalt tool within the NCBI site [10]. The second alignment was necessary as UGENE lacks a nexus file format option when exporting alignments. The nexus file format was more easily imported to MS Word, where it was annotated.

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). Generated CSV file was imported into MSExcel, annotated, and exported as a PDF. 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 ASCII gene alignment, all sequences were examined for SARS-CoV-2/ACEII gene interaction sites using the 20 known human sites as the reference [1, 2]. Amino acid changes were annotated and represented graphically using MSWord and SnagIt Editor [11]. The number of matching sites was denoted into an MS Excel spreadsheet and exported as PDF.

Using human (NP_001358344) reference sequence, amino acids that differ from human SARS-CoV-2 interaction sites were queried manually and recorded in MS Word 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.

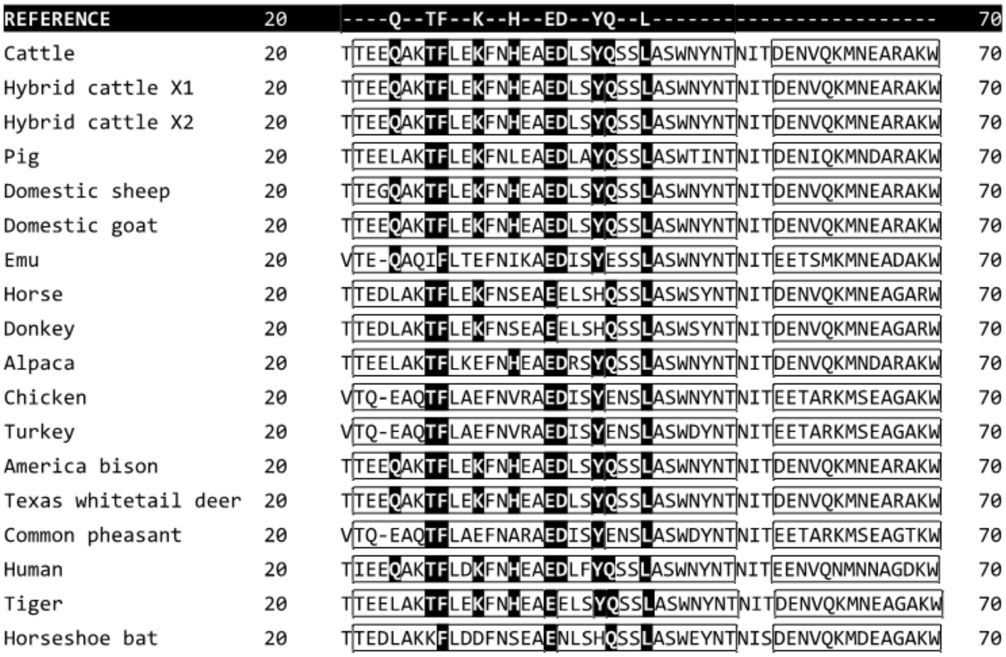
3. Results

Amino acid alignment of the ACEII binding domain (Figure 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 ≥78% whole gene identity to all species in this study except the four birds: emu 65%, turkey 55%, chicken 66%, and pheasant 66% (Fig. 2a-c). When only the 20 ACEII interaction sites are analyzed, all species except the four birds, emu 53%, turkey 58%, chicken 58%, and pheasant 58%, in addition to bat (55%), demonstrate ≥75% similarity (Fig. 3a-c).

Our study noted the following AA residue differencesin 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 (Figure 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 ten interaction sites. The known host tiger and cattle, sheep, goat, bison, and deer contained the most SARS-CoV-2 interaction sites outside humans (Table 2). 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.



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Hybrid cattle X1      20    TTEEQAKTFLEKFNHEAEDLSYQSSLASWNYNTNITDENVQKMNEARAKW    70
Hybrid cattle X2      20    TTEEQAKTFLEKFNHEAEDLSYQSSLASWNYNTNITDENVQKMNEARAKW    70
Pig                   20    TTEELAKTFLEKFNLEAEDLAYQSSLASWTINTNITDENIQKMNDARAKW    70
Domestic sheep        20    TTEGQAKTFLEKFNHEAEDLSYQSSLASWNYNTNITDENVQKMNEARAKW    70
Domestic goat         20    TTEEQAKTFLEKFNHEAEDLSYQSSLASWNYNTNITDENVQKMNEARAKW    70
Emu                   20    VTE-QAQIFLTEFNIKAEDISYESSLASWNYNTNITEETSMKMNEADAKW    70
Horse                 20    TTEDLAKTFLEKFNSEAEELSHQSSLASWSYNTNITDENVQKMNEAGARW    70
Donkey                20    TTEDLAKTFLEKFNSEAEELSHQSSLASWSYNTNITDENVQKMNEAGARW    70
Alpaca                20    TTEELAKTFLKEFNHEAEDRSYQSSLASWNYNTNITDENVQKMNDARAKW    70
Chicken               20    VTQ-EAQTFLAEFNVRAEDISYENSLASWNYNTNITEETARKMSEAGAKW    70
Turkey                20    VTQ-EAQTFLAEFNVRAEDISYENSLASWDYNTNITEETARKMSEAGAKW    70
America bison         20    TTEEQAKTFLEKFNHEAEDLSYQSSLASWNYNTNITDENVQKMNEARAKW    70
Texas whitetail deer  20    TTEEQAKTFLEKFNHEAEDLSYQSSLASWNYNTNITDENVQKMNEARAKW    70
Common pheasant       20    VTQ-EAQTFLAEFNARAEDISYENSLASWDYNTNITEETARKMSEAGTKW    70
Human                 20    TIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMNNAGDKW    70
Tiger                 20    TTEELAKTFLEKFNHEAEELSYQSSLASWNYNTNITDENVQKMNEAGAKW    70
Horseshoe bat         20    TTEDLAKKFLDDFNSEAENLSHQSSLASWEYNTNISDENVQKMDEAGAKW    70
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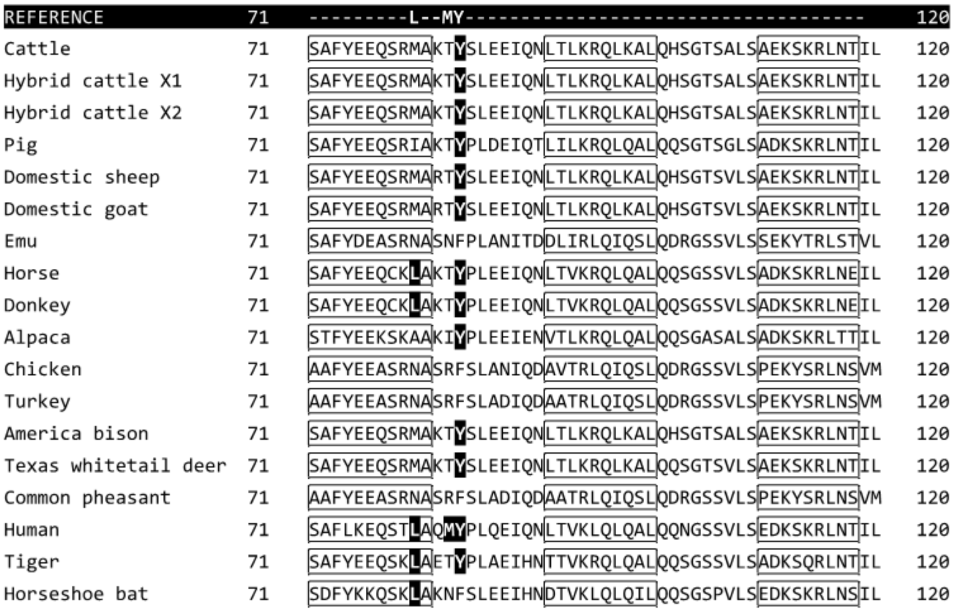
Figure 1a



REFERENCE	71	---------L--MY-----------------------------------	120
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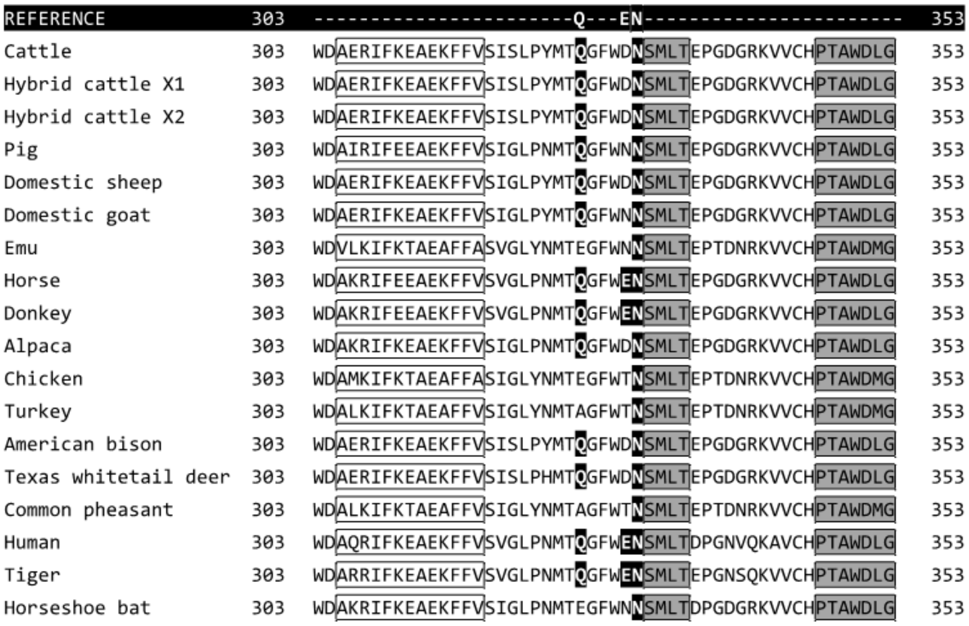
Hybrid cattle X1	71	SAFYEEQSRMAKTYSLEEIQNLTLKRQLKALQHSGTSALSAEKSKRLNTIL	120
Hybrid cattle X2	71	SAFYEEQSRMAKTYSLEEIQNLTLKRQLKALQHSGTSALSAEKSKRLNTIL	120
Pig	71	SAFYEEQSRIAKTYPLDEIQTLILKRQLQALQQSGTSGLSADKSKRLNTIL	120
Domestic sheep	71	SAFYEEQSRMARTYSLEEIQNLTLKRQLKALQHSGTSVLSAEKSKRLNTIL	120
Domestic goat	71	SAFYEEQSRMARTYSLEEIQNLTLKRQLKALQHSGTSVLSAEKSKRLNTIL	120
Emu	71	SAFYDEASRNASNFPLANITDDLIRLQIQSLQDRGSSVLSSEKYTRLSTVL	120
Horse	71	SAFYEEQCKLAKTYPLEEIQNLTVKRQLQALQQSGSSVLSADKSKRLNEIL	120
Donkey	71	SAFYEEQCKLAKTYPLEEIQNLTVKRQLQALQQSGSSVLSADKSKRLNEIL	120
Alpaca	71	STFYEEKSKAAKIYPLEEIENVTLKRQLQALQQSGASALSADKSKRLTTIL	120
Chicken	71	AAFYEEASRNASRFSLANIQDAVTRLQIQSLQDRGSSVLSPEKYSRLNSVM	120
Turkey	71	AAFYEEASRNASRFSLADIQDAATRLQIQSLQDRGSSVLSPEKYSRLNSVM	120
America bison	71	SAFYEEQSRMAKTYSLEEIQNLTLKRQLKALQHSGTSALSAEKSKRLNTIL	120
Texas whitetail deer	71	SAFYEEQSRMAKTYSLEEIQNLTLKRQLKALQQSGTSVLSAEKSKRLNTIL	120
Common pheasant	71	AAFYEEASRNASRFSLADIQDAATRLQIQSLQDRGSSVLSPEKYSRLNSVM	120
Human	71	SAFLKEQSTLAQMYPLQEIQNLTVKLQLQALQQNGSSVLSEDKSKRLNTIL	120
Tiger	71	SAFYEEQSKLAETYPLAEIHNTTVKRQLQALQQSGSSVLSADKSQRLNTIL	120
Horseshoe bat	71	SDFYKKQSKLAKNFSLEEIHNDTVKLQLQILQQSGSPVLSEDKSKRLNSIL	120

Figure 1b



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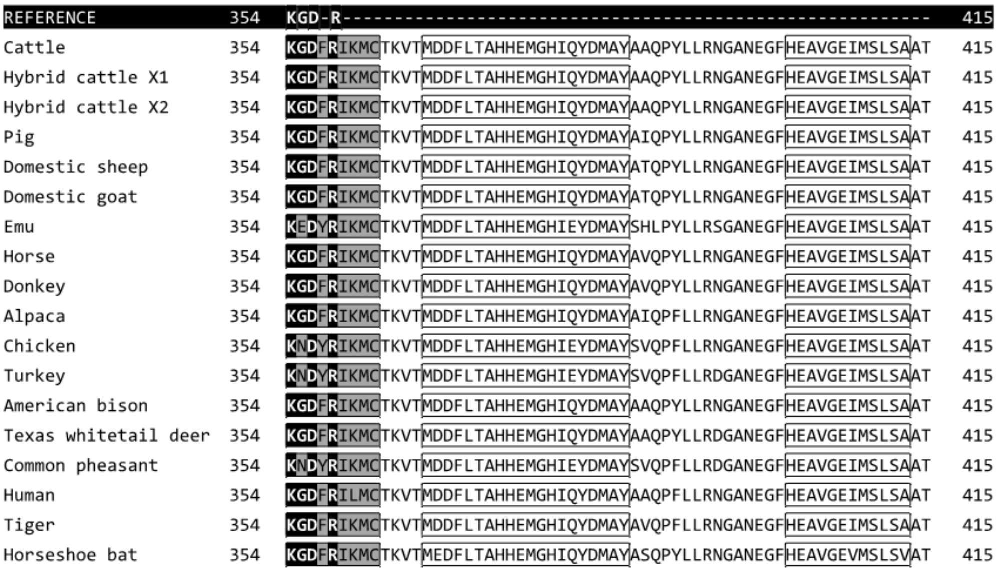
Hybrid cattle X1	303	WDAERIFKEAEKFFVSISLPYMTQGFWDNSMLTEPGDGRKVVCHPTAWDLG	353
Hybrid cattle X2	303	WDAERIFKEAEKFFVSISLPYMTQGFWDNSMLTEPGDGRKVVCHPTAWDLG	353
Pig	303	WDAIRIFEEAEKFFVSIGLPNMTQGFWNNSMLTEPGDGRKVVCHPTAWDLG	353
Domestic sheep	303	WDAERIFKEAEKFFVSIGLPYMTQGFWDNSMLTEPGDGRKVVCHPTAWDLG	353
Domestic goat	303	WDAERIFKEAEKFFVSIGLPYMTQGFWNNSMLTEPGDGRKVVCHPTAWDLG	353
Emu	303	WDVLKIFKTAEAFFASVGLYNMTEGFWNNSMLTEPTDNRKVVCHPTAWDMG	353
Horse	303	WDAKRIFEEAEKFFVSVGLPNMTQGFWENSMLTEPGDGRKVVCHPTAWDLG	353
Donkey	303	WDAKRIFEEAEKFFVSVGLPNMTQGFWENSMLTEPGDGRKVVCHPTAWDLG	353
Alpaca	303	WDAKRIFKEAEKFFVSIGLPNMTQGFWDNSMLTEPGDGRKVVCHPTAWDLG	353
Chicken	303	WDAMKIFKTAEAFFASIGLYNMTEGFWTNSMLTEPTDNRKVVCHPTAWDMG	353
Turkey	303	WDALKIFKTAEAFFVSIGLYNMTAGFWTNSMLTEPTDNRKVVCHPTAWDMG	353
American bison	303	WDAERIFKEAEKFFVSISLPYMTQGFWDNSMLTEPGDGRKVVCHPTAWDLG	353
Texas whitetail deer	303	WDAERIFKEAEKFFVSISLPHMTQGFWDNSMLTEPGDGRKVVCHPTAWDLG	353
Common pheasant	303	WDALKIFKTAEAFFVSIGLYNMTAGFWTNSMLTEPTDNRKVVCHPTAWDMG	353
Human	303	WDAQRIFKEAEKFFVSVGLPNMTQGFWENSMLTDPGNVQKAVCHPTAWDLG	353
Tiger	303	WDARRIFKEAEKFFVSVGLPNMTQGFWENSMLTEPGNSQKVVCHPTAWDLG	353
Horseshoe bat	303	WDAKRIFKEAEKFFVSIGLPNMTEGFWNNSMLTDPGDGRKVVCHPTAWDLG	353

Figure 1c



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Hybrid cattle X2     354  KGDFRIKMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPYLLRNGANEGFHEAVGEIMSLSAAT  415
Pig                  354  KGDFRIKMCTKVTMDDFLTAHHEMGHIQYDMAYAIQPYLLRNGANEGFHEAVGEIMSLSAAT  415
Domestic sheep       354  KGDFRIKMCTKVTMDDFLTAHHEMGHIQYDMAYATQPYLLRNGANEGFHEAVGEIMSLSAAT  415
Domestic goat        354  KGDFRIKMCTKVTMDDFLTAHHEMGHIQYDMAYATQPYLLRNGANEGFHEAVGEIMSLSAAT  415
Emu                  354  KEDYRIKMCTKVTMDDFLTAHHEMGHIEYDMAYSHLPYLLRSGANEGFHEAVGEIMSLSAAT  415
Horse                354  KGDFRIKMCTKVTMDDFLTAHHEMGHIQYDMAYAVQPYLLRNGANEGFHEAVGEIMSLSAAT  415
Donkey               354  KGDFRIKMCTKVTMDDFLTAHHEMGHIQYDMAYAVQPYLLRNGANEGFHEAVGEIMSLSAAT  415
Alpaca               354  KGDFRIKMCTKVTMDDFLTAHHEMGHIQYDMAYAIQPFLLRNGANEGFHEAVGEIMSLSAAT  415
Chicken              354  KNDYRIKMCTKVTMDDFLTAHHEMGHIEYDMAYSVQPFLLRNGANEGFHEAVGEIMSLSAAT  415
Turkey               354  KNDYRIKMCTKVTMDDFLTAHHEMGHIEYDMAYSVQPFLLRDGANEGFHEAVGEIMSLSAAT  415
American bison       354  KGDFRIKMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPYLLRNGANEGFHEAVGEIMSLSAAT  415
Texas whitetail deer 354  KGDFRIKMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPYLLRDGANEGFHEAVGEIMSLSAAT  415
Common pheasant      354  KNDYRIKMCTKVTMDDFLTAHHEMGHIEYDMAYSVQPFLLRDGANEGFHEAVGEIMSLSAAT  415
Human                354  KGDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNGANEGFHEAVGEIMSLSAAT  415
Tiger                354  KGDFRIKMCTKVTMDDFLTAHHEMGHIQYDMAYAVQPFLLRNGANEGFHEAVGEIMSLSAAT  415
Horseshoe bat        354  KGDFRIKMCTKVTMEDFLTAHHEMGHIQYDMAYASQPYLLRNGANEGFHEAVGEVMSLSVAT  415
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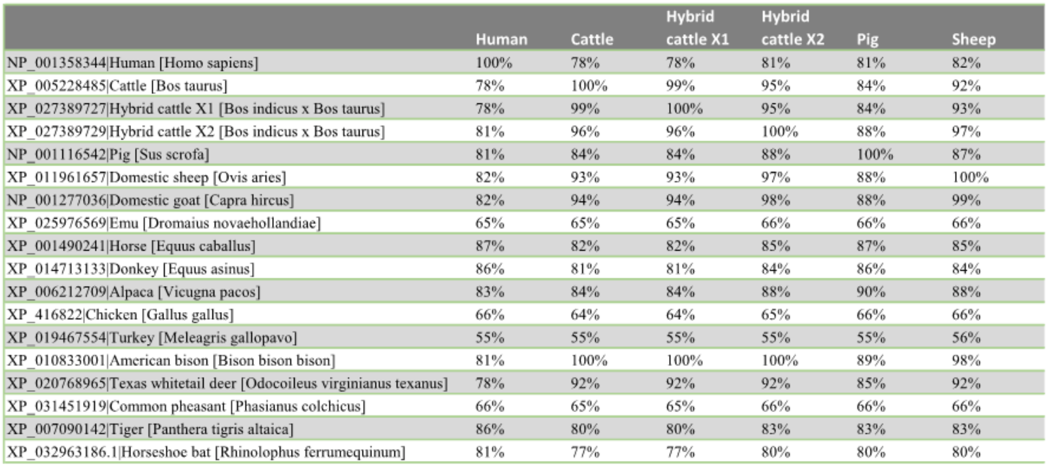
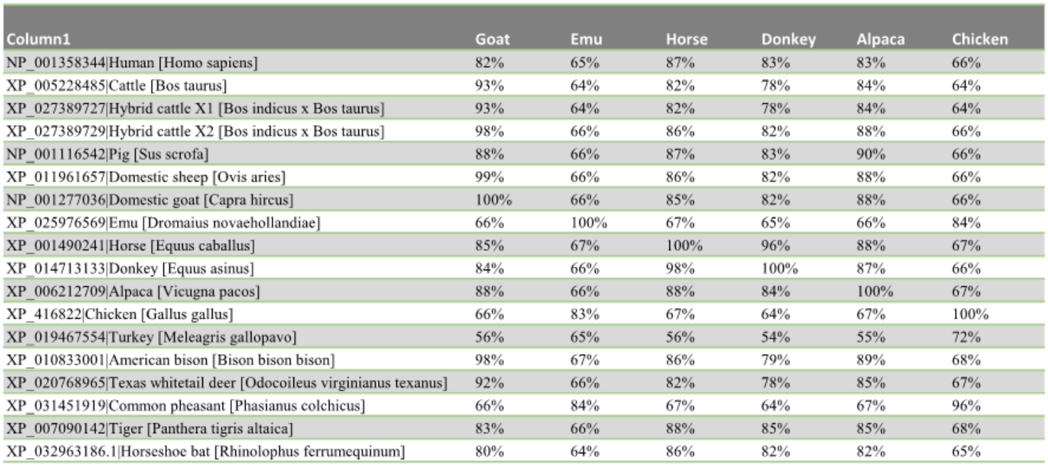
Figure 1d

Figure 1a, 1b, 1c, 1d: ACEII protein sequence alignment. 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.



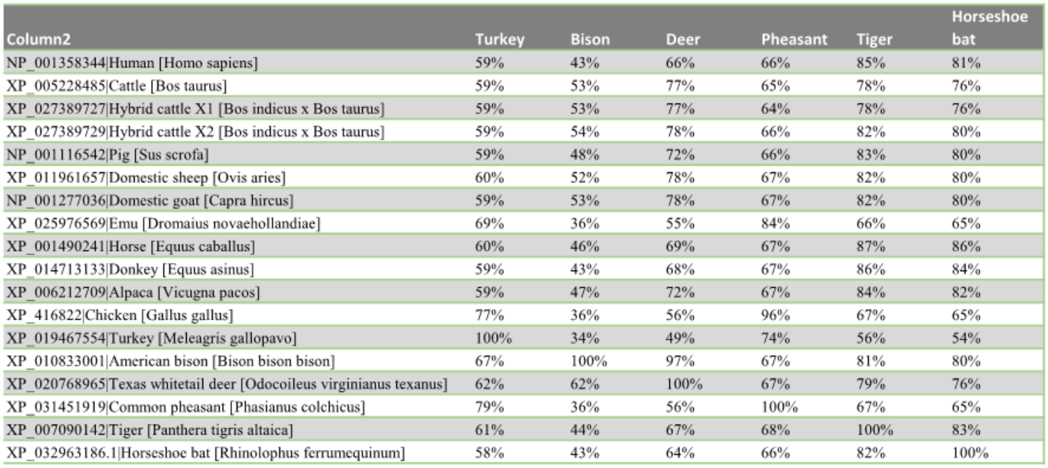
	Human	Cattle	Hybrid cattle X1	Hybrid cattle X2	Pig	Sheep
NP_001358344|Human [Homo sapiens]	100%	78%	78%	81%	81%	82%
XP_005228485|Cattle [Bos taurus]	78%	100%	99%	95%	84%	92%
XP_027389727|Hybrid cattle X1 [Bos indicus x Bos taurus]	78%	99%	100%	95%	84%	93%
XP_027389729|Hybrid cattle X2 [Bos indicus x 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%

Figure 2a



Column1	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%

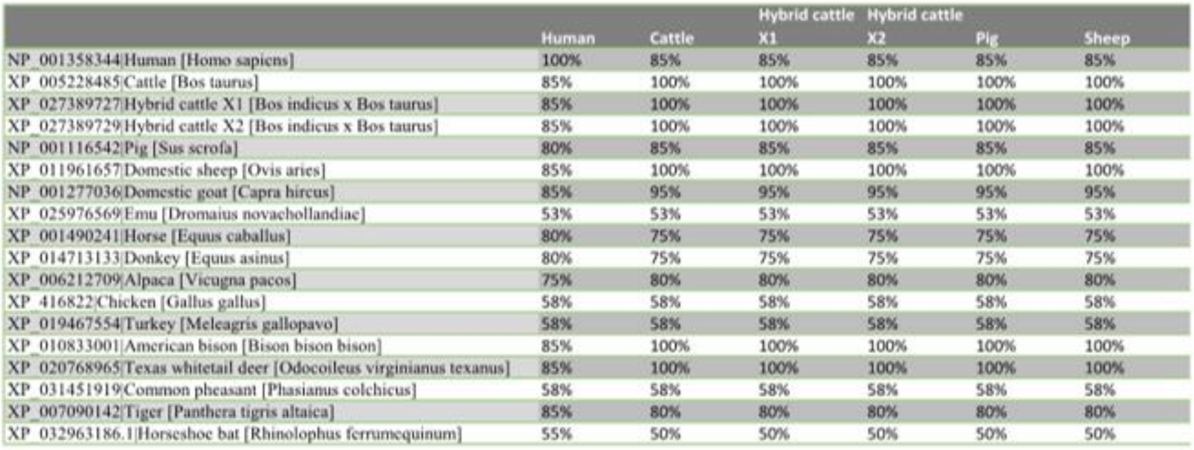
Figure 2b



Column2	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%

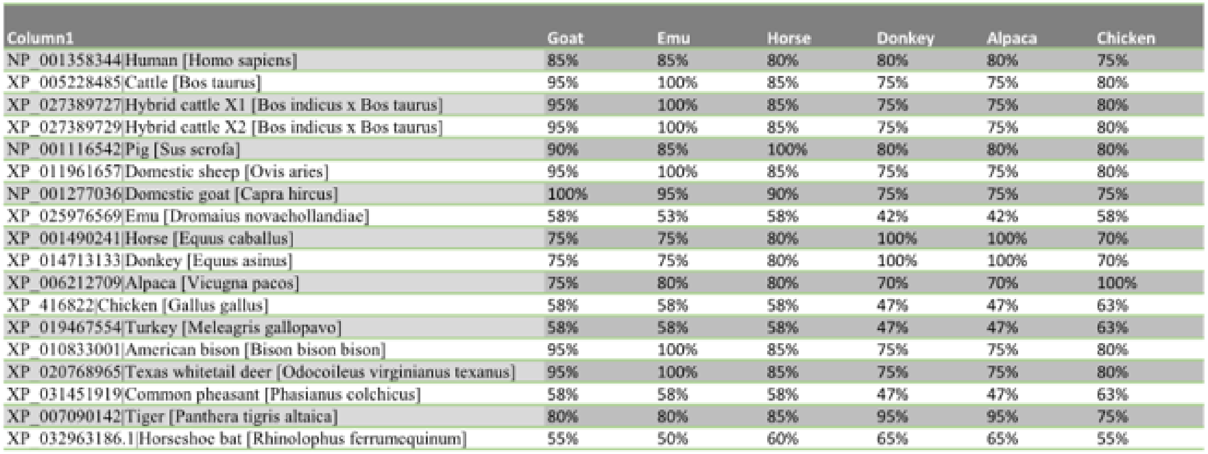
Figure 2c

Figure 2a, 2b, 2c: Distance matrix



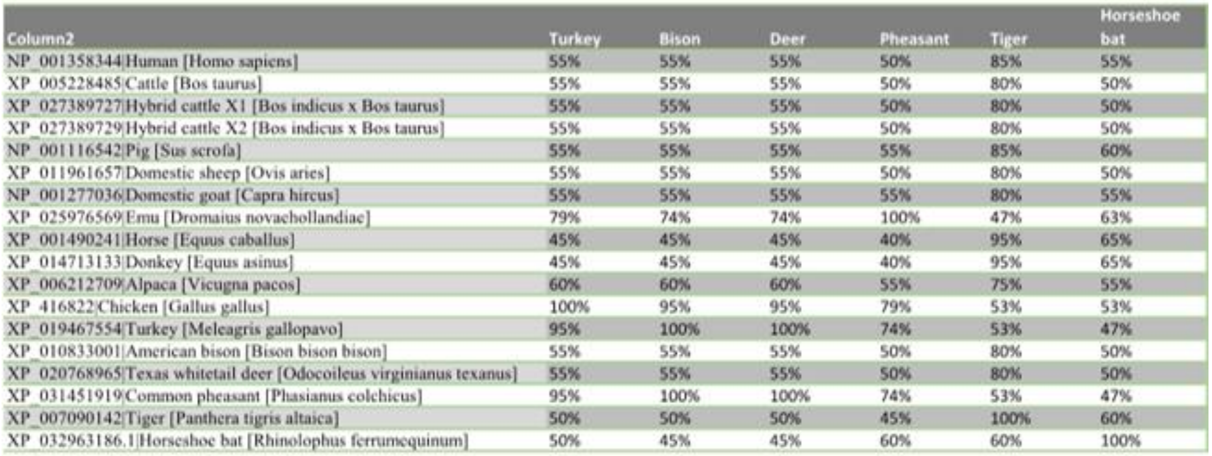
	Human	Cattle	Hybrid cattle X1	Hybrid cattle X2	Pig	Sheep
NP_001358344|Human [Homo sapiens]	100%	85%	85%	85%	85%	85%
XP_005228485|Cattle [Bos taurus]	85%	100%	100%	100%	100%	100%
XP_027389727|Hybrid cattle X1 [Bos indicus x Bos taurus]	85%	100%	100%	100%	100%	100%
XP_027389729|Hybrid cattle X2 [Bos indicus 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%

Figure 3a



Column1	Goat	Emu	Horse	Donkey	Alpaca	Chicken
NP_001358344|Human [Homo sapiens]	85%	85%	80%	80%	80%	75%
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%

Figure 3b



Column2	Turkey	Bison	Deer	Pheasant	Tiger	Horseshoe bat
NP_001358344|Human [Homo sapiens]	55%	55%	55%	50%	85%	55%
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%

Figure 3c

Figure 3a, 3b, 3c: Matrix table using only 20 known SARS-CoV-2/ACEII interaction sites.

Accession number|common name [scientific name]
NP_001358344|Human [Homo sapiens]
XP_005228485|Cattle [Bos taurus]
XP_027389727|Hybrid cattle X1 [Bos indicus x Bos taurus]
XP_027389729|Hybrid cattle X2 [Bos indicus x Bos taurus]
NP_001116542|Pig [Sus scrofa]
XP_011961657|Domestic sheep [Ovis aries]
NP_001277036|Domestic goat [Capra hircus]
XP_025976569|Emu [Dromaius novaehollandiae]
XP_001490241|Horse [Equus caballus]

XP_014713133|Donkey [Equus asinus]
XP_006212709|Alpaca [Vicugna pacos]
XP_416822|Chicken [Gallus gallus]
XP_019467554|Turkey [Meleagris gallopavo]
XP_010833001|American bison [Bison bison bison]
XP_020768965|Texas whitetail deer [Odocoileus virginianus texanus]
XP_031451919|Common pheasant [Phasianus colchicus]
XP_007090142|Tiger [Panthera tigris altaica]
XP_032963186.1|Horseshoe bat [Rhinolophus ferrumequinum]

Table 1: Accession list.

Accession number|Common name	SARS-CoV-2 Binding Sites (20 possible)
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**

Table 2: SARS-CoV-2/ACEII site interaction values.Accessions in the black background are known as SARS-CoV2 hosts.

4. Discussion

4.1 Structural analysis

It has been previously described that the unique SARS-CoV-2 F486 site can bind deep in the ACEII hydrophobic pockets created by F28, L79, Y83, and L97 [1]. The interspecies AA residue differences of those sites noted in this study conserve the hydrophobic pockets in the ACEII complex structure. It retains the hydrophobic pockets by the use of other hydrophobic residues. Of note is the p.Y83F substitution in birds and bats, which creates π-stacking of aromatic residues, an effect that can alter drug effectiveness/design. It also establishes a disulfide bond with F486 increasing virus binding affinity (k=6) [12]. 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 fewer 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. The number of interaction sites may or may not cause concern, but it certainly leads to a high index of suspicion to their SARS-CoV-2 host viability.

4.2 Historical coronavirus evidence in agricultural species

It is essential to understand that ALL species in this study have been previously identified as coronavirus hosts; cattle [13] chicken, pheasant [14], turkey [15] pig [16], sheep [17], goat, bison [13], emu [18], donkey, horse, [19-21], alpaca, [22, 23] bison [24, 25] and deer [24]. While not SARS-CoV-2, they were of the same closely related viral coronavirus genus. Therefore, we cannot disregard the possibility of SARS-CoV-2 until proven otherwise.

It is still not known what the pathogenic effect, if any, will be in SARS-CoV-2 infected animals. However, looking at historical data from bovine coronavirus (BCoV) infections [26], it is conceivable that SARS-CoV-2 could overcome BCoV as the leading cause of morbidity and mortality in cattle < 2years old.

4.3 SARS-CoV-2 impact on agricultural security

“Agricultural animals are typically kept in large numbers and in close proximity” to each other. As a result, the possibility of one animal infecting another is exceptionally 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 consumption or process handling. However, the absence of this data does not eliminate this prospect.

SARS-C0V-2 has demonstrated surface survivability of up to 9 days [27]. In excreted body fluids, it can survive >7 days [28]. Our understanding is that survivability in biological tissues has yet to be determined, but we can infer survivability of the virus is also >7 days from excreted fluids. Within biological tissues, the virus' survivability could increase beyond 20 days when refrigerated [29], a vital process to advert food spoilage. A commonly used method to increase food preservation is gamma irradiation [30, 31]. However, this process may not inactivate enough of the virus to prevent viral transmission, as demonstrated from gamma irradiation studies with the Ebola virus [32]. In that study, it was determined that "no dosage could be considered to inactivate 100% of a sample" [32]. Principally, the 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 100 days at 4ºC and approximately ten days at 23ºC [33]. This study also indicates the possibility of human-to-animal transmission through an open water source.

4.4 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. However, 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 vector, or a contaminated food source.

Understanding the potential impact of SARS-CoV-2 on animals is paramount to agricultural and national security [34-37]. Until now, there has been no data to support a threat to the global food supply. Existing control measures are not adequate to mitigate SARS-CoV-2 propagation. Agricultural industries should begin preparing for a worst-case scenario before they are forced to respond to one.

Using the human ACEII gene sequence as a reference limit this study since actual SARS-CoV-2/ACEII animal complex structure interactions could differ in animals. Another limitation is that SARS-CoV-2 survivability in tissues and water must be inferred from other coronaviruses and may vary under identical environmental conditions. Finally, the lack of animal testing precludes a definitive viral susceptibility analysis. Nevertheless, 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.

5. 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 should be considered at serious 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 after processing.

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Author contributions

Michael Ruhl was performed data query, protein analysis, literature review, developed the initial manuscript, and produced tables and graphics. Tracie Jenkins was the primary editor and contributed to genomic analysis.

Competing interests

Authors have no competing interests to declare.

Data and materials availability

All accessions are publicly available and listed in Table 1. All software used is publicly available, and citations are provided in body text.

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