Predicting the susceptibility of animals to COVID-19

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The SARS-CoV-2 virus has spread rapidly across the globe, with millions of people now testing positive for COVID-19. The disease was thought to have started in Wuhan, Hubei Province, People's Republic of China, with the virus considered to have originated in a bat, passing to humans, possibility through a secondary species, which has been suggested to be pangolins. For a recent overviews of the SARS-CoV-2 and COVID-19 see Russell *et al.* (2020) or Singhal (2020).

The SARS-CoV-2 virus is a RNA containing enveloped virus which when viewed at high magnification resembles a crown or corona, hence the name as coronavirus. It is not totally unique, as it closely resembles viruses which caused the severe acute respiratory syndrome (SARS) outbreak of 2003 and Middle East respiratory syndrome (MERS) in 2015. The action of the virus requires it to recognise its host cells, and then invade, hijacking the molecular machinery of the host's cells to create more virus particles. These new virus particles can then invade further cells in the host, rapidly progressing the disease, or the new virus can be released from the host to invade new hosts.

UK government policy (https://www.gov.uk/coronavirus) is based on the risk to humans and the spread of the disease. However, as this is believed to be a zoonotic disease with its origin in animals, there is a potential risk from and to animals too. Animals may be a contact source of the virus, being simply carried on their fur or feathers, but they may also be able to act as a viral host. Several species are known to be vulnerable. Shi *et al.* (2020) reported that cats and ferrets are susceptible to COVID-19 but that the virus does not replicate well in pigs, dogs, chickens and ducks. COVID-19 has been a problem in mink farms in the Netherlands (Enserink, 2020) and more recently in Spain. Coronaviruses have been reported in the past to affect dogs (van Nguyen *et al.*, 2017), cats (Tekes and Thiel, 2016) and even marine mammals (Mihindukulasuriya *et al.*, 2008). Therefore, there is a potential for the SARS-CoV-2 virus to affect animals either kept in captivity or in the wild.

For the virus to be able to invade its host, it needs to have a way to recognise the host cells and a mechanism to then get into those cells. It is known that the SARS-

CoV-2 virus recognises a protein in humans cells called angiotensin-converting-enzyme-2 (ACE2) (Wan *et al.*, 2020). This is one of a group of proteins known as peptidases, recognised to be the targets of coronaviruses. ACE2 has a normal function in human cells, but its presence is hijacked by the virus as a means of cell entry. The ACE2 protein is composed of 805 amino acids (in humans), which are arranged in a three dimensional structure. ACE2 is the target of one of the spike proteins projecting from the virus envelop, and therefore there must be a protein-to-protein recognition event. There will be certain regions on both proteins which come together and enable the proteins to bind, sticking the virus to the cell surface, an event which will initiate the mechanisms for viral entry into the cell. Therefore, there has been a considerable amount of work which has studied the structure of both proteins to determine which particular areas of each are important for this interaction. However, it will not necessarily be amino acids which are directly next to each other in a sequence that may be involved, as it must be remembered that both proteins have a complex three dimensional structure, so amino acids which are far apart in a sequence can in reality be close together in three dimensions. An example of such a study of the SARS-CoV-2/ACE2 interaction is by Shang *et al.* (2020).

Using the human sequence and structural analysis of the ACE2 protein, it has been determined that there are 25 amino acids (out of the total of 805) which are likely to be important for the SARS-CoV-2 binding (see Damas *et al.*, 2020). These are clustered in regions along the protein sequence. Interestingly, some of them are able to be modified by the addition of sugars, i.e. they are glycosylated. Glycosylated proteins are known to have their sugar moieties outside the cell surface, and are often involved in the interaction of the cell with its environment; sometimes that would be another cell in the tissue. Therefore, the involvement of such amino acids in the SARS-Cov-2 interaction comes as no great surprise.

The question here, is whether such human derived information can be translated to animal species, and whether it can be predicted if specific animal species are susceptible to SARS-CoV-2. If animals were found to be vulnerable this might have a profound influence how we should manage future human-to-animal contact, and therefore animal welfare. Humans could unwittingly give COVID-19 to animals, or, on the other hand, if it was known that certain species were inclined to get the disease, such transmission may be able to be avoided or at least mitigated.

The amino acid sequence of ACE2 proteins from a wide range of animal species are held in genetic databases around the world, and these are publically available (for example at NCBI: https://www.ncbi.nlm.nih.gov/). It is easy to therefore download such sequences and compare them to the human sequence. Comparisons can then be easily carried out with publically available programmes such as *Clustal Omega* (Sievers *et al.*, 2011). The human sequence is known to bestow on us our inclination to contract the disease, so if an animal has the same sequence then it too may be susceptible, at least to SARS-Cov-2 binding to the surface of some of its cells. If the sequence is dramatically different it suggests that SARS-Cov-2 binding is not possible, and without the virus and cells being able to interact then the disease cannot take hold. This is a rather naïve view as the initiation and progression of the disease is complex, but it gives an indication of whether an animal is likely to be attacked by the virus.

Taking this approach here, the ACE2 amino acid sequences of twelve animal species were aligned and then the amino acids suggested to be important from other studies were highlighted, as shown in Figure 1 (note: only the first 46 amino acids at the N-terminal end of ACE2 are shown here (human numbering)). By doing this type of alignment it can easily be seen that primates such as gorillas and macaques have an identical sequence to humans and therefore appear to have the protein machinery to allow SARS-CoV-2 to bind to their cells. On the other hand, birds and fish have quite disparate sequences and many of the vital amino acids are different in the relevant sequence positions. This analysis, which is relatively easy and quick to carry out, matches the points made by Shi *et al.* (2020) who said that the virus replicates badly in chickens and ducks.

This ACE2 analysis is rather simple and naïve, but others have taken the idea to the next level (Damas *et al.*, 2020; Pach *et al.*, 2020; Sun *et al.*, 2020). By considering the actual three dimensional structure of the ACE2 protein, and then designed algorithms to analyse the differences, an opinion as to whether particular animals are likely to catch COVID-19 can be obtained. An excellent example of this is the study by Damas *et al.* (2020) where they analysed the ACE2 sequences from 410 vertebrates, including 252 mammals, 65 fish, 72 birds, 4 amphibians and 17 reptiles. They could then rank the animals for the likelihood of being infected, in categories of very high, high, medium, low or very low. As predicted from the simple analysis here, Damas *et al.* (2020) also found that species such as gorillas, macaques and monkeys

are in the very high group. In the high category are some marine mammals, such as the beluga whale, killer whale and the common bottlenose dolphin. Considering animals with which people are more likely to interact, sheep, cattle, goats, cats and rabbits were in the medium group, whilst dogs, donkey and horse were all categorised as low. Some bats were also classed as low. In the very low likelihood class are many rats, mice and shrews, as well as the hedgehog. Interestingly, many bat species came out in the very low group. Damas *et al.* (2020) suggested that it may be possible that SARS-CoV-2 was using a different cell surface protein in some animals such as bats, which of course throws doubt on the rationale of this approach of analysing ACE2. Having said that, this approach could be used to analyse any future proteins which may be deemed to be important in SARS-Cov-2 host invasion.

Damas *et al.* (2020) also looked at 158 non-mammalian vertebrates and reported that all birds, fish, amphibians and reptiles could be classed as very low in their likelihood to be vulnerable to COVID-19, based on their ACE2 sequences.

In conclusion, future animal welfare may need to consider whether current practices of handling and managing animal species (including companion and farm/zoo animals), and interactions with populations of wild animals, is safe for both animals and humans during the present COVID-19 pandemic. Infection with the COVID-19 virus appears to require the presence of a cell surface protein, ACE2. By analysing the amino acid composition of the ACE2 protein from different animal species an indication of the vulnerability of animal species can be gleaned. Primates seem to be particularly at risk, with birds, fish and reptiles all being at very low risk. Many non-primate mammals were categorised with a likelihood of high or medium vulnerability. Therefore, such information should be taken into account in future animal welfare practices. Animals in captivity and in the wild should not be assumed to be safe from COVID-19, although the severity of the disease is not known for most animals. But it is likely that even asymptomatic animals may carry and spread the disease. However, some animals are at more risk than others, and by analysing the proteins involved in SARS-COV-2 infection mechanisms, an estimation of the risk of certain animal species can be gained which can inform animal management practice.

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Figure 1. Alignment of amino acid sequences of ACE2 receptor proteins for 12 animal species, highlighting the amino acids used for SARS-CoV-2 binding.
Yellow highlight; amino acids suggested by Shang *et al*. (2020): green highlight; the amino acids suggested by Shang *et al*. (2020) and by Sun *et al*. (2020). Pink highlight; different amino acids also suggested by Damas *et al*. (2020). Grey highlights are to show human sequence to clarity. Animal sequences chosen were representative example of animal groups. Specifically they were: Fish: cod (*Gadus morhua*) Ac: XP_030232530.1; Bird, Rock Pigeon (*Columba livia*) Ac: XP_021154486.1; Bat (*Myotis lucifugus*) Ac: XP_023609437.1; Rodent (*Ictidomys tridecemlineatus*) Ac: XP_005316051.3; Rabbit (*Oryctolagus cuniculus*) Ac: QHX39726.1; Macaque (*Macaca nemestrina*) Ac: XP_011733505.1; Humans (*Homo sapiens*) Ac: NP_001358344.1; Gorilla (*Gorilla gorilla gorilla*) Ac: XP_018874749.1; Cat (*Lynx pardinus*) Ac: VFV30336.1; Dog (*Canis lupus familiaris*) Ac: QJS40032.1; Camel (*Camelus ferus*) Ac: XP_006194263.1; Dolphin; Common bottlenose (*Tursiops truncates*) Ac: XP_019781177.2.

Output from Clustal Omega, including consensus analysis as the last row.

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Fish      MSTA--GRV-AAGAAAMLLLVVALLTPGLRAQVDTETRARAFLEKFSTEASVKMYDYSLA   57
Bird      --------MDMLVCIWLLCG----LIAVVSPQT-VTQQAQMFLEEFNKRAEDINYESSLA   47
Bat       ----------MSGSSWLFLS----LVAVAAAQSSTEEKAKIFLENFNSKAEDLSHESALA   46
Rodent    MGSCPGARGKMLGSSWLLLS----FVAVTAAQSTIEELAKTFLDKFNQEAEDLDYQRSLA   56
Rabbit    ----------MSGSSWLLLS----LVAVTAAQSTIEELAKTFLEKFNQEAEDLSYQSALA   46
Macaque   ----------MSGSSWLLLS----LVAVTAAQSTIEEQAKTFLDKFNHEAEDLFYQSSLA   46
Human     ----------MSSSSWLLLS----LVAVTAAQSTIEEQAKTFLDKFNHEAEDLFYQSSLA   46
Gorilla   ----------MSGSSWLLLS----LVAVTAAQSTIEEQAKTFLDKFNHEAEDLFYQSSLA   46
Cat       ---------MSGSFWLLLS----FAALTAAQSTTEELAKTFLEKFNHEAEELSYQSSLA    46
Dog       ---------MSGSSWLLPS----LAALTAAQS-TEDLVKTFLEKFNYEAEELSYQSSLA    45
Camel     ---------MSGSFWLLLS----LVAVTAAQSTTEELAKTFLEEFNHEAEDLSYQSSLA    46
Dolphin   ---------MSGSFWLLLS----LVAVTAAQSATEERAKTFLQKFDREAEDLSYQSSLA    46
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