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Review of "Identifying SARS-CoV-2 related coronaviruses in Malayan pangolins"

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One-Minute Summary

- This study used genomic and phylogenetic analyses to investigate Malayan pangolins (*Manis javanica*) as a possible host for the virus that causes coronavirus disease 2019 (COVID-19).
- Using high-throughput metagenomic sequencing, coronavirus (CoV) RNA was identified in 6 of 43 tissue samples from 5 of 18 pangolins from Guangxi, China.
 - Phylogenetic analysis showed that the 6 near complete genome sequences belonged to the **COVID-19 virus lineage** (family *Coronaviridae*, genus *Betacoronavirus*) and had similar genomic organization compared to COVID-19 virus.
- Two additional pangolin-CoV sequences were identified in Guangzhou and Guangdong, China.
- Collectively, the 8 pangolin-CoV partial genomes had **85.5-92.4% sequence similarity to COVID-19**, and **represent two distinct sub-lineages** of COVID-19-related viruses.
- Recombination analysis revealed **putative recombination sites between pangolin-CoVs, bat-CoVs and COVID-19**.
- Despite COVID-19 being more closely related to bat-CoV (RaTG13), the receptor-binding domain (RBD) of COVID-19 showed high (97.4%) amino acid similarity to the Guangdong pangolin-CoV, compared to 89.2% to bat-CoV. Further, COVID-19 and Guangdong pangolin-CoV have identical amino acids at the 5 critical residues for binding to the human angiotensin converting enzyme 2 (ACE2) receptor, whereas bat-CoV only shares one with COVID-19.
- Phylogenetic analysis of the RBD suggests that the similarity between COVID-19 and the Guangdong pangolin-CoV may not have occurred through recombination, but rather through independent evolution. This is supported by a higher ACE2 sequence similarity between humans and pangolins (84.8%) compared to bats (80.8-81.4%).
- The authors conclude that pangolins are potential animal hosts of COVID-19; however, their role in the emergence of COVID-19 remains uncertain.

Additional Information

• Malayan pangolins are solitary animals that are found in the forests of Southeast Asia. Pangolins are a critically endangered species that are illegally smuggled into China, where they are used as a food source and their scales used in traditional Chinese medicine.

- Frozen samples (lung, intestine, blood) were obtained from pangolins found during antismuggling customs operations in Guangxi (August 2017 to January 2018). Additional CoV sequences were identified from 1 of 5 archived pangolin samples collected in Guangzhou (March 2018; unknown number of pangolins) and another from assembly of sequences from a previous study in Guangdong (Liu P. et al.; March 2019; 21 samples from 11 pangolins).
- Using the identified sequences, a real-time PCR assay was developed and used to test 19 archived pangolin samples from 12 animals (collected in Guangxi; May-July 2018), of which 3 tested positive.
- Virus was successfully isolated in cell culture from one tissue sample from Guangxi.

PHO Reviewer's Comments

• Further research is needed to understand COVID-19 transmission among pangolins and the risks pangolins pose to future zoonoses.

Citation

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