More efforts are needed for background surveys of zoonotic coronaviruses in animals

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SUMMARY

Recently, a novel dog-origin coronavirus has been found in humans. The low similarity between the receptor-binding domain from this novel virus and other human-infecting coronaviruses in genus Alphacoronavirus suggests it might use a novel receptor or mechanism to enter human cells and also might trigger a novel immune response.

"Host jump" of coronaviruses from animals to humans

Seven types of coronaviruses (CoVs) have been found to infect humans, including human coronavirus (HCoV)-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2. Among them, HCoV-OC43 and HCoV-HKU1 were originated from rodents, while others were derived from bats.1 After spillover to different intermediate hosts and then accumulating genetic mutations and/or recombination, coronaviruses can occasionally acquire the ability to infect humans. In addition, coronaviruses that can infect humans (like SARS-CoV-2, belonging to genus Betacoronavirus) have also been found to infect both domesticated and wild animals.2,3 Until now, limited animals are suspected (Palm civets for SARS-CoV, domesticated animals for HCoV-OC43, or camellids for HCoV-229E) or confirmed (dromedary camels for MERS-CoV) as intermediate hosts for these coronaviruses. However, there are still many coronaviruses infecting humans whose intermediate hosts are still unknown. For example, despite a large amount of work undertaken, the intermediate host of SARS-CoV-2 is still unknown. Due to the high frequency of contact between human and animals in some regions, the zoonotic coronaviruses have been posing a potential threat to public health. As close companions of humans, there are a huge number of dogs all over the world. Canine coronavirus (CCoV, belonging to genus Alphacoronavirus) infection generally causes mild or asymptomatic enteritis, but highly virulent isolates have also gradually been found.4 In addition, previous study has also documented that dogs can be infected with SARS-CoV-2 via humans, as angiotensin-converting enzyme 2 (ACE2, the receptor for SARS-CoV-2) of dogs and humans are as similar as 81% at the amino-acid level.5 However, the cross-species transmission in the “opposite direction” (canine coronavirus infecting humans) has not been found before.

The emergence of canine coronaviruses infecting humans indicates a novel viral entry mechanism

Recently, Vlasova and colleagues isolated and sequenced a novel coronavirus from a hospitalized pneumonia patient in Malaysia, and they also showed that this novel coronavirus was from CCoV and belonged to CCoV genotype II (CCoV-II) in the Alphacoronavirus genus.6 After that, Lednicky and colleagues also isolated a coronavirus with extremely high genomic similarity (99.4%) to the above strain from a member of a medical team returned from Haiti.7 Since coronaviruses from CCoV-II used host aminopeptidase N (hAPN) as a receptor, which was also a receptor for HCoV-229E (belonging to genus Alphacoronavirus), we therefore reasonably speculate that this novel CCoV still used human APN (hAPN) as a receptor. The protein sequences of receptor-binding domain (RBD) are identical between these two novel CCoVs, indicating that they might use the same receptor and mechanism to enter human cells. However, further analysis of RBD in S gene, which determines virus attachment, host cell entry, and “host jump” of coronaviruses,8 showed that nine out of ten of the key residues in the RBD of HCoV-229E directly in contact with its hAPN were found have mutated in this novel CCoV (Figure 1A). In this case, the novel CCoV might not use a similar molecular mechanism to enter human cells like HCoV-229E, or it may not even use hAPN as its receptor. Then, we speculated that this novel CCoV might use ACE2, which is also the receptor for HCoV-NL63 (another coronavirus belonging to genus Alphacoronavirus that infects humans), as its receptor. Our analysis showed that nine out of eleven of the key residues in the RBD of HCoV-NL63 directly in contact with its human receptor (ACE2) were also found to have mutated in this novel...
CCoV (Figure 1A). Since this novel CCoV had been isolated from human, these results suggested that it might either use a novel receptor (other than hAPN and hACE2) or use a novel molecular mechanism to enter human cells through hAPN. Furthermore, RBD has been known to be a major target for neutralizing antibody (nAb), which is responsible for interfering with the binding of the virus to its host receptor. Therefore, currently available nAbs that prevent HCoV-229E and HCoV-NL63 from entering human cells might not work against this novel CCoV. In addition to containing neutralizing epitopes, RBD also contains T cell epitopes. Therefore, the identification of epitopes of this novel CCoV recognized by human T cell response is important for monitoring immune response, vaccine development, and facilitating assessment of immunogenicity for vaccines.

**Several types of coronaviruses may also have the potential to infect humans**

We use BLAST (the basic local alignment search tool) to detect the similarity between the RBD region of this novel CCoV and other viruses of the species Alphacoronavirus 1 and CoVs that infect humans. The RBD of the novel CCoV has an extremely high similarity at amino-acid level to other viruses of species Alphacoronavirus 1 (belonging to genus Alphacoronavirus), but low similarity was found when it was compared to HCoV-NL63 and HCoV-229E (Figure 1B). Since Alphacoronavirus 1 contains several types of coronaviruses, like canine coronavirus, feline coronavirus, transmissible gastroenteritis virus and swine enteric coronavirus, we suspected that the feline coronavirus and swine enteric coronavirus might have acquired the ability to enter human cells already or through limited mutations via a molecular mechanism similar to that of this novel CCoV. Previous study speculated that pig might serve as a mixing vessel for coronaviruses. Given the fact that genetic recombination frequently occurs among coronaviruses and other close-related CoVs (like feline and porcine strains), we speculated that dog and cat might also serve as potential mixing vessels for coronaviruses, which could generate more novel coronaviruses with unknown risk. Recently, porcine deltacoronavirus from genus Deltacorona-\textbf{virus} has been detected in humans. This has also expanded our knowledge of the phylogenetic range and intermediate hosts of coronaviruses that can infect humans, which were considered to all belong to genus Alphacoronavirus (HCoV-229E, HCoV-NL63) and Betacoronavirus (HCoV-OC43, HCoV-HKU1, SARS-CoV, SARS-CoV-2, and MERS-CoV) so far. Since cats, dogs, and pigs are all in close contact with humans, once a novel coronavirus that can infect humans is accidentally generated within them, it will be quickly transmitted to humans, posing a potential threat to public health.

**Concluding remarks**

The identification of novel CCoV in humans expanded our knowledge of the host range of dog-origin coronavirus and also the intermediate hosts for human-infecting coronaviruses. Since the volume of the population of dogs is very high (900 million globally) and their contact with humans and other animals is very frequent, there is a high probability of cross-species transmission of viruses. Despite considerable progress in characterizing the cross-species transmission for coronaviruses, several areas also need to be resolved, including: (i) confirmation of the human receptor for this novel CCoV; (ii) the molecular mechanism
of this novel CCoV used to enter human cells; (iii) human immune response triggered by this novel CCoV; (iv) development of a vaccine against this novel CCoV to deal with the potential risk of outbreaks in humans in the future. In-depth study of the molecular and cellular mechanism of viral entry into human cells of this novel CCoV and development of vaccines against it should be performed immediately to answer these urgent questions.

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AUTHOR CONTRIBUTIONS

L.W., Y.B., and G.F.G designed and coordinated the study, L.W., J.Y., and K.S. collected data and performed the analysis, L.W., J.Y., K.S., Y.B., and G.F.G contributed to the critical interpretation of the results. L.W. wrote the paper. L.W., J.Y., K.S., Y.B., and G.F.G, revised the manuscript. All authors reviewed and edited the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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