Cell Reports Medicine

CellPress
OPEN ACCESS

Commentary

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

Liang Wang,[1,4,*] Jing Yang,[1,4] Kangtai Sun,[2,4] Yuhai Bi,[1,3,*] and George F. Gao[1,3,*]

[1]CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Center for Influenza Research and Early-warning (CASCIRE), CAS-TWAS Center of Excellence for Emerging Infectious Diseases (CEEID), Chinese Academy of Sciences, Beijing 100101, China

[2]China Rural Technology Development Center, Beijing 100038, China

[3]University of Chinese Academy of Sciences, Beijing 101409, China

[4]These authors contributed equally

*Correspondence: wangliang@im.ac.cn (L.W.), beeyh@im.ac.cn (Y.B.), gaof@im.ac.cn (G.F.G.)

https://doi.org/10.1016/j.xcrm.2022.100524

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 camelids 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 (APN) 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

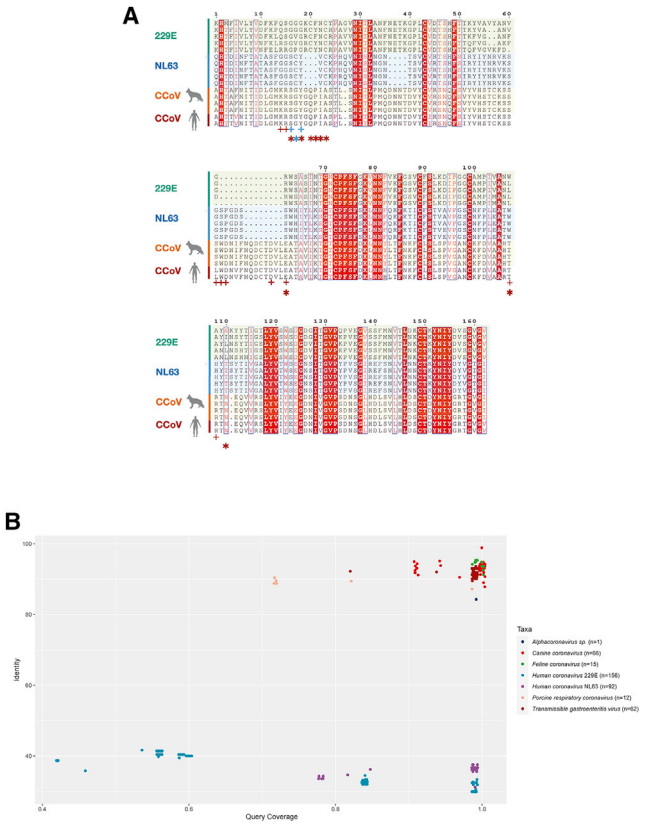

Figure 1. Sequence similarity overview of the novel canine coronavirus infecting humans

(A) Alignment of the receptor binding domain in the S gene. Residues in direct contact with the human receptor for HCoV-NL63 are indicated by a (+) sign. If a substitution occurred in novel CCoV compared to HCoV-NL63, the color of the (+) sign is red; otherwise, the color of the (+) sign is blue. Residues in direct contact with the human receptor for HCoV-229E are indicated by a (*) sign. If a substitution occurred in novel CCoV compared to HCoV-229E, the color of the (*) sign is red; otherwise, the color of the (*) sign is blue. Sequences from novel CCoV, CCoV, HCoV-299E, and HCoV-NL63 are indicated by different colors. (B) The sequence similarity between RBD of novel CCoV and other coronaviruses. Protein BLAST was performed using RBD from novel CCoV as a query against the nonredundant protein database, HCoV-NL63, and HCoV-229E. Only sequences with both coverage ≥ 70% and identity ≥ 80% were retained when compared to the whole nonredundant protein database. The number of sequences belonging to each species used in this study are listed within parenthesis behind the virus species name in the figure.

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.[9] 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 that belong to the genus *Alphacoronavirus* at the protein level. 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.[10] Given the fact that genetic recombination frequently occurs among coronaviruses[11,12] and Spike has been found among CCoV and other close-related CoVs (like feline and porcine strains),[13] 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 *Deltacoronavirus* has been detected in humans.[14] 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[15]) 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.

ACKNOWLEDGMENTS

We gratefully acknowledge the authors from the originating laboratories and the submitting laboratories where genetic sequence data were generated, enabling this research. This work was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (Grant No. XDB29010202 and XDB29010102) and the intramural special grant for SARS-CoV-2 research from the Chinese Academy of Sciences, National Natural Science Foundation of China (NSFC) (Grant No. 32041010 and 31900155). Y.B. is supported by the NSFC Outstanding Young Scholars (Grant number 31822055) and Youth Innovation Promotion Association of CAS (Grant number 2017122).

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.

REFERENCES

1. Cui, J., Li, F., and Shi, Z.L. (2019). Origin and evolution of pathogenic coronaviruses. Nat. Rev. Microbiol. *17*, 181–192. https://doi.org/10.1038/s41579-018-0118-9.
2. Wang, L., Didelot, X., Bi, Y., and Gao, G.F. (2021). Assessing the extent of community spread caused by mink-derived SARS-CoV-2 variants. Innovation *2*, 100128. https://doi.org/10.1016/j.xinn.2021.100128.
3. Gao, G.F., and Wang, L. (2021). COVID-19 expands its territories from humans to animals. China CDC Wkly *3*, 855–858. https://doi.org/10.46234/ccdcw2021.210.
4. Decaro, N., and Buonavoglia, C. (2008). An update on canine coronaviruses: viral evolution and pathobiology. Vet. Microbiol. *132*, 221–234. https://doi.org/10.1016/j.vetmic.2008.06.007.
5. Sit, T.H.C., Brackman, C.J., Ip, S.M., Tam, K.W.S., Law, P.Y.T., To, E.M.W., Yu, V.Y.T., Sims, L.D., Tsang, D.N.C., Chu, D.K.W., et al. (2020). Infection of dogs with SARS-CoV-2. Nature *586*, 776–778. https://doi.org/10.1038/s41586-020-2334-5.
6. Vlasova, A.N., Diaz, A., Damtie, D., Xiu, L., Toh, T.H., Lee, J.S., Saif, L.J., and Gray, G.C. (2021). Novel canine coronavirus isolated from a hospitalized pneumonia patient, East Malaysia. Clin. Infect. Dis., ciab456. https://doi.org/10.1093/cid/ciab456.
7. Lednicky, J.A., Tagliamonte, M.S., White, S.K., Blohm, G.M., Alam, M.M., Iovine, N.M., Salemi, M., Mavian, C., and Morris, J.G., Jr. (2021). Isolation of a novel recombinant canine coronavirus from a visitor to Haiti: further evidence of transmission of coronaviruses of zoonotic origin to humans. Clin. Infect. Dis., ciab924. https://doi.org/10.1093/cid/ciab924.
8. Lu, G., Wang, Q., and Gao, G.F. (2015). Bat-to-human: spike features determining 'host jump' of coronaviruses SARS-CoV, MERS-CoV, and beyond. Trends Microbiol. *23*, 468–478. https://doi.org/10.1016/j.tim.2015.06.003.
9. Dai, L., and Gao, G.F. (2021). Viral targets for vaccines against COVID-19. Nat. Rev. Immunol. *21*, 73–82. https://doi.org/10.1038/s41577-020-00480-0.
10. Wang, L., Su, S., Bi, Y., Wong, G., and Gao, G.F. (2018). Bat-origin coronaviruses expand their host range to pigs. Trends Microbiol. *26*, 466–470. https://doi.org/10.1016/j.tim.2018.03.001.
11. Su, S., Wong, G., Shi, W., Liu, J., Lai, A.C.K., Zhou, J., Liu, W., Bi, Y., and Gao, G.F. (2016). Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends Microbiol. *24*, 490–502. https://doi.org/10.1016/j.tim.2016.03.003.
12. Chan, J.F., To, K.K., Tse, H., Jin, D.Y., and Yuen, K.Y. (2013). Interspecies transmission and emergence of novel viruses: lessons from bats and birds. Trends Microbiol. *21*, 544–555. https://doi.org/10.1016/j.tim.2013.05.005.
13. Licitra, B.N., Duhamel, G.E., and Whittaker, G.R. (2014). Canine enteric coronaviruses: emerging viral pathogens with distinct recombinant spike proteins. Viruses *6*, 3363–3376. https://doi.org/10.3390/v6083363.
14. Lednicky, J.A., Tagliamonte, M.S., White, S.K., Elbadry, M.A., Alam, M.M., Stephenson, C.J., Bonny, T.S., Loeb, J.C., Telisma, T., Chavannes, S., et al. (2021). Independent infections of porcine deltacoronavirus among Haitian children. Nature *600*, 133–137. https://doi.org/10.1038/s41586-021-04111-z.
15. Lescureux, N., and Linnell, J.D.C. (2014). Warring brothers: the complex interactions between wolves (Canis lupus) and dogs (Canis familiaris) in a conservation context. Biol. Conserv. *171*, 232–245. https://doi.org/10.1016/j.biocon.2014.01.032.