

Retrospective detection reveals absence of SARS-CoV-2 infection in human throat swab samples collected during Jan-May 2019 in the influenza epidemic season

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), along with Severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) are three emerging coronaviruses with huge public health in the 21st century. Especially, SARS-CoV-2 causes an unprecedented global pandemic and has spread in over than 200 countries. Clinically, SARS-CoV-2 could co-infected with respiratory pathogens including influenza virus and adenovirus in some present cases. This suggests that the differential diagnosis cannot be ignored. To investigate whether SARS-CoV-2 exists earlier and identify possible co-infection in human with respiratory disease, we performed the detections of SARS-CoV-2 and influenza virus using real-time RT-PCR method and colloidal gold test strip in 534 throat swab samples collected in influenza epidemic season (from January to May of 2019). 336 samples (62.9%) were tested positive for influenza virus, which involved into 230 single influenza A virus (IAV)-positive samples, 4 single influenza B virus (IBV)-positive samples, and 102 IAV and IBV co-infection samples. Unfortunately, we found no positive signal of SARS-CoV-2 in 534 samples. Our finding reveals absence of SARS-CoV-2 infection in human archived samples in before the outbreak of SARS-CoV-2 in China. In addition, this study suggests that IAV was still major respiratory pathogen responsible for respiratory disease in the influenza season.

1. Introduction

Since December 2019, the severe acute respiratory syndrome coronavirus (SARS-CoV-2) causing severe lower respiratory tract infections (COVID-19) was firstly reported in Wuhan China (Zhu et al., 2020). In the past

three months, SARS-CoV-2 has spread to over than 200 countries rapidly. On March 11, 2020, the WHO has declared a global pandemic.

The clinical symptom of COVID-19, including fever, cough and myalgia, has similarities to that of other infectious diseases such as influenza (Bordi et al., 2020; Guan et al., 2020). The SARS-CoV-2 also co-infected with influenza A virus (IAV) (Wu et al., 2020). And some people who seemingly died from influenza were later tested positive for SARS-CoV-2 in the United States (Redfield, 2020). Maybe, SARS-CoV-2 has appeared among human for a long time and been misdiagnosed as influenza virus. In order to trace the occurrence time of SARS-CoV-2, we conducted a retrospective detection of SARS-CoV-2 and influenza virus in human throat swab samples in influenza epidemic season (from January to May of 2019) in Guangzhou, China.

2. Materials and methods

2.1 Sampling

A total of 534 throat swab specimens were collected from human with respiratory disease in hospitals from January to May of 2019. It was approved by the ethics committee of the third affiliated hospital of Sun Yat-sen university. Each throat swab was placed in a EP tube with 2 ml PBS and stored at -80°C until use.

2.2 Detection of influenza virus

These samples were tested for human influenza A and B viruses (IAV/IBV) by colloidal gold test strip of Flu A and B (Wondfo Biotech Inc., Guangzhou).

2.2 Detection of SARS-CoV-2

200 μl sample supernatant was used for extraction of nucleic acids in GeneRotex 96 nucleic acid extraction instruments (TianLong Technology, Xi'an, China) in the Biosafety level 2 laboratory. Then RNA detection was done using qTower³G thermocycler (Analytikjena, Germany) by real-time RT-PCR methods. The primers and probes targeting the N and Orf1ab genes of SARS-CoV-2 were used (Zhu et al., 2020). The amplification was performed with the One Step PrimeScript RT-PCR kit (TaKaRa, Japan). The amplification condition was as follows: 42°C for 5 min, 95°C for 10 s, followed by 45 cycles of 95°C for 5 s and 58°C for 30 s.

3. Results and Discussion

336 samples (62.9%) were tested positive for influenza virus, which involved into 230 single influenza A virus (IAV)-positive samples, 4 single influenza B virus (IBV)-positive samples, and 102 IAV and IBV co-infection samples. Unfortunately, we found no positive signal of SARS-CoV-2 in 534 samples.

Until now, the source of SARS-CoV-2 was questionable. The early human cases could be epidemiologically linked to the exposure of the Huanan Seafood Market in Wuhan City, but there were several early cases that did not have the exposure of this market (Huang et al., 2020). This indicated that the seafood market was not the only source of SARS-CoV-2 infection. The genetic analysis showed that SARS-CoV-2 was likely to be a bat-origin coronavirus, exhibiting high genome identity with a β -CoV from *Rhinolophus affinis* bats (Zhou et al., 2020). This virus may still need an intermediate host to transmit from bats to humans. However, the intermediate host transmitted from bats to humans has not been fully understood. Several research groups have provided evidence based on sequence analysis that suggests pangolins are potential hosts of SARS-CoV-2 (Lam et al., 2020). And their pangolins samples were collected from foreign countries smuggling into Guangxi province and Guangdong province of China during 2017 to 2019.

Based on the similar symptoms between the influenza and COVID-19, we retrospectively detected over than 534 throat swab samples collected in influenza epidemic season in Guangzhou, however, no SARS-CoV-2 was discovered by the method recommended by authority. The results indicated that SARS-CoV-2 had not emerged in humans in Guangzhou in January to May of 2019, though asymptomatic infection could occur in humans (Rothe et al., 2020), and suggested that IAV was still major respiratory pathogen responsible for

respiratory disease in the influenza season. Another possibility was that the viral load in these throat swab specimens was limited, because SARS-CoV-2 infection starts at the lungs, not in the upper respiratory tract (Huang et al., 2020). And there have been some cases of false negatives for RT-PCR of COVID-19 reported recently (Li et al., 2020). The first case of COVID-19 was reported in Wuhan, China, but its primary source may not come from this city. We think that more influenza samples from other places in 2019 or before need to be screened for SARS-CoV-2.

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Conflict of interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in this manuscript.

References:

Bordi, L., Nicastri, E., Scorzolini, L., Di Caro, A., Capobianchi, M. R., Castilletti, C.,... On, B. O. I. C. (2020). Differential diagnosis of illness in patients under investigation for the novel coronavirus (SARS-CoV-2), Italy, February 2020. *Euro Surveill*, 25(8). doi:10.2807/1560-7917.ES.2020.25.8.2000170

Guan, W., Ni, Z., Hu, Y., Liang, W., Ou, C., He, J.,... Zhong, N. (2020). Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med* . doi:10.1056/NEJMoa2002032

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y.,... Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*, 395(10223),497-506.doi:10.1016/S0140-6736(20)30183-5

Lam, T. T., Shum, M. H., Zhu, H., Tong, Y., Ni, X., Liao, Y.,... Guan, Y. (2020). Identifying SARS-CoV-2 related coronaviruses in Malayan pangolins. *Nature* . doi:10.1038/s41586-020-2169-0

Li, Z., Yi, Y., Luo, X., Xiong, N., Liu, Y., Li, S.,... Ye, F. (2020). Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. *J Med Virol* . doi:10.1002/jmv.25727

Redfield R. (2020). US CDC director responds issues about COVID-19 at hearing. Retriever from <https://www.chinadaily.com>

Rothe, C., Schunk, M., Sothmann, P., Bretzel, G., Froeschl, G., Wallrauch, C.,... Hoelscher, M. (2020). Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. *N Engl J Med*,382(10), 970-971.doi:10.1056/NEJMc2001468

Wu, X., Cai, Y., Huang, X., Yu, X., Zhao, L., Wang, F.,... Zhan, Q. (2020). Co-infection with SARS-CoV-2 and influenza A virus in patient with pneumonia, China. *Emerg Infect Dis*,26(6).doi:10.3201/eid2606.200299

Zhou, P., Yang, X. L., Wang, X. G., Hu, B., Zhang, L., Zhang, W.,... Shi, Z. L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, 579(7798),270-273.doi:10.1038/s41586-020-2012-7

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J.,... Tan, W. (2020). A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* .doi:10.1056/NEJMoa2001017