附件 3 论文著作全文目录

3.1 Na Zhu, Dingyu Zhang, Wenling Wang, Xingwang Li, Bo Yang,Jingdong Song, Xiang Zhao,Baoying Huang, Weifeng Shi, Roujian Lu, Peihua Niu, Faxian Zhan, Xuejun Ma, Dayan Wang, Wenbo Xu, **Guizhen Wu***, George F Gao*, Wenjie Tan*; China Novel Coronavirus Investigating and Research Team. A Novel Coronavirus from Patients with Pneumonia in China, 2019.The New England Journal of Medicine. 2020 Feb 20;382(8):727-733.

3.2 Roujian Lu, Xiang Zhao, Juan Li, Peihua Niu, Bo Yang, Honglong Wu, Wenling Wang, Hao Song, Baoying Huang, Na Zhu, Yuhai Bi, Xuejun Ma, Faxian Zhan, Liang Wang, Tao Hu, Hong Zhou, Zhenhong Hu, Weimin Zhou, Li Zhao, Jing Chen, Yao Meng, Ji Wang, Yang Lin, Jianying Yuan, Zhihao Xie, Jinmin Ma, William J Liu, Dayan Wang, Wenbo Xu, Edward C Holmes, George F Gao, **Guizhen Wu***, Weijun Chen*, Weifeng Shi*, Wenjie Tan*. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020 Feb 22;395(10224):565-574.

3.3 William J Liu*, Peipei Liu , Wenwen Lei, Zhiyuan Jia, Xiaozhou He, Weifeng Shi, Yun Tan, Shumei Zou, Gary Wong, Ji Wang, Feng Wang, Gang Wang, Kun Qin, Rongbao Gao, Jie Zhang, Min Li, Wenling Xiao, Yuanyuan Guo, Ziqian Xu, Yingze Zhao, Jingdong Song, Jing Zhang, Wei Zhen, Wenting Zhou, Beiwei Ye, Juan Song, Mengjie Yang, Weimin Zhou, Yuting Dai, Gang Lu, Yuhai Bi, Wenjie Tan, Jun Han, George F Gao*, **Guizhen Wu***. Surveillance of SARS-CoV-2 at the Huanan Seafood Market. Nature. 2023 Apr 5. PMID: 37019149.

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BRIEF REPORT

A Novel Coronavirus from Patients with Pneumonia in China, 2019

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SUMMARY

In December 2019, a cluster of patients with pneumonia of unknown cause was linked to a seafood wholesale market in Wuhan, China. A previously unknown betacoronavirus was discovered through the use of unbiased sequencing in samples from patients with pneumonia. Human airway epithelial cells were used to isolate a novel coronavirus, named 2019-nCoV, which formed a clade within the subgenus sarbecovirus, Orthocoronavirinae subfamily. Different from both MERS-CoV and SARS-CoV, 2019-nCoV is the seventh member of the family of coronaviruses that infect humans. Enhanced surveillance and further investigation are ongoing. (Funded by the National Key Research and Development Program of China and the National Major Project for Control and Prevention of Infectious Disease in China.)

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This article was published on January 24, 2020, and updated on January 29, 2020, at NEJM.org.

N Engl J Med 2020;382:727-33.
DOI: 10.1056/NEJMoa2001017

Emerging and reemerging pathogens are global challenges for public health.[1] Coronaviruses are enveloped RNA viruses that are distributed broadly among humans, other mammals, and birds and that cause respiratory, enteric, hepatic, and neurologic diseases.[2,3] Six coronavirus species are known to cause human disease.[4] Four viruses — 229E, OC43, NL63, and HKU1 — are prevalent and typically cause common cold symptoms in immunocompetent individuals.[4] The two other strains — severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) — are zoonotic in origin and have been linked to sometimes fatal illness.[5] SARS-CoV was the causal agent of the severe acute respiratory syndrome outbreaks in 2002 and 2003 in Guangdong Province, China.[6-8] MERS-CoV was the pathogen responsible for severe respiratory disease outbreaks in 2012 in the Middle East.[9] Given the high prevalence and wide distribution of coronaviruses, the large genetic diversity and frequent recombination of their genomes, and increasing human–animal interface activities, novel coronaviruses are likely to emerge periodically in humans owing to frequent cross-species infections and occasional spillover events.[5,10]

In late December 2019, several local health facilities reported clusters of patients with pneumonia of unknown cause that were epidemiologically linked to a seafood and wet animal wholesale market in Wuhan, Hubei Province, China.[11] On December 31, 2019, the Chinese Center for Disease Control and Prevention (China CDC) dispatched a rapid response team to accompany Hubei provincial and Wuhan city health authorities and to conduct an epidemiologic and etiologic investigation. We report the results of this investigation, identifying the source of the pneumonia

clusters, and describe a novel coronavirus detected in patients with pneumonia whose specimens were tested by the China CDC at an early stage of the outbreak. We also describe clinical features of the pneumonia in two of these patients.

METHODS

VIRAL DIAGNOSTIC METHODS

Four lower respiratory tract samples, including bronchoalveolar-lavage fluid, were collected from patients with pneumonia of unknown cause who were identified in Wuhan on December 21, 2019, or later and who had been present at the Huanan Seafood Market close to the time of their clinical presentation. Seven bronchoalveolar-lavage fluid specimens were collected from patients in Beijing hospitals with pneumonia of known cause to serve as control samples. Extraction of nucleic acids from clinical samples (including uninfected cultures that served as negative controls) was performed with a High Pure Viral Nucleic Acid Kit, as described by the manufacturer (Roche). Extracted nucleic acid samples were tested for viruses and bacteria by polymerase chain reaction (PCR), using the RespiFinderSmart22kit (PathoFinder BV) and the LightCycler 480 real-time PCR system, in accordance with manufacturer instructions.[12] Samples were analyzed for 22 pathogens (18 viruses and 4 bacteria) as detailed in the Supplementary Appendix. In addition, unbiased, high-throughput sequencing, described previously,[13] was used to discover microbial sequences not identifiable by the means described above. A real-time reverse transcription PCR (RT-PCR) assay was used to detect viral RNA by targeting a consensus RdRp region of pan β-CoV, as described in the Supplementary Appendix.

ISOLATION OF VIRUS

Bronchoalveolar-lavage fluid samples were collected in sterile cups to which virus transport medium was added. Samples were then centrifuged to remove cellular debris. The supernatant was inoculated on human airway epithelial cells,[13] which had been obtained from airway specimens resected from patients undergoing surgery for lung cancer and were confirmed to be special-pathogen-free by NGS.[14]

Human airway epithelial cells were expanded on plastic substrate to generate passage-1 cells and were subsequently plated at a density of 2.5×10^5 cells per well on permeable Transwell-COL (12-mm diameter) supports. Human airway epithelial cell cultures were generated in an air–liquid interface for 4 to 6 weeks to form well-differentiated, polarized cultures resembling in vivo pseudostratified mucociliary epithelium.[13]

Prior to infection, apical surfaces of the human airway epithelial cells were washed three times with phosphate-buffered saline; 150 μl of supernatant from bronchoalveolar-lavage fluid samples was inoculated onto the apical surface of the cell cultures. After a 2-hour incubation at 37°C, unbound virus was removed by washing with 500 μl of phosphate-buffered saline for 10 minutes; human airway epithelial cells were maintained in an air–liquid interface incubated at 37°C with 5% carbon dioxide. Every 48 hours, 150 μl of phosphate-buffered saline was applied to the apical surfaces of the human airway epithelial cells, and after 10 minutes of incubation at 37°C the samples were harvested. Pseudostratified mucociliary epithelium cells were maintained in this environment; apical samples were passaged in a 1:3 diluted vial stock to new cells. The cells were monitored daily with light microscopy, for cytopathic effects, and with RT-PCR, for the presence of viral nucleic acid in the supernatant. After three passages, apical samples and human airway epithelial cells were prepared for transmission electron microscopy.

TRANSMISSION ELECTRON MICROSCOPY

Supernatant from human airway epithelial cell cultures that showed cytopathic effects was collected, inactivated with 2% paraformaldehyde for at least 2 hours, and ultracentrifuged to sediment virus particles. The enriched supernatant was negatively stained on film-coated grids for examination. Human airway epithelial cells showing cytopathic effects were collected and fixed with 2% paraformaldehyde–2.5% glutaraldehyde and were then fixed with 1% osmium tetroxide dehydrated with grade ethanol embedded with PON812 resin. Sections (80 nm) were cut from resin block and stained with uranyl acetate and lead citrate, separately. The negative stained grids and ultrathin sections were observed under transmission electron microscopy.

VIRAL GENOME SEQUENCING

RNA extracted from bronchoalveolar-lavage fluid and culture supernatants was used as a template to clone and sequence the genome. We used a combination of Illumina sequencing and nanopore sequencing to characterize the virus genome. Sequence reads were assembled into contig maps (a set of overlapping DNA segments) with the use of CLC Genomics software, version 4.6.1 (CLC Bio). Specific primers were subsequently designed for PCR, and 5′- or 3′-RACE (rapid amplification of cDNA ends) was used to fill genome gaps from conventional Sanger sequencing. These PCR products were purified from gels and sequenced with a BigDye Terminator v3.1 Cycle Sequencing Kit and a 3130XL Genetic Analyzer, in accordance with the manufacturers' instructions.

Multiple-sequence alignment of the 2019-nCoV and reference sequences was performed with the use of Muscle. Phylogenetic analysis of the complete genomes was performed with RAxML (13) with 1000 bootstrap replicates and a general time-reversible model used as the nucleotide substitution model.

RESULTS

PATIENTS

Three adult patients presented with severe pneumonia and were admitted to a hospital in Wuhan on December 27, 2019. Patient 1 was a 49-year-old woman, Patient 2 was a 61-year-old man, and Patient 3 was a 32-year-old man. Clinical profiles were available for Patients 1 and 2. Patient 1 reported having no underlying chronic medical conditions but reported fever (temperature, 37°C to 38°C) and cough with chest discomfort on December 23, 2019. Four days after the onset of illness, her cough and chest discomfort worsened, but the fever was reduced; a diagnosis of pneumonia was based on computed tomographic (CT) scan. Her occupation was retailer in the seafood wholesale market. Patient 2 initially reported fever and cough on December 20, 2019; respiratory distress developed 7 days after the onset of illness and worsened over the next 2 days (see chest radiographs, Fig. 1), at which time mechanical ventilation was started. He had been a frequent visitor to the seafood wholesale market. Patients 1 and 3 recovered and were discharged from the hospital on January 16, 2020. Patient 2 died on January 9, 2020. No biopsy specimens were obtained.

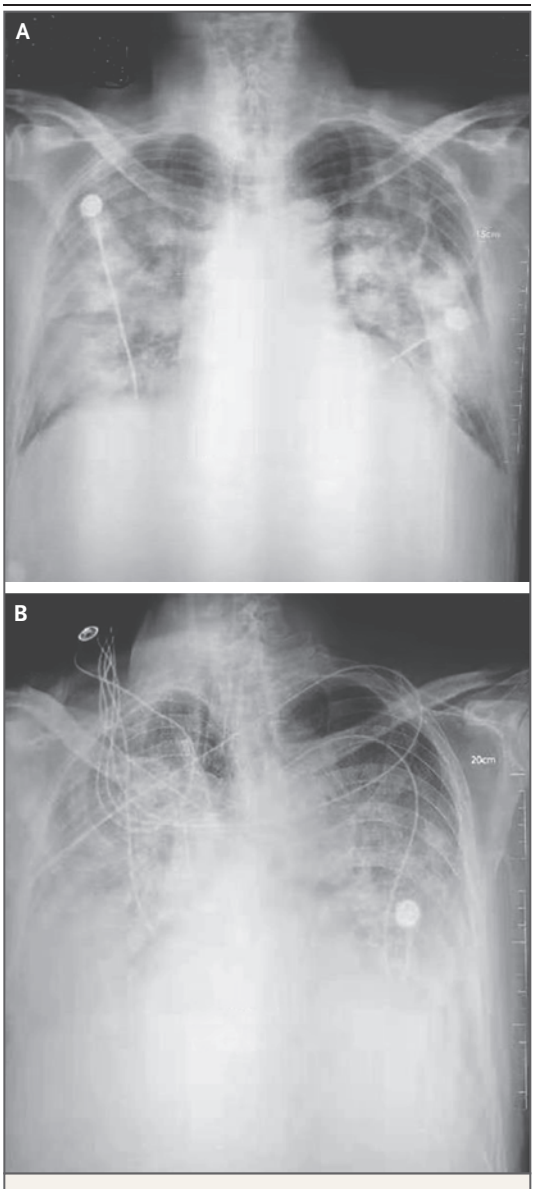

Figure 1. Chest Radiographs.

Shown are chest radiographs from Patient 2 on days 8 and 11 after the onset of illness. The trachea was intubated and mechanical ventilation instituted in the period between the acquisition of the two images. Bilateral fluffy opacities are present in both images but are increased in density, profusion, and confluence in the second image; these changes are most marked in the lower lung fields. Changes consistent with the accumulation of pleural liquid are also visible in the second image.

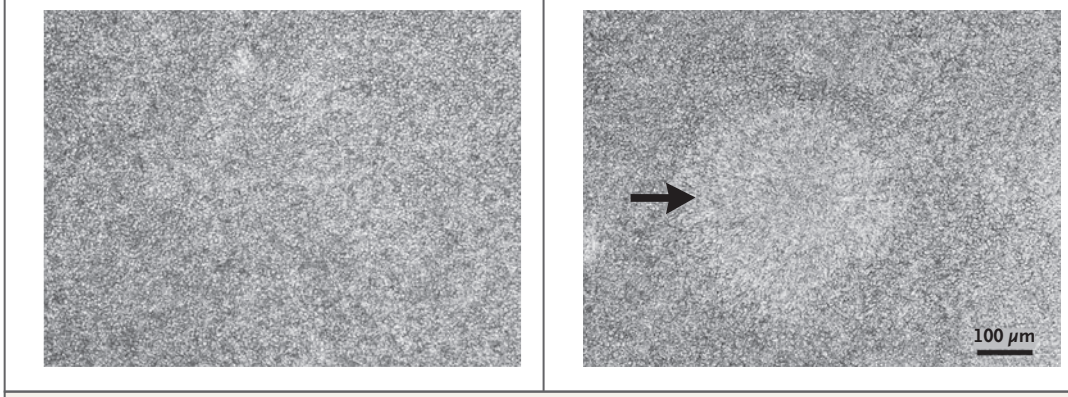

Figure 2. Cytopathic Effects in Human Airway Epithelial Cell Cultures after Inoculation with 2019-nCoV.

DETECTION AND ISOLATION OF A NOVEL CORONAVIRUS

Three bronchoalveolar-lavage samples were collected from Wuhan Jinyintan Hospital on December 30, 2019. No specific pathogens (including HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1) were detected in clinical specimens from these patients by the RespiFinderSmart-22kit. RNA extracted from bronchoalveolar-lavage fluid from the patients was used as a template to clone and sequence a genome using a combination of Illumina sequencing and nanopore sequencing. More than 20,000 viral reads from individual specimens were obtained, and most contigs matched to the genome from lineage B of the genus betacoronavirus — showing more than 85% identity with a bat SARS-like CoV (bat-SL-CoVZC45, MG772933.1) genome published previously. Positive results were also obtained with use of a real-time RT-PCR assay for RNA targeting to a consensus RdRp region of pan β-CoV (although the cycle threshold value was higher than 34 for detected samples). Virus isolation from the clinical specimens was performed with human airway epithelial cells and Vero E6 and Huh-7 cell lines. The isolated virus was named 2019-nCoV.

To determine whether virus particles could be visualized in 2019-nCoV–infected human airway epithelial cells, mock-infected and 2019-nCoV–infected human airway epithelial cultures were examined with light microscopy daily and with transmission electron microscopy 6 days after inoculation. Cytopathic effects were observed 96 hours after inoculation on surface layers of human airway epithelial cells; a lack of cilium beating was seen with light microcopy in the center of the focus (Fig. 2). No specific cytopathic effects were observed in the Vero E6 and Huh-7 cell lines until 6 days after inoculation.

Electron micrographs of negative-stained 2019-nCoV particles were generally spherical with some pleomorphism (Fig. 3). Diameter varied from about 60 to 140 nm. Virus particles had quite distinctive spikes, about 9 to 12 nm, and gave virions the appearance of a solar corona. Extracellular free virus particles and inclusion bodies filled with virus particles in membrane-bound vesicles in cytoplasm were found in the human airway epithelial ultrathin sections. This observed morphology is consistent with the Coronaviridae family.

To further characterize the virus, de novo sequences of 2019-nCoV genome from clinical specimens (bronchoalveolar-lavage fluid) and human airway epithelial cell virus isolates were obtained by Illumina and nanopore sequencing. The novel coronavirus was identified from all three patients. Two nearly full-length coronavirus sequences were obtained from bronchoalveolar-lavage fluid (BetaCoV/Wuhan/IVDC-HB-04/2020, BetaCoV/Wuhan/IVDC-HB-05/2020|EPI_ISL_402121), and one full-length sequence was obtained from a virus isolated from a patient (BetaCoV/Wuhan/IVDC-HB-01/2020|EPI_ISL_402119). Complete genome sequences of the three novel coronaviruses were submitted to GISAID (BetaCoV/Wuhan/IVDC-HB-01/2019, accession ID: EPI_ISL_402119; BetaCoV/Wuhan/IVDC-HB-04/2020, accession ID: EPI_ISL_402120; BetaCoV/Wuhan/IVDC-HB-05/2019,

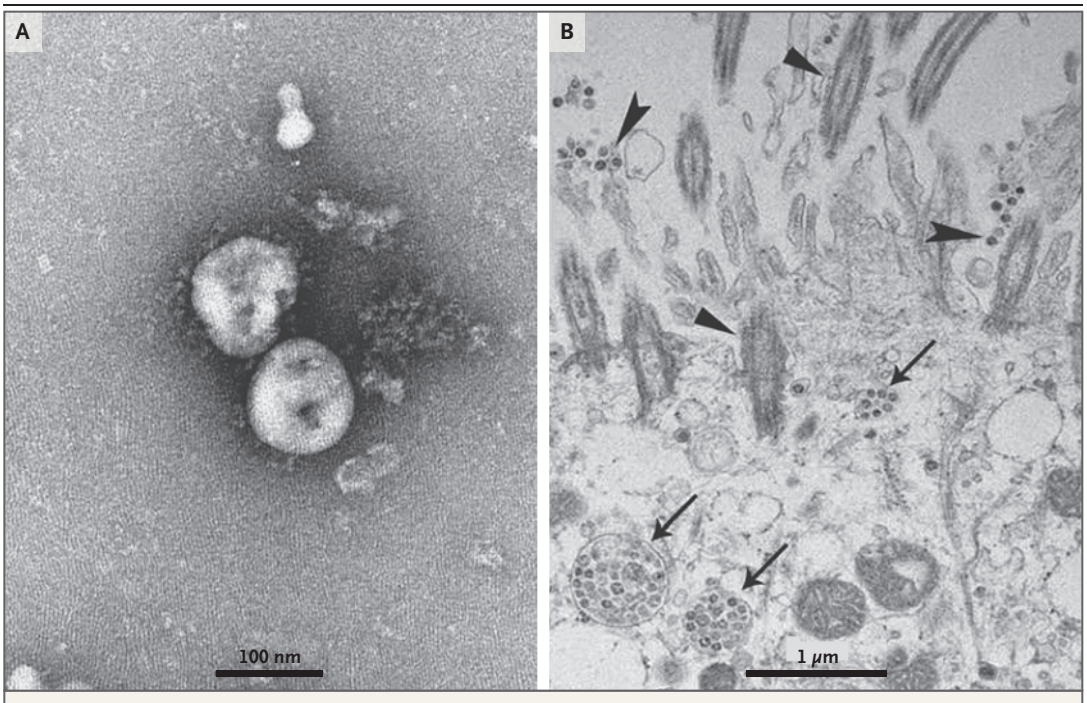

Figure 3. Visualization of 2019-nCoV with Transmission Electron Microscopy.

Negative-stained 2019-nCoV particles are shown in Panel A, and 2019-nCoV particles in the human airway epithelial cell ultrathin sections are shown in Panel B. Arrowheads indicate extracellular virus particles, arrows indicate inclusion bodies formed by virus components, and triangles indicate cilia.

accession ID: EPI_ISL_402121) and have a 86.9% nucleotide sequence identity to a previously published bat SARS-like CoV (bat-SL-CoVZC45, MG772933.1) genome. The three 2019-nCoV genomes clustered together within the sarbecovirus subgenus, which shows the typical betacoronavirus organization: a 5′ untranslated region (UTR), replicase complex (orf1ab), S gene, E gene, M gene, N gene, 3′ UTR, and several unidentified nonstructural open reading frames.

Although 2019-nCoV is similar to some betacoronaviruses detected in bats (Fig. 4), it is distinct from SARS-CoV and MERS-CoV. The three 2019-nCoV coronaviruses from Wuhan, together with two bat-derived SARS-like strains, ZC45 and ZXC21, form a distinct clade. SARS-CoV strains from humans and genetically similar SARS-like coronaviruses from bats collected from southwestern China formed another clade within the subgenus sarbecovirus. Since the sequence identity in conserved replicase domains (ORF 1ab) is less than 90% between 2019-nCoV and other members of betacoronavirus, the 2019-nCoV — the likely causative agent of the viral pneumonia in Wuhan — is a novel betacoronavirus belonging to the sarbecovirus subgenus of Coronaviridae family.

DISCUSSION

We report a novel CoV (2019-nCoV) that was identified in hospitalized patients in Wuhan, China, in December 2019 and January 2020. Evidence for the presence of this virus includes identification in bronchoalveolar-lavage fluid in three patients by whole-genome sequencing, direct PCR, and culture. The illness likely to have been caused by this CoV was named "novel coronavirus-infected pneumonia" (NCIP). Complete genomes were submitted to GISAID. Phylogenetic analysis revealed that 2019-nCoV falls into the genus betacoronavirus, which includes coronaviruses (SARS-CoV, bat SARS-like CoV, and others) discovered in humans, bats, and other wild animals.[15] We report isolation of the virus and the initial description of its specific cytopathic effects and morphology.

Molecular techniques have been used successfully to identify infectious agents for many years. Unbiased, high-throughput sequencing is

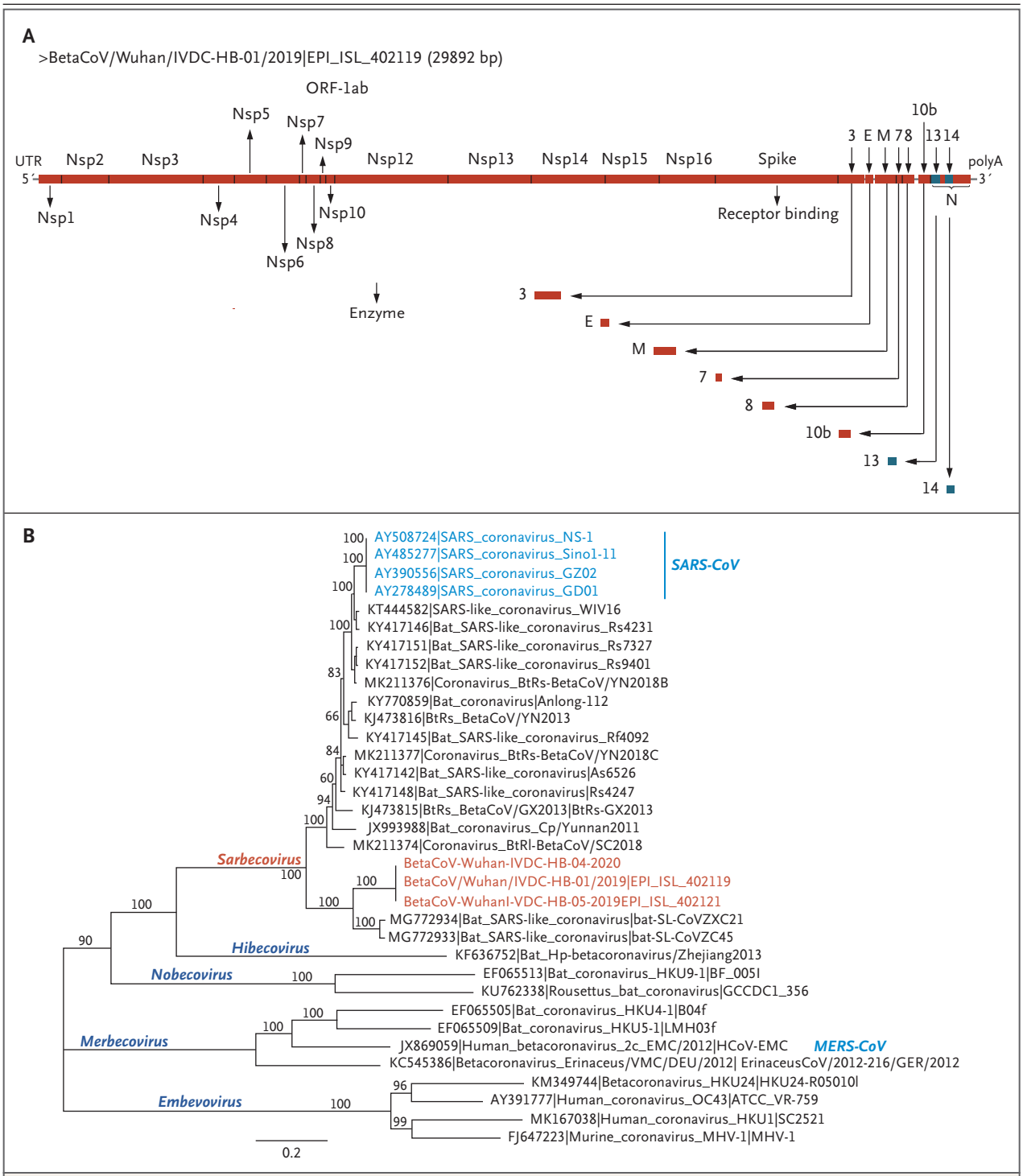

Figure 4. Schematic of 2019-nCoV and Phylogenetic Analysis of 2019-nCoV and Other Betacoronavirus Genomes.

Shown are a schematic of 2019-nCoV (Panel A) and full-length phylogenetic analysis of 2019-nCoV and other betacoronavirus genomes in the Orthocoronavirinae subfamily (Panel B).

a powerful tool for the discovery of pathogens.[14,16] Next-generation sequencing and bioinformatics are changing the way we can respond to infectious disease outbreaks, improving our understanding of disease occurrence and transmission, accelerating the identification of pathogens, and promoting data sharing. We describe in this report the use of molecular techniques and unbiased DNA sequencing to discover a novel betacoronavirus that is likely to have been the cause of severe pneumonia in three patients in Wuhan, China.

Although establishing human airway epithelial cell cultures is labor intensive, they appear to be a valuable research tool for analysis of human respiratory pathogens.[13] Our study showed that initial propagation of human respiratory secretions onto human airway epithelial cell cultures, followed by transmission electron microscopy and whole genome sequencing of culture supernatant, was successfully used for visualization and detection of new human coronavirus that can possibly elude identification by traditional approaches.

Further development of accurate and rapid methods to identify unknown respiratory pathogens is still needed. On the basis of analysis of three complete genomes obtained in this study, we designed several specific and sensitive assays targeting ORF1ab, N, and E regions of the 2019-nCoV genome to detect viral RNA in clinical specimens. The primer sets and standard operating procedures have been shared with the World Health Organization and are intended for surveillance and detection of 2019-nCoV infection globally and in China. More recent data show 2019-nCoV detection in 830 persons in China.[17]

Although our study does not fulfill Koch's postulates, our analyses provide evidence implicating 2019-nCoV in the Wuhan outbreak. Additional evidence to confirm the etiologic significance of 2019-nCoV in the Wuhan outbreak include identification of a 2019-nCoV antigen in the lung tissue of patients by immunohistochemical analysis, detection of IgM and IgG antiviral antibodies in the serum samples from a patient at two time points to demonstrate seroconversion, and animal (monkey) experiments to provide evidence of pathogenicity. Of critical importance are epidemiologic investigations to characterize transmission modes, reproduction interval, and clinical spectrum resulting from infection to inform and refine strategies that can prevent, control, and stop the spread of 2019-nCoV.

This work was supported by grants from the National Key Research and Development Program of China (2016YFD0500301) and the National Major Project for Control and Prevention of Infectious Disease in China (2018ZX10101002).

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank Dr. Zhongjie Li, Dr. Guangxue He, Dr. Lance Rodewald, Yu Li, Fei Ye, Li Zhao, Weimin Zhou, Jun Liu, Yao Meng, Huijuan Wang, and many staff members at the China CDC for their contributions and assistance in this preparation and submission of an earlier version of the manuscript.

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Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding

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Summary

Background In late December, 2019, patients presenting with viral pneumonia due to an unidentified microbial agent were reported in Wuhan, China. A novel coronavirus was subsequently identified as the causative pathogen, provisionally named 2019 novel coronavirus (2019-nCoV). As of Jan 26, 2020, more than 2000 cases of 2019-nCoV infection have been confirmed, most of which involved people living in or visiting Wuhan, and human-to-human transmission has been confirmed.

Methods We did next-generation sequencing of samples from bronchoalveolar lavage fluid and cultured isolates from nine inpatients, eight of whom had visited the Huanan seafood market in Wuhan. Complete and partial 2019-nCoV genome sequences were obtained from these individuals. Viral contigs were connected using Sanger sequencing to obtain the full-length genomes, with the terminal regions determined by rapid amplification of cDNA ends. Phylogenetic analysis of these 2019-nCoV genomes and those of other coronaviruses was used to determine the evolutionary history of the virus and help infer its likely origin. Homology modelling was done to explore the likely receptor-binding properties of the virus.

Findings The ten genome sequences of 2019-nCoV obtained from the nine patients were extremely similar, exhibiting more than 99·98% sequence identity. Notably, 2019-nCoV was closely related (with 88% identity) to two bat-derived severe acute respiratory syndrome (SARS)-like coronaviruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21, collected in 2018 in Zhoushan, eastern China, but were more distant from SARS-CoV (about 79%) and MERS-CoV (about 50%). Phylogenetic analysis revealed that 2019-nCoV fell within the subgenus Sarbecovirus of the genus Betacoronavirus, with a relatively long branch length to its closest relatives bat-SL-CoVZC45 and bat-SL-CoVZXC21, and was genetically distinct from SARS-CoV. Notably, homology modelling revealed that 2019-nCoV had a similar receptor-binding domain structure to that of SARS-CoV, despite amino acid variation at some key residues.

Interpretation 2019-nCoV is sufficiently divergent from SARS-CoV to be considered a new human-infecting betacoronavirus. Although our phylogenetic analysis suggests that bats might be the original host of this virus, an animal sold at the seafood market in Wuhan might represent an intermediate host facilitating the emergence of the virus in humans. Importantly, structural analysis suggests that 2019-nCoV might be able to bind to the angiotensin-converting enzyme 2 receptor in humans. The future evolution, adaptation, and spread of this virus warrant urgent investigation.

Funding National Key Research and Development Program of China, National Major Project for Control and Prevention of Infectious Disease in China, Chinese Academy of Sciences, Shandong First Medical University.

Introduction

Viruses of the family Coronaviridae possess a single-strand, positive-sense RNA genome ranging from 26 to 32 kilobases in length.[1] Coronaviruses have been identified in several avian hosts,[2,3] as well as in various mammals, including camels, bats, masked palm civets, mice, dogs, and cats. Novel mammalian coronaviruses are now regularly identified.[1] For example, an HKU2-related coronavirus of bat origin was responsible for a fatal acute diarrhoea syndrome in pigs in 2018.[4]

Among the several coronaviruses that are pathogenic to humans, most are associated with mild clinical symptoms,[1] with two notable exceptions: severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV), a novel betacoronavirus that emerged in Guangdong, southern China, in November, 2002,[5] and resulted in more than 8000 human infections and 774 deaths in 37 countries during 2002–03;[6] and Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV), which was first detected in Saudi Arabia in 2012[7] and was responsible

Lancet 2020; 395: 565–74

Published **Online** January 29, 2020 https://doi.org/10.1016/S0140-6736(20)30251-8

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Research in context

Evidence before this study

The causal agent of an outbreak of severe pneumonia in Wuhan, China, is a novel coronavirus, provisionally named 2019 novel coronavirus (2019-nCoV). The first cases were reported in December, 2019.

Added value of this study

We have described the genomic characteristics of 2019-nCoV and similarities and differences to other coronaviruses, including the virus that caused the severe acute respiratory syndrome epidemic of 2002–03. Genome sequences of 2019-nCoV sampled from nine patients who were among the early cases of this severe infection are almost genetically identical, which suggests very recent emergence of this virus in humans and that the outbreak was detected relatively rapidly. 2019-nCoV is most closely related to other betacoronaviruses of bat origin, indicating that these animals are the likely reservoir hosts for this emerging viral pathogen.

Implications of all the available evidence

By documenting the presence of 2019-nCoV in a sample of patients, our study extends previous evidence that this virus has led to the novel pneumonia that has caused severe disease in Wuhan and other geographical localities. Currently available data suggest that 2019-nCoV infected the human population from a bat reservoir, although it remains unclear if a currently unknown animal species acted as an intermediate host between bats and humans.

for 2494 laboratory-confirmed cases of infection and 858 fatalities since September, 2012, including 38 deaths following a single introduction into South Korea.[8,9]

In late December, 2019, several patients with viral pneumonia were found to be epidemiologically associated with the Huanan seafood market in Wuhan, in the Hubei province of China, where a number of non-aquatic animals such as birds and rabbits were also on sale before the outbreak. A novel, human-infecting coronavirus,[10,11] provisionally named 2019 novel coronavirus (2019-nCoV), was identified with use of next-generation sequencing. As of Jan 28, 2020, China has reported more than 5900 confirmed and more than 9000 suspected cases of 2019-nCoV infection across 33 Chinese provinces or municipalities, with 106 fatalities. In addition, 2019-nCoV has now been reported in Thailand, Japan, South Korea, Malaysia, Singapore, and the USA. Infections in medical workers and family clusters were also reported and human-to-human transmission has been confirmed.[12] Most of the infected patients had a high fever and some had dyspnoea, with chest radiographs revealing invasive lesions in both lungs.[12,13]

We report the epidemiological data of nine inpatients, from at least three hospitals in Wuhan, who were diagnosed with viral pneumonia of unidentified cause. Using next-generation sequencing of bronchoalveolar lavage fluid samples and cultured isolates from these patients, 2019-nCoV was found. We describe the genomic characterisation of ten genomes of this novel virus, providing important information on the origins and cell receptor binding of the virus.

Methods

Patients and samples

Nine patients with viral pneumonia and negative for common respiratory pathogens, who presented to at least three hospitals in Wuhan, were included in this study. Eight of the patients had visited the Huanan seafood market before the onset of illness, and one patient (WH04) did not visit the market but stayed in a hotel near the market between Dec 23 and Dec 27, 2019 (table). Five of the patients (WH19001, WH19002, WH19004, WH19008, and YS8011) had samples collected by the Chinese Center for Disease Control and Prevention (CDC) which were tested for 18 viruses and four bacteria using the RespiFinderSmart22 Kit (PathoFinder, Maastricht, Netherlands) on the LightCycler 480 Real-Time PCR system (Roche, Rotkreuz, Switzerland). Presence of SARS-CoV and MERS-CoV was tested using a previously reported method.[14] All five CDC samples were negative for all common respiratory pathogens screened for. Four of the patients (WH01, WH02, WH03, and WH04) had samples collected by BGI (Beijing, China), and were tested for five viruses and one bacterium using the RespiPathogen 6 Kit (Jiangsu Macro & Micro Test, Nantong, China) on the Applied Biosystems ABI 7500 Real-Time PCR system (ThermoFisher Scientific, Foster City, CA, USA). All four samples were negative for the targeted respiratory pathogens.

Virus isolation

Special-pathogen-free human airway epithelial (HAE) cells were used for virus isolation. Briefly, bronchoalveolar lavage fluids or throat swabs from the patients were inoculated into the HAE cells through the apical surfaces. HAE cells were maintained in an air–liquid interface incubated at 37°C. The cells were monitored daily for cytopathic effects by light microscopy and the cell supernatants were collected for use in quantitative RT-PCR assays. After three passages, apical samples were collected for sequencing.

BGI sequencing strategy

All collected samples were sent to BGI for sequencing. 140 µL bronchoalveolar lavage fluid samples (WH01 to WH04) were reserved for RNA extraction using the QIAamp Viral RNA Mini Kit (52904; Qiagen, Heiden, Germany), according to the manufacturer's recommendations. A probe-captured technique was used to remove human nucleic acid. The remaining RNA was

	Patient information			Sample information			Genome sequence obtained
	Exposure to Huanan seafood market	Date of symptom onset	Admission date	Sample type	Collection date	Ct value	
Samples WH19001 and WH19005	Yes	Dec 23, 2019	Dec 29, 2019	BALF and cultured virus	Dec 30, 2019	30·23	Complete
Sample WH19002	Yes	Dec 22, 2019	NA	BALF	Dec 30, 2019	30·50	Partial (27 130 nucleotides)
Sample WH19004	Yes	NA	NA	BALF	Jan 1, 2020	32·14	Complete
Sample WH19008	Yes	NA	Dec 29, 2019	BALF	Dec 30, 2019	26·35	Complete
Sample YS8011	Yes	NA	NA	Throat swab	Jan 7, 2020	22·85	Complete
Sample WH01	Yes	NA	NA	BALF	Dec 26, 2019	32·60	Complete
Sample WH02	Yes	NA	NA	BALF	Dec 31, 2019	34·23	Partial (19 503 nucleotides)
Sample WH03	Yes	Dec 26, 2019	NA	BALF	Jan 1, 2020	25·38	Complete
Sample WH04	No*	Dec 27, 2019	NA	BALF	Jan 5, 2020	25·23	Complete

Ct=threshold cycle. BALF=bronchoalveolar lavage fluid. NA=not available. 2019-nCoV=2019 novel coronavirus. *Patient stayed in a hotel near Huanan seafood market from Dec 23 to Dec 27, 2019, and reported fever on Dec 27, 2019.

Table: **Information about samples taken from nine patients infected with 2019-nCoV**

reverse-transcribed into cDNA, followed by the second-strand synthesis. Using the synthetic double-stranded DNA, a DNA library was constructed through DNA-fragmentation, end-repair, adaptor-ligation, and PCR amplification. The constructed library was qualified with an Invitrogen Qubit 2.0 Fluorometer (ThermoFisher, Foster City, CA, USA), and the qualified double-stranded DNA library was transformed into a single-stranded circular DNA library through DNA-denaturation and circularisation. DNA nanoballs were generated from single-stranded circular DNA by rolling circle amplification, then qualified with Qubit 2.0 and loaded onto the flow cell and sequenced with PE100 on the DNBSEQ-T7 platform (MGI, Shenzhen, China).

After removing adapter, low-quality, and low-complexity reads, high-quality genome sequencing data were generated. Sequence reads were first filtered against the human reference genome (hg19) using Burrows-Wheeler Alignment.[15] The remaining data were then aligned to the local nucleotide database (using Burrows-Wheeler Alignment) and non-redundant protein database (using RapSearch),[16] downloaded from the US National Center for Biotechnology Information website, which contain only coronaviruses that have been published. Finally, the mapped reads were assembled with SPAdes[17] to obtain a high-quality coronavirus genome sequence.

Primers were designed with use of OLIGO Primer Analysis Software version 6.44 on the basis of the assembled partial genome, and were verified by Primer-Blast (for more details on primer sequencs used please contact the corresponding author). PCR was set up as follows: 4·5 μL of 10X buffer, 4 μL of dNTP mix (2·5 μmol/L), 1 μL of each primer (10 μmol/L), and 0·75 units of HS Ex Taq (Takara Biomedical Technology, Beijing, China), in a total volume of 30 μL. The cDNAs reverse transcribed from clinical samples were used as templates, and random primers were used. The following program was run on the thermocycler: 95°C for 5 min; 40 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min as determined by product size; 72°C for 7 min; and a 4°C hold. Finally, the PCR products were separated by agarose gel electrophoresis, and products of the expected size were sequenced from both ends on the Applied Biosystems 3730 DNA Analyzer platform (Applied Biosystems, Life Technologies, Foster City, CA, USA; for more details on expected size please contact the corresponding author).

Chinese CDC sequencing strategy

The whole-genome sequences of 2019-nCoV from six samples (WH19001, WH19005, WH19002, WH19004, WH19008, and YS8011) were generated by a combination Sanger, Illumina, and Oxford nanopore sequencing. First, viral RNAs were extracted directly from clinical samples with the QIAamp Viral RNA Mini Kit, and then used to synthesise cDNA with the SuperScript III Reverse Transcriptase (ThermoFisher, Waltham, MA, USA) and N6 random primers, followed by second-strand synthesis with DNA Polymerase I, Large (Klenow) Fragment (ThermoFisher). Viral cDNA libraries were prepared with use of the Nextera XT Library Prep Kit (Illumina, San Diego, CA, USA), then purified with Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA), followed by quantification with an Invitrogen Qubit 2.0 Fluorometer. The resulting DNA libraries were sequenced on either the MiSeq or iSeq platforms (Illumina) using a 300-cycle reagent kit. About 1·2–5 GB of data were obtained for each sample.

The raw fastQ files for each virus sample were filtered using previously described criteria,[18] then subjected to de novo assembly with the CLCBio software version 11.0.1. Mapped assemblies were also done using the bat-derived SARS-like coronavirus isolate bat-SL-CoVZC45 (accession number MG772933.1) as a reference. Variant calling, genome alignments, and sequence illustrations were generated with CLCBio software, and the

For the **National Center for Biotechnology Information website** see https://www.ncbi.nlm.nih.gov/

assembled genome sequences were confirmed by Sanger sequencing.

Rapid amplification of cDNA ends (RACE) was done to obtain the sequences of the 5′ and 3′ termini, using the Invitrogen 5′ RACE System and 3′ RACE System (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. Gene-specific primers (appendix p 1) for 5′ and 3′ RACE PCR amplification were designed to obtain a fragment of approximately 400–500 bp for the two regions. Purified PCR products were cloned into the pMD18-T Simple Vector (TaKaRa, Takara Biotechnology, Dalian, China) and chemically competent *Escherichia coli* (DH5α cells; TaKaRa), according to the manufacturer's instructions. PCR products were sequenced with use of M13 forward and reverse primers.

See Online for appendix

Virus genome analysis and annotation

Reference virus genomes were obtained from GenBank using Blastn with 2019-nCoV as a query. The open reading frames of the verified genome sequences were predicted using Geneious (version 11.1.5) and annotated using the Conserved Domain Database.[19] Pairwise sequence identities were also calculated using Geneious. Potential genetic recombination was investigated using SimPlot software (version 3.5.1)[20] and phylogenetic analysis.

For **Genbank** see https://www.ncbi.nlm.nih.gov/genbank

For the **China National Microbiological Data Center website** see http://nmdc.cn/

For the **data from BGI on the China National GeneBank** see https://db.cngb.org/datamart/disease/DATAdis19/

Phylogenetic analysis

Sequence alignment of 2019-nCoV with reference sequences was done with Mafft software (version 7.450).[21] Phylogenetic analyses of the complete genome and major coding regions were done with RAxML software (version 8.2.9)[22] with 1000 bootstrap replicates, employing the general time reversible nucleotide substitution model.

Development of molecular diagnostics for 2019-nCoV

On the basis of the genome sequences obtained, a real-time PCR detection assay was developed. PCR primers and probes were designed using Applied Biosystems Primer Express Software (ThermoFisher Scientific, Foster City, CA, USA) on the basis of our sequenced virus genomes. The specific primers and probe set (labelled with the reporter 6-carboxyfluorescein [FAM] and the quencher Black Hole Quencher 1 [BHQ1]) for *orf1a* were as follows: forward primer 5′-AGAAGATTGGTTAGATGATGATAGT-3′; reverse primer 5′-TTCCATCTCTAATTGAGGTTGAACC-3′; and probe 5′-FAM-TCCTCACTGCCGTCTTGTTGACCA-BHQ1-3′. The human *GAPDH* gene was used as an internal control (forward primer 5′-TCAAGAAGGTGGTGAAGCAGG-3′; reverse primer 5′-CAGCGTCAAAGGTGGAGGAGT-3′; probe 5′-VIC-CCTCAAGGGCATCCTGGGCTACACT-BHQ1-3′). Primers and probes were synthesised by BGI (Beijing, China). RT-PCR was done with an Applied Biosystems 7300 Real-Time PCR System (ThermoScientific), with 30 µL reaction volumes consisting of 14 µL of diluted RNA, 15 µL of 2X Taqman One-Step RT-PCR Master Mix Reagents (4309169; Applied Biosystems, ThermoFisher), 0·5 µL of 40X MultiScribe and RNase inhibitor mixture, 0·75 µL forward primer (10 µmol/L), 0·75 µL reverse primer (10 µmol/L), and 0·375 µL probe (10 µmol/L). Thermal cycling parameters were 30 min at 42°C, followed by 10 min at 95°C, and a subsequent 40 cycles of amplification (95°C for 15 s and 58°C for 45 s). Fluorescence was recorded during the 58°C phase.

Role of the funding source

The funder of the study had no role in data collection, data analysis, data interpretation, or writing of report. GFG and WS had access to all the data in the study, and GFG, WS, WT, WC, and GW were responsible for the decision to submit for publication.

Results

From the nine patients' samples analysed, eight complete and two partial genome sequences of 2019-nCoV were obtained. These data have been deposited in the China National Microbiological Data Center (accession number NMDC10013002 and genome accession numbers NMDC60013002-01 to NMDC60013002-10) and the data from BGI have been deposited in the China National GeneBank (accession numbers CNA0007332–35).

Based on these genomes, we developed a real-time PCR assay and tested the original clinical samples from the BGI (WH01, WH02, WH03, and WH04) again to determine their threshold cycle (Ct) values (table). The remaining samples were tested by a different real-time PCR assay developed by the Chinese CDC, with Ct values ranging from 22·85 to 32·41 (table). These results confirmed the presence of 2019-nCoV in the patients.

Bronchoalveolar lavage fluid samples or cultured viruses of nine patients were used for next-generation sequencing. After removing host (human) reads, de novo assembly was done and the contigs obtained used as queries to search the non-redundant protein database. Some contigs identified in all the samples were closely related to the bat SARS-like betacoronavirus bat-SL-CoVZC45 betacoronavirus.[23] Bat-SL-CoVZC45 was then used as the reference genome and reads from each pool were mapped to it, generating consensus sequences corresponding to all the pools. These consensus sequences were then used as new reference genomes. Eight complete genomes and two partial genomes (from samples WH19002 and WH02; table) were obtained. The de novo assembly of the clean reads from all the pools did not identify any other long contigs that corresponded to other viruses at high abundance.

The eight complete genomes were nearly identical across the whole genome, with sequence identity above 99·98%, indicative of a very recent emergence into the human population (figure 1A). The largest nucleotide difference was four mutations. Notably, the sequence identity between the two virus genomes from the same patient (WH19001, from bronchoalveolar lavage fluid, and WH19005, from cell culture) was more than 99·99%,

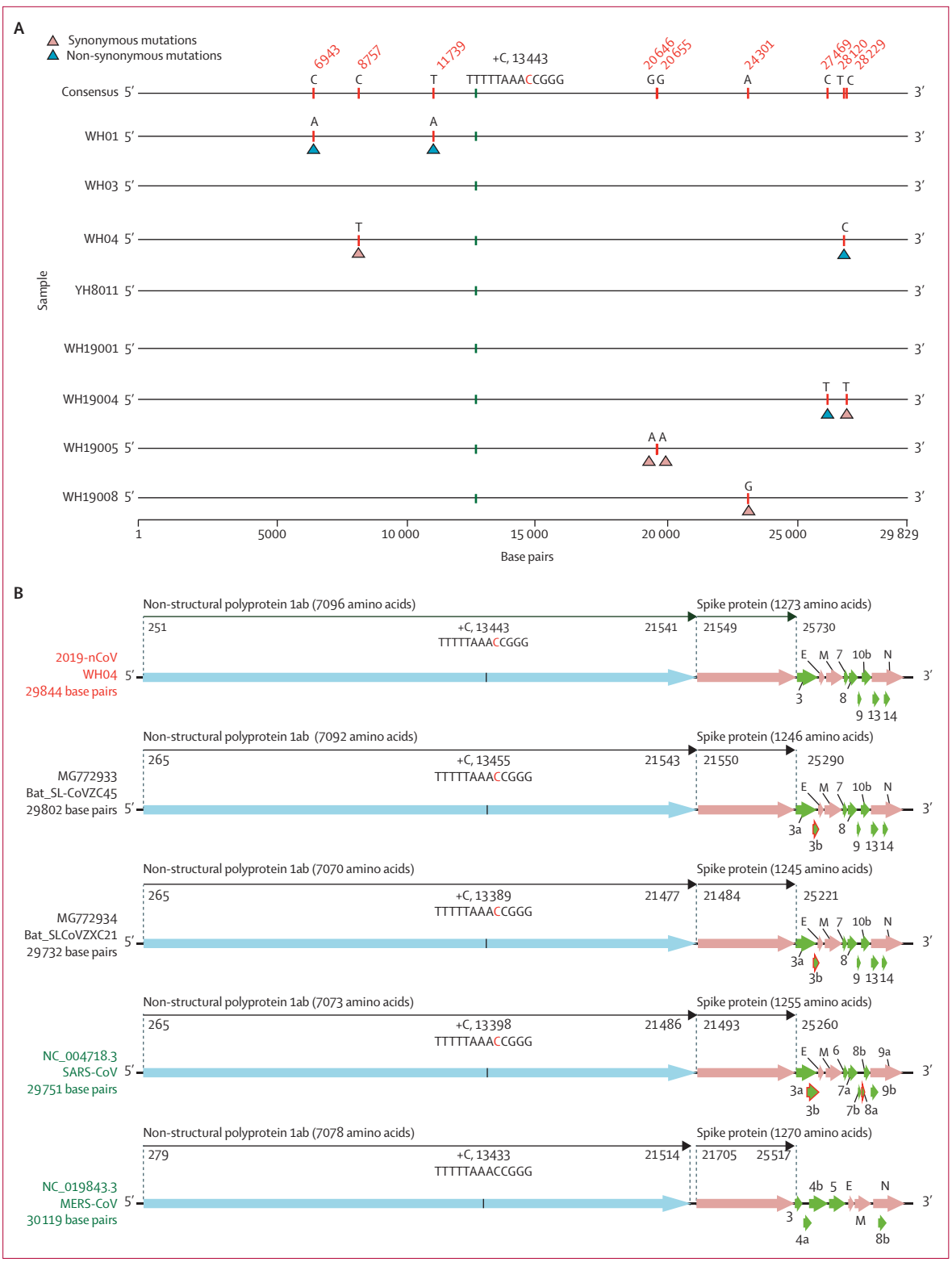

Figure 1: **Sequence comparison and genomic organisation of 2019-nCoV**
(A) Sequence alignment of eight full-length genomes of 2019-nCoV, 29 829 base pairs in length, with a few nucleotides truncated at both ends of the genome. (B) Coding regions of 2019-nCoV, bat-SL-CoVZC45, bat-SL-CoVZXC21, SARS-CoV, and MERS-CoV. Only open reading frames of more than 100 nucleotides are shown. 2019-nCoV=2019 novel coronavirus. SARS-CoV=severe acute respiratory syndrome coronavirus. MERS-CoV=Middle East respiratory syndrome coronavirus.

A

	Strain	Complete genome (%)	Gene region (%)											
			1ab	1a	1b	S	3	E	M	7	8	10b	N	13	14
Nucleotide sequences	Bat-SL-CoVZC45	87·6	88·9	90·7	86·0	75·2	87·8	98·7	93·4	95·2	88·8	88·5	91·1	89·1	96·7
	Bat-SL-CoVZXC21	87·5	88·7	90·3	86·1	74·7	88·9	98·7	93·4	95·2	89·1	88·5	91·2	89·5	96·7
	SARS-CoVGZ02	79·0	79·5	75·4	86·3	72·7	75·6	93·5	85·1	74·5	82·1	..	88·1	..	..
Amino acid sequences	Bat-SL-CoVZC45	..	95·6	95·6	95·8	80·2	90·9	100·0	98·6	93·4	87·6	94·2	94·3	73·2	92·9
	Bat-SL-CoVZXC21	..	95·2	95·1	95·5	79·6	92·0	100·0	98·6	93·4	88·4	94·2	94·3	73·2	92·9
	SARS-CoVGZ02	..	86·2	80·5	95·6	76·2	73·1	94·7	90·1	68·9	85·2	..	90·3	..	..

B

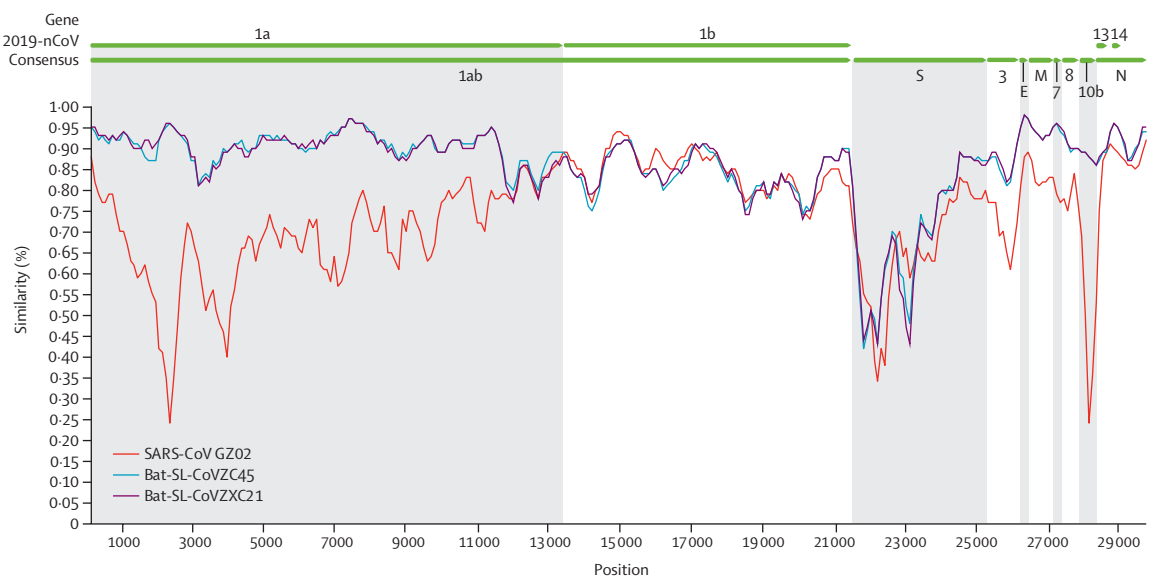

Figure 2: **Sequence identity between the consensus of 2019-nCoV and representative betacoronavirus genomes**
(A) Sequence identities for 2019-nCoV compared with SARS-CoV GZ02 (accession number AY390556) and the bat SARS-like coronaviruses bat-SL-CoVZC45 (MG772933) and bat-SL-CoVZXC21 (MG772934). (B) Similarity between 2019-nCoV and related viruses. 2019-nCoV=2019 novel coronavirus. SARS-CoV=severe acute respiratory syndrome coronavirus.

with 100% identity at the amino acid level. In addition, the partial genomes from samples WH02 and WH19002 also had nearly 100% identity to the complete genomes across the aligned gene regions.

A Blastn search of the complete genomes of 2019-nCoV revealed that the most closely related viruses available on GenBank were bat-SL-CoVZC45 (sequence identity 87·99%; query coverage 99%) and another SARS-like betacoronavirus of bat origin, bat-SL-CoVZXC21 (accession number MG772934;[23] 87·23%; query coverage 98%). In five gene regions (E, M, 7, N, and 14), the sequence identities were greater than 90%, with the highest being 98·7% in the E gene (figure 2A). The S gene of 2019-nCoV exhibited the lowest sequence identity with bat-SL-CoVZC45 and bat-SL-CoVZXC21, at only around 75%. In addition, the sequence identity in 1b (about 86%) was lower than that in 1a (about 90%; figure 2A). Most of the encoded proteins exhibited high sequence identity between 2019-nCoV and the related bat-derived coronaviruses (figure 2a). The notable exception was the spike protein, with only around 80% sequence identity, and protein 13, with 73·2% sequence identity. Notably, the 2019-nCoV strains were less genetically similar to SARS-CoV (about 79%) and MERS-CoV (about 50%). The similarity between 2019-nCoV and related viruses was visualised using SimPlot software, with the 2019-nCoV consensus sequence employed as the query (figure 2B).

Comparison of the predicted coding regions of 2019-nCoV showed that they possessed a similar genomic organisation to bat-SL-CoVZC45, bat-SL-CoVZXC21, and SARS-CoV (figure 1B). At least 12 coding regions were predicted, including 1ab, S, 3, E, M, 7, 8, 9, 10b, N, 13, and 14 (figure 1B). The lengths of most of the proteins encoded by 2019-nCoV, bat-SL-CoVZC45, and bat-SL-CoVZXC21 were similar, with only a few minor insertions or deletions. A notable difference was a longer spike protein encoded by 2019-nCoV compared with the bat SARS-like coronaviruses, SARS-CoV, and MERS-CoV (figure 1B).

Phylogenetic analysis of 2019-nCoV and its closely related reference genomes, as well as representative betacoronaviruses, revealed that the five subgenera formed five well supported branches (figure 3). The subgenus

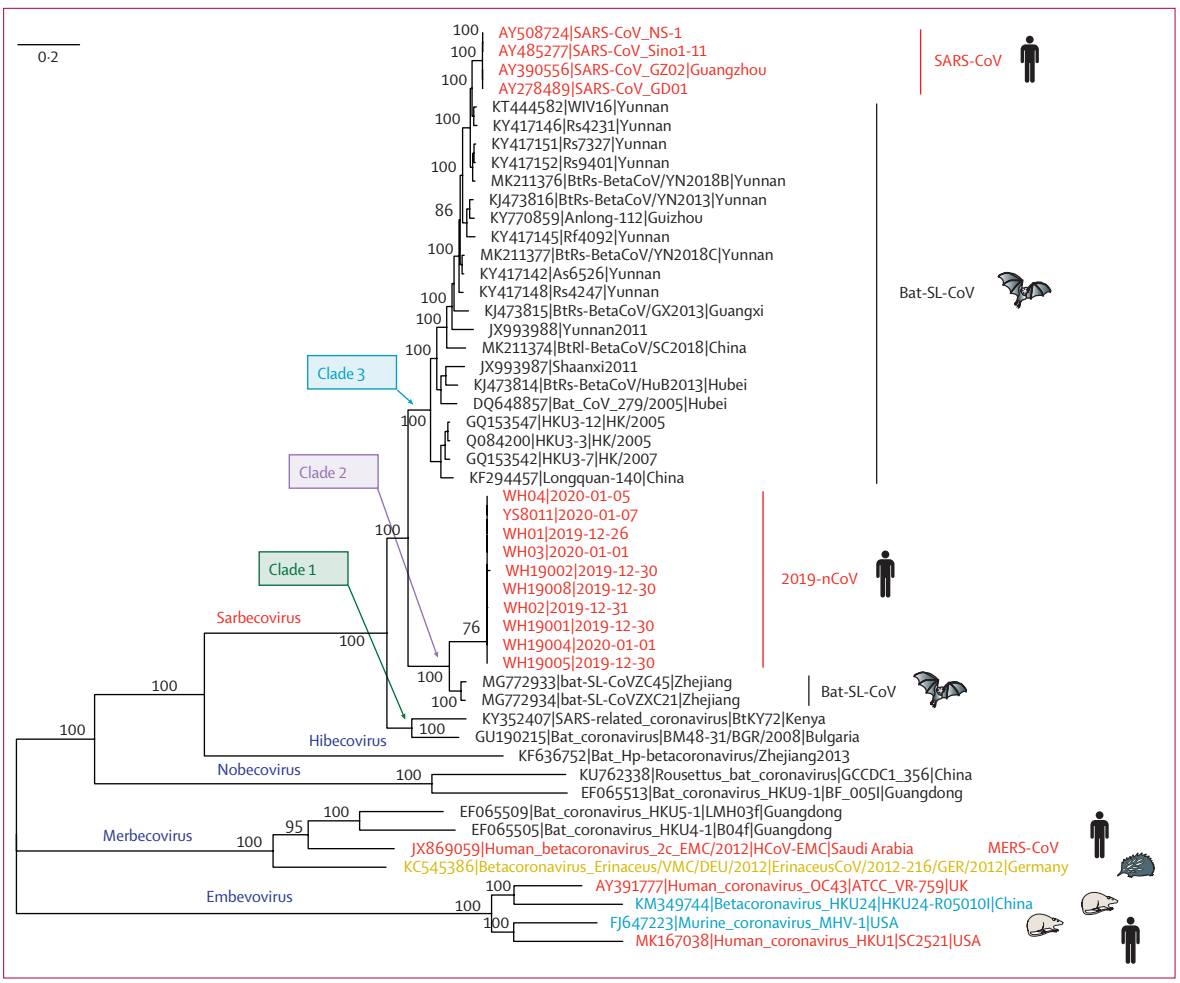

Figure 3: **Phylogenetic analysis of full-length genomes of 2019-nCoV and representative viruses of the genus Betacoronavirus**
2019-nCoV=2019 novel coronavirus. MERS-CoV=Middle East respiratory syndrome coronavirus. SARS-CoV=severe acute respiratory syndrome coronavirus.

Sarbecovirus could be classified into three well supported clades: two SARS-CoV-related strains from *Rhinolophus* sp from Bulgaria (accession number GU190215) and Kenya (KY352407) formed clade 1; the ten 2019-nCoV from Wuhan and the two bat-derived SARS-like strains from Zhoushan in eastern China (bat-SL-CoVZC45 and bat-SL-CoVZXC21) formed clade 2, which was notable for the long branch separating the human and bat viruses; and SARS-CoV strains from humans and many genetically similar SARS-like coronaviruses from bats collected from southwestern China formed clade 3, with bat-derived coronaviruses also falling in the basal positions (figure 3). In addition, 2019-nCoV was distinct from SARS-CoV in a phylogeny of the complete RNA-dependent RNA polymerase (*RdRp*) gene (appendix p 2). This evidence indicates that 2019-nCoV is a novel betacoronavirus from the subgenus Sarbecovirus.

As the sequence similarity plot revealed changes in genetic distances among viruses across the 2019-nCoV genome, we did additional phylogenetic analyses of the major encoding regions of representative members of the subgenus Sarbecovirus. Consistent with the genome phylogeny, 2019-nCoV, bat-SL-CoVZC45, and bat-SL-CoVZXC21 clustered together in trees of the 1a and spike genes (appendix p 3). By contrast, 2019-nCoV did not cluster with bat-SL-CoVZC45 and bat-SL-CoVZXC21 in the 1b tree, but instead formed a distinct clade with SARS-CoV, bat-SL-CoVZC45, and bat-SL-CoVZXC21 (appendix p 3), indicative of potential recombination events in 1b, although these probably occurred in the bat coronaviruses rather than 2019-nCoV. Phylogenetic analysis of the 2019-nCoV genome excluding 1b revealed similar evolutionary relationships as the full-length viral genome (appendix p 3).

The envelope spike (S) protein mediates receptor binding and membrane fusion[24] and is crucial for determining host tropism and transmission capacity.[25,26] Generally, the spike protein of coronaviruses is functionally divided into the S1 domain (especially positions 318–510 of SARS-CoV), responsible for receptor binding,

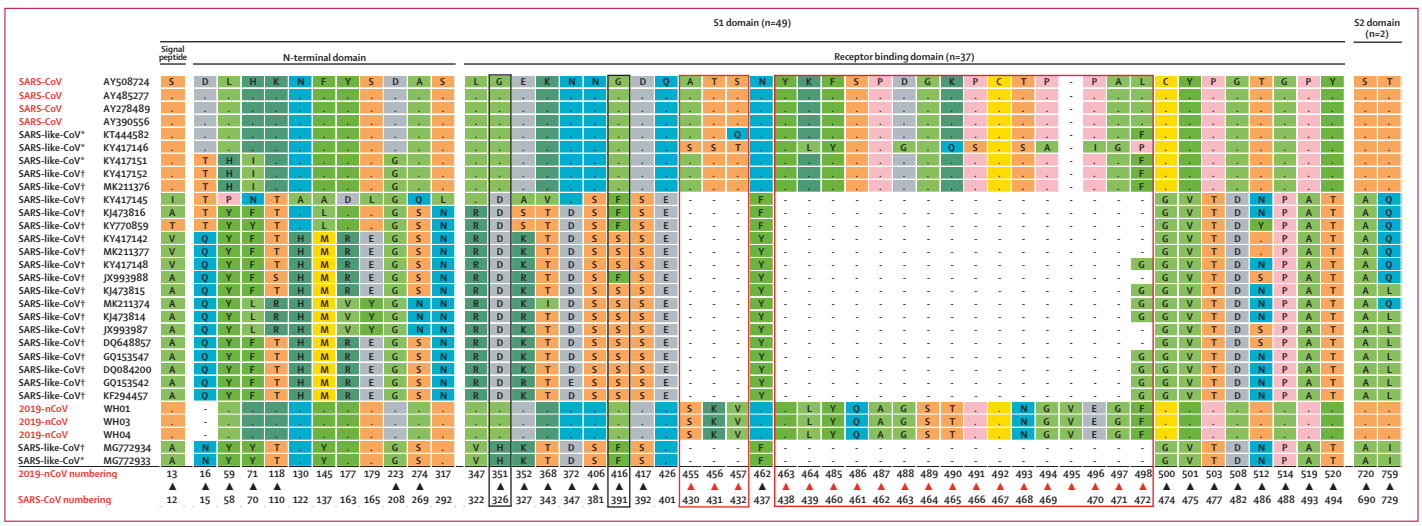

Figure 4: **Specific amino acid variations among the spike proteins of the subgenus sarbecovirus**
Viruses are ordered by the tree topology (as shown in figure 3) from top to bottom. One-letter codes are used for amino acids. CoV=coronavirus. 2019-nCoV=2019 novel coronavirus. SARS=severe acute respiratory syndrome. *Bat-derived SARS-like viruses that can grow in human cell lines or in mice. †Bat-derived SARS-like viruses without experimental data available.

and the S2 domain, responsible for cell membrane fusion.[27] The 2019-nCoV S2 protein showed around 93% sequence identity with bat-SL-CoVZC45 and bat-SL-CoVZXC21—much higher than that of the S1 domain, which had only around 68% identity with these bat-derived viruses. Both the N-terminal domain and the C-terminal domain of the S1 domain can bind to host receptors.[28] We inspected amino acid variation in the spike protein among the Sarbecovirus coronaviruses (figure 4). Although 2019-nCoV and SARS-CoV fell within different clades (figure 3), they still possessed around 50 conserved amino acids in S1, whereas most of the bat-derived viruses displayed mutational differences (figure 4). Most of these positions in the C-terminal domain (figure 4). In addition, a number of deletion events, including positions 455–457, 463–464, and 485–497, were found in the bat-derived strains (figure 4).

The receptor-binding domain of betacoronaviruses, which directly engages the receptor, is commonly located in the C-terminal domain of S1, as in SARS-CoV[29] for lineage B, and MERS-CoV[30,31] and BatCoV HKU4,[32] for lineage C (figure 5). Through phylogenetic analysis of the receptor-binding domain of four different lineages of betacoronaviruses (appendix p 4), we found that, although 2019-nCoV was closer to bat-SL-CoVZC45 and bat-SL-CoVZXC21 at the whole-genome level, the receptor-binding domain of 2019-nCoV fell within lineage B and was closer to that of SARS-CoV (figure 5A). The three-dimensional structure of 2019-nCoV receptor-binding domain was modelled using the Swiss-Model program[33] with the SARS-CoV receptor-binding domain structure (Protein Data Bank ID 2DD8)[34] as a template. This analysis suggested that, like other betacoronaviruses, the receptor-binding domain was composed of a core and an external subdomain (figure 5B–D). Notably, the external subdomain of the 2019-nCoV receptor-binding domain was more similar to that of SARS-CoV. This result suggests that 2019-nCoV might also use angiotensin-converting enzyme 2 (ACE2) as a cell receptor. However, we also observed that several key residues responsible for the binding of the SARS-CoV receptor-binding domain to the ACE2 receptor were variable in the 2019-nCoV receptor-binding domain (including Asn439, Asn501, Gln493, Gly485 and Phe486; 2019-nCoV numbering).

Discussion

From genomic surveillance of clinical samples from patients with viral pneumonia in Wuhan, China, a novel coronavirus (termed 2019-nCoV) has been identified.[10,11] Our phylogenetic analysis of 2019-nCoV, sequenced from nine patients' samples, showed that the virus belongs to the subgenus Sarbecovirus. 2019-nCoV was more similar to two bat-derived coronavirus strains, bat-SL-CoVZC45 and bat-SL-CoVZXC21, than to known human-infecting coronaviruses, including the virus that caused the SARS outbreak of 2003.

Epidemiologically, eight of the nine patients in our study had a history of exposure to the Huanan seafood market in Wuhan, suggesting that they might have been in close contact with the infection source at the market. However, one patient had never visited the market, although he had stayed in a hotel near the market before the onset of their illness. This finding suggests either possible droplet transmission or that the patient was infected by a currently unknown source. Evidence of clusters of infected family members and medical workers has now confirmed the presence of human-to-human transmission.[12] Clearly, this infection is a major public health concern, particularly as this outbreak coincides with the peak of the Chinese

Spring Festival travel rush, during which hundreds of millions of people will travel through China.

As a typical RNA virus, the average evolutionary rate for coronaviruses is roughly 10^{-4} nucleotide substitutions per site per year,[1] with mutations arising during every replication cycle. It is, therefore, striking that the sequences of 2019-nCoV from different patients described here were almost identical, with greater than 99·9% sequence identity. This finding suggests that 2019-nCoV originated from one source within a very short period and was detected relatively rapidly. However, as the virus transmits to more individuals, constant surveillance of mutations arising is needed.

Phylogenetic analysis showed that bat-derived coronaviruses fell within all five subgenera of the genus Betacoronavirus. Moreover, bat-derived coronaviruses fell in basal positions in the subgenus Sarbecovirus, with 2019-nCoV most closely related to bat-SL-CoVZC45 and bat-SL-CoVZXC21, which were also sampled from bats.[23] These data are consistent with a bat reservoir for coronaviruses in general and for 2019-nCoV in particular. However, despite the importance of bats, several facts suggest that another animal is acting as an intermediate host between bats and humans. First, the outbreak was first reported in late December, 2019, when most bat species in Wuhan are hibernating. Second, no bats were sold or found at the Huanan seafood market, whereas various non-aquatic animals (including mammals) were available for purchase. Third, the sequence identity between 2019-nCoV and its close relatives bat-SL-CoVZC45 and bat-SL-CoVZXC21 was less than 90%, which is reflected in the relatively long branch between them. Hence, bat-SL-CoVZC45 and bat-SL-CoVZXC21 are not direct ancestors of 2019-nCoV. Fourth, in both SARS-CoV and MERS-CoV, bats acted as the natural reservoir, with another animal (masked palm civet for SARS-CoV[35] and dromedary camels for MERS-CoV)[36] acting as an intermediate host, with humans as terminal hosts. Therefore, on the basis of current data, it seems likely that the 2019-nCoV causing the Wuhan outbreak might also be initially hosted by bats, and might have been transmitted to humans via currently unknown wild animal(s) sold at the Huanan seafood market.

Previous studies have uncovered several receptors that different coronaviruses bind to, such as ACE2 for SARS-CoV[29] and CD26 for MERS-CoV.[30] Our molecular modelling showed structural similarity between the receptor-binding domains of SARS-CoV and 2019-nCoV. Therefore, we suggest that 2019-nCoV might use ACE2 as the receptor, despite the presence of amino acid mutations in the 2019-nCoV receptor-binding domain. Although a previous study using HeLa cells expressing ACE2 proteins showed that 2019-nCoV could employ the ACE2 receptor,[37] whether these mutations affect ACE2 binding or change receptor tropism requires further study.

Recombination has been seen frequently in coronaviruses.[1] As expected, we detected recombination in the Sarbecoviruses analysed here. Our results suggest that recombination events are complex and are more likely occurring in bat coronaviruses than in 2019-nCoV. Hence, despite its occurrence, recombination is probably not the reason for emergence of this virus, although this inference might change if more closely related animal viruses are identified.

In conclusion, we have described the genomic structure of a seventh human coronavirus that can cause severe pneumonia and have shed light on its origin and

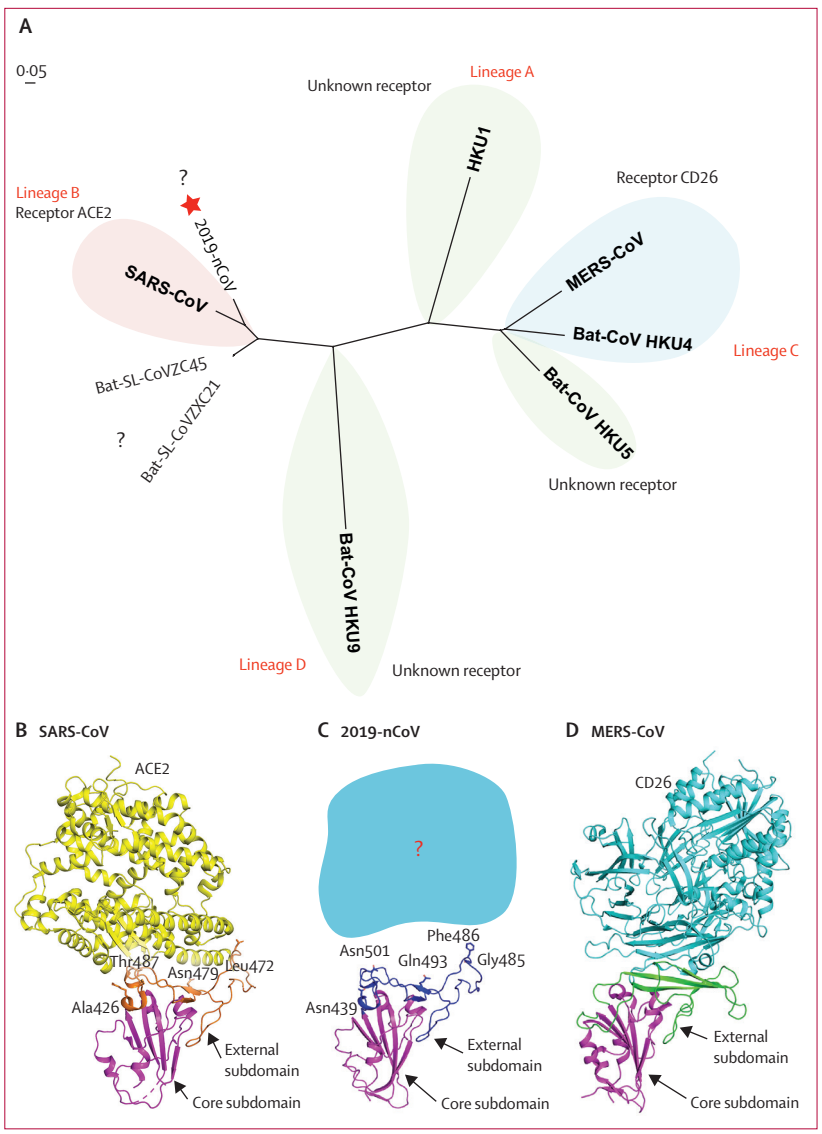

Figure 5: **Phylogenetic analysis and homology modelling of the receptor-binding domain of the 2019-nCoV, SARS-CoV, and MERS-CoV**

(A) Phylogenetic analysis of the receptor-binding domain from various betacoronaviruses. The star highlights 2019-nCoV and the question marks means that the receptor used by the viruses remains unknown. Structural comparison of the receptor-binding domain of SARS-CoV (B), 2019-nCoV (C), and MERS-CoV (D) binding to their own receptors. Core subdomains are magenta, and the external subdomains of SARS-CoV, 2019-nCoV, and MERS CoV are orange, dark blue, and green, respectively. Variable residues between SARS-CoV and 2019-nCoV in the receptor-binding site are highlighted as sticks. CoV=coronavirus. 2019-nCoV=2019 novel coronavirus. SARS-CoV=severe acute respiratory syndrome coronavirus. MERS=Middle East respiratory syndrome coronavirus.

receptor-binding properties. More generally, the disease outbreak linked to 2019-nCoV again highlights the hidden virus reservoir in wild animals and their potential to occasionally spill over into human populations.

Contributors

GFG, WT, WS, WC, WX, and GW designed the study. RL, XZ, PN, HW, WW, BH, NZ, XM, WZ, LZ, JC, YM, JW, YL, JY, ZX, JM, WJL, and DW did the experiments. BY, FZ, and ZH provided samples. WS, WC, WT, JL, HS, YB, LW, TH, and HZ analysed data. WS, WT, and JL wrote the report. ECH and GFG revised the report.

Declaration of interests

We declare no competing interests.

Data sharing

Data are available on various websites and have been made publicly available (more information can be found in the first paragraph of the Results section).

Acknowledgments

This work was supported by the National Key Research and Development Programme of China (2016YFD0500301, 2020YFC0840800, 2020YFC0840900), the National Major Project for Control and Prevention of Infectious Disease in China (2017ZX10104001, 2018ZX10101002, 2018ZX10101004, and 2018ZX10732-401), the State Key Research Development Program of China (2019YFC1200501), the Strategic Priority Research Programme of the Chinese Academy of Sciences (XDB29010102), and the Academic Promotion Programme of Shandong First Medical University (2019QL006 and 2019PT008). WS was supported by the Taishan Scholars Programme of Shandong Province (ts201511056).

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nature

https://doi.org/10.1038/s41586-023-06043-2

Accelerated Article Preview

Surveillance of SARS-CoV-2 at the Huanan Seafood Market

Received: 17 February 2022

Accepted: 3 April 2023

Accelerated Article Preview

Cite this article as: Liu, W. J. et al. Surveillance of SARS-CoV-2 at the Huanan Seafood Market. *Nature* https://doi.org/10.1038/s41586-023-06043-2 (2023)

William J. Liu, Peipei Liu, Wenwen Lei, Zhiyuan Jia, Xiaozhou He, Weifeng Shi, Yun Tan, Shumei Zou, Gary Wong, Ji Wang, Feng Wang, Gang Wang, Kun Qin, Rongbao Gao, Jie Zhang, Min Li, Wenling Xiao, Yuanyuan Guo, Ziqian Xu, Yingze Zhao, Jingdong Song, Jing Zhang, Wei Zhen, Wenting Zhou, Beiwei Ye, Juan Song, Mengjie Yang, Weimin Zhou, Yuting Dai, Gang Lu, Yuhai Bi, Wenjie Tan, Jun Han, George F. Gao & Guizhen Wu

Surveillance of SARS-CoV-2 at the Huanan Seafood Market

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Abstract SARS-CoV-2, the causative agent of COVID-19, emerged in December 2019. Its origins remain uncertain. It has been reported that a number of the early human cases had a history of contact with the Huanan Seafood Market. Here we present the results of surveillance for SARS-CoV-2 within the market. From January 1st 2020, after closure of the market, 923 samples were collected from the environment. From 18th January, 457 samples were collected from 18 species of animals, comprising of unsold contents of refrigerators and freezers, swabs from stray animals, and the contents of a fish tank. Using RT-qPCR, SARS-CoV-2 was detected in 73 environmental samples, but none of the animal samples. Three live viruses were successfully isolated. The viruses from the market shared nucleotide identity of 99.99% to 100% with the human isolate HCoV-19/Wuhan/IVDC-HB-01/2019. SARS-CoV-2 lineage A (8782T and 28144C) was found in an environmental sample. RNA-seq analysis of SARS-CoV-2 positive and negative environmental samples showed an abundance of different vertebrate genera at the market. In summary, this study provides information about the distribution and prevalence of SARS-CoV-2 in the Huanan Seafood Market during the early stages of the COVID-19 outbreak.

Keywords:
COVID-19, SARS-CoV-2, Huanan Seafood Market, origin, high-throughput sequencing, virus isolation, sewage

Infections with novel human coronavirus 2019 (HCoV-19) [1,2], named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses (ICTV) [3], can result in coronavirus disease 2019 (COVID-19), characterized by various clinical outcomes from asymptomatic infections to severe pneumonia and even death [4,5]. Globally, as of Feb 28th 2023, over 758 million confirmed cases and over 6.8 million deaths have been reported (covid19.who.int).

Human cases with COVID-19 were first reported in late December 2019, in Wuhan, China, as pneumonia of unknown etiology (PUE). A majority of these early cases were found to be linked to the Huanan Seafood Market (HSM) in Wuhan [4,6], where various animal meats, exotic seafood and live animals were available for purchase. The HSM has been suspected to be the source of the COVID-19 pandemic [7]. Not all of the early human cases had epidemiological links to the market[6,8] and alternative hypotheses for the market association, for example entry of virus into the market via humans or the cold-chain, also exist.

SARS-CoV-2 has high similarity with a few coronaviruses derived from bats in Asian countries including China, Laos, Japan, Cambodia and Thailand, and some scientists have proposed that bats might be the original source of SARS-CoV-2 [1,8-14]. Whether another animal might have acted as an intermediate host to facilitate virus spillover from bats to humans is still unknown [15,16]. An important finding was the discovery of SARS-CoV-2-related coronaviruses from pangolins, in which the spike proteins contained receptor-binding domains (RBD) showing high similarity to the RBD of SARS-CoV-2 [17-19]. Pangolins might be involved in the ecology of coronaviruses, but whether they are the intermediate host for SARS-CoV-2 is unknown, given the current data[20]. A recent study documented the animal species in the HSM between May 2017 and November 2019 and noted that no pangolins or bats were present, but some hypothesized sarbecovirus-susceptible animals, such as raccoon dogs were present [21]. Thus far, the origins of SARS-CoV-2 [22,23] and the role of the HSM in the origins and

spread of SARS-CoV-2 remain unclear. The data from the HSM may provide important information.

The HSM is located in the Jianghan District in the downtown area of Wuhan, the capital city of Hubei Province, and is approximately 800 m away from Hankou Railway Station, a major railway travel hub. It occupies >50,000 m^2, with 678 stalls located close to each other in extremely crowded conditions (Fig. 1A). The market is separated into two zones, the East and West Zones, with seafood and animals mainly sold in the West Zone and livestock meat in the East Zone. Among the 678 stalls of the market, 10 stalls selling domesticated wildlife (1.5%) were identified according to sale records [24], located in the south-western corner of West Zone (8/10) and the north-western corner of East Zone (2/10), respectively (Fig. 1A). According to sale records, during late December 2019, animals or animal products were sold in these 10 animal stalls. Animals included snakes, avian species (chickens, ducks, gooses, pheasants and doves), Sika deer, badgers, rabbits, bamboo rats, porcupines, hedgehogs, salamanders, giant salamanders, bay crocodiles and Siamese crocodiles, etc., among which snakes, salamanders and crocodiles were traded as live animals (described in detail in the Report of WHO-convened global study of origins of SARS-CoV-2 [24]).

The market was closed in the morning of January 1st, 2020, shortly after the identification of the PUE. On the same day, in the early morning, the Chinese Center for Disease Control and Prevention (China CDC) dispatched an epidemiological team, together with experts from Hubei Provincial CDC and Wuhan Municipal CDC, to the HSM to collect environmental samples in order to investigate the potential introduction of SARS-CoV-2 to the market (Fig. 1B). From January 1st 2020 until March 2nd 2020, a total of 923 environmental samples from different locations within and around the market and 457 animal samples, including dead animals in refrigerators and freezers and stray animals and their feces, were collected, with some stray animals sampled until March 30th (Extended Data Tables 1, 2, 3 and Supplementary Table 1). After the closure

of the market, the outside surface of the rolling shutter doors of the stalls and the corridors were disinfected (with 1% bleach mixed with water) throughout January and February 2020. The goods inside the stalls were completely cleared and disinfected until early March 2020.

Out of the 923 environmental samples collected in and around the HSM, 73 were found by the real-time polymerase chain reactions (RT-PCR) to be positive for SARS-CoV-2 with positivity rate of 7.9%. Cycle threshold (CT) values for the RT-PCR ranged from 23.9 to 41.7 (Supplementary Table 2). Among the 828 samples inside the HSM, 64 samples (7.7%) were positive. Of the 64 SARS-CoV-2 positive samples collected inside the HSM, 87.5% (56/64) were collected in the West Zone of the market, in particular streets from no. 1 to 8, with 71.4% (40/56) positive samples identified herein (Fig. 1A). Among the 14 samples from warehouses related to the HSM, five tested positive. This may reflect the nature of SARS-CoV-2 presence in the market during the early phase of the outbreak. Among the 51 samples from sewerage wells (Supplementary Table 1) in the surrounding areas outside the HSM, three tested positive (Supplementary Table 2). Notably, one sample (Env_0601), a floor surface swab, out of the 30 environmental samples collected from Dongxihu Market in Wuhan on January 22nd 2020, also tested positive (Supplementary Table 2, Extended Data Table 4).

Of the 110 samples collected from sewers or sewerage wells in the market, 24 samples were positive for SARS-CoV-2 nucleic acid. All four sewerage wells in the market tested positive. During the onsite investigation of the overground drainage pathway in the HSM, we found that the wastewater in the overground drainage led into the underground drainage inside the market and then flowed into the wells on the edge of the market. We then did a spot-check sampling across all the overground drains according to the principles described in the Methods (Extended Data Fig. 1). Excreta of the upper respiratory tract of infected humans and the potential animal waste would be mixed together into the overground drainage. Thus, these data suggested that either

infected people and/or animals in the market contaminated the sewage or that the contaminated sewage may have further played a role in furthering the virus transmission within the case cluster in the market.

The merchants' activities were assessed against the PCR results of the environmental samples. The sampling covered 19.8% (134/678) of the shops in the market (95% confidence interval (CI): 16.8-23.0%). Of the positive samples, 44 were distributed among 21 shops in the market, 19 of whom were located in the West Zone with the remaining two located in the east area (Fig. 1A). Some vendors sold more than one type of product. While the results provided some indication of the association of cases with different products, no significant differences were observed between different shops, including those selling poultry (22%, 8/37: 95% CI: 9.8-38.2%), cold-chain products (18.4%, 16/87, 95% CI: 10.9-28.1%), aquatic products (17.8%, 13/73, 95% CI: 9.8-28.5%), livestock (14%, 5/36: 95% CI: 4.7-29.5%), seafood products (11%, 6/56: 95% CI: 4-21.9%), wildlife products (11%, 1/9: 95% CI: 0.3-48.2%), and vegetables (25%, 2/8: 95% CI: 3.2-65%) (Extended Data Fig. 2, Extended Data Table 5). The detection of SARS-CoV-2 in multiple shops selling different product types suggested that SARS-CoV-2 may have been circulating in the market, especially the West Zone, for a while in December 2019, leading to an extensive distribution of the virus within the market, which may have been facilitated by the crowded buyers and the contaminated environment.

The 457 animal samples included 188 individuals belonging to 18 species (with some stray animals sampled until March 30th) (Extended Data Table 6). The sources of the samples included unsold goods kept in refrigerators and freezers in the stalls of the HSM, and goods kept in warehouses and refrigerators related to the HSM. Three Chinese giant salamanders, which were found in a fish tank, were alive and swab samples were collected and tested. Samples from stray animals in the market were also collected, comprising swab samples from 10 stray cats, 27 samples of cat feces, one

dog, one weasel, and 10 rats. All the 457 animal samples tested negative for SARS-CoV-2 nucleic acid.

To determine whether there was live virus in the HSM, we inoculated 27 SARS-CoV-2 positive environmental samples collected on January 1st, 2020, into cell lines, including Vero E6 and Huh7.5 cells. Cytopathic effects (CPE) were observed 3 days post inoculation with sample Env_0313 on Vero E6 cells. CPE was also observed 5 days post inoculation on Huh7.5 cells. The electron micrographs of Vero E6 cells after 5 days of post inoculation showed that virus particles were present in both the supernatant and the cells. Negative-stained virus particles and ultra-thin cultured cell sections showed typical coronavirus morphology (Fig. 2). Live viruses were isolated from samples Env_0313, Env_0354 and Env_0126, which were the only three samples with CT values <30 in the PCR. Env_0354 and Env_0126 were swab samples from the ground and Env_0313 were swab samples from a wall. Notably, samples Env_0313 and Env_0354 were from the stalls with confirmed patients. All the results of successful virus isolation from the original samples with low CT values revealed the existence of live SARS-CoV-2 with high titers in the environment of the HSM. Do the high CT values, we did not perform virus isolation based on the samples collected from later time points due.

During later sampling in the HSM in February, we collected samples to investigate the virus RNA persistence in the market. Some of these samples tested positive, especially in the sewage well and even on the walls (Supplementary Table 2). Within the 73 PCR positive samples, 35 samples (27 within the HSM and 8 from the surrounding area) collected in February were still positive for SARS-CoV-2. The long persistence of its genetic material in the environment might reflect high levels of environmental contamination before the market was closed. For the sample Env_0838, collected from a wall on February 20th 2020, a 3-plex PCR test was performed. The viral RNA segment was undetectable in one PCR channel targeting N gene, but could be amplified in the

other two channels targeting the RdRp and E genes, with CT values of 32.59 and 37.34, respectively. This result is reasonable considering the degradation of the viral genome. However, the results also indicate a long persistence of the viral RNA in the environment.

We further performed high-throughput sequencing (Supplementary Table 3) and successfully obtained seven complete or near complete SARS-CoV-2 genome sequences, including three sequences from three environmental samples (Env_0313, Env_0354 and Env_0020), and four sequences from cell supernatants of Env_0313, Env_0354 and Env_0126 (Fig. 3, Supplementary Table 4). A few samples were re-sequenced using a multiplex PCR approach, including Env_0020_seq01, Env_0313_seq04, Env_0313_seq05, Env_0126_seq06, and Env_0354_seq07 (Supplementary Table 3 and 4). The genome sequences of three environmental samples, Env_0126, Env_0313 and Env_0354, were found to be completely identical to the reference strain HCoV-19/Wuhan/IVDC-HB-01/2019 (IVDC-HB-01, GISAID accession number: EPI_ISL_402119) and the human strain Wuhan-Hu-1 (GenBank: NC_045512) (Fig. 3A). The genome sequence of the isolated virus from environmental sample Env_0354 had two synonymous mutations compared to HCoV-19/Wuhan/IVDC-HB-01/2019, with sequence identity of 99.99% (Fig. 3A). Therefore, the SARS-CoV-2 sequences from environmental samples were highly similar to the clinical strains obtained during the early stages of the COVID-19 outbreak.

Previously, SARS-CoV-2 has been proposed to be classified into two major lineages based on the two highly-linked single nucleotide polymorphisms (SNPs): A lineage (8782T and 28144C, or S lineage in another nomenclature of SARS-CoV-2) and B lineage (8782C and 28144T, or L lineage). It has been proposed that A/S lineage most likely is the ancestral lineage, because all of the SARS-CoV-2 related coronaviruses from bats and pangolins possessed 8782T and 28144C [25,26], while Pekar et al. also presented a possibility that both lineages represent separate introduction events[27].

Phylogenetic analysis revealed that most of the environmental strains belong to the B/L lineage and they cluster together with the human strains circulating in the early stage of the pandemic (Fig. 3B, Supplementary Fig. 1). The phylogenetic analysis did not involve the environmental sample Env_0020, the A/S lineage of which was confirmed by the high number of reads mapped to positions 8782 and 28144 in Env_0020 (Supplementary Table 5). However, it should be noted that the genome of Env_0020 is of low quality and there are many discontinuous gaps in the assembled genome. Indeed, though it is difficult to root the SARS-CoV-2 phylogenetic tree, our analysis indicated that the environmental viruses clustered together with the human strains circulating in the early stages of the pandemic.

We conducted RNA-seq analysis using 60 SARS-CoV-2 PCR-positive and 112 SARS-CoV-2 PCR-negative environmental samples from the HSM (Fig. 4A and Supplementary Table 3), in which the bias of sampling and RNA-seq should be considered. We used two approaches for genera identification. The Kraken2 method with all available genes/genomes in the database was used for the identification of all genera, including bacteria, viruses, eukaryota, and archaea. Additionally, the barcoding method using mitochondrial cytochrome c oxidase subunit I (COI) sequences was used specifically for the identification of Chordata genera. Bacteria were the most abundant species in almost all samples and mammal species could be found in most samples, which fit the feature of samples collected from the environment (Fig. 4B and Supplementary Table 6 and 7). *Gallus*, *Homo*, *Anas*, *Sus*, *Bos*, and *Canis* could be detected in most samples (Fig. 4C and Supplementary Table 8), which was in accordance with the environmental feature of the seafood markets in China. We analyzed the mammalian genera in all sequenced samples with kranken2 (detailed in the methods) using different thresholds. A total of 70 mammal genera, which existed in more than 2% samples, were identified with a threshold of 100 reads per million (Fig. 4D). It is important to highlight that the results of the kraken2 analysis (Figure 4D) and the BOLD analysis (Extended Data Figure 3) differ. In particular, the proportion of

reads assigned as raccoon dog differ considerably with the two methods used. This may be due to the heterogeneity of the reference data used by the two methods (BOLD, as for mitochondria, and kraken2 for whole genome). It should be noted that the genera identified using current approaches might be updated with additional reference genomes. As such, this list is not definitive and further in-depth analysis with other methods will be required to provide more information regarding the wildlife species present at the market.

Particularly, we analyzed three samples (Env_0126, Env_0313 and Env_0354) collected on 1st Jan 2020 with high levels of SARS-CoV-2 (Ct value <30) (Fig. 4E). The identified mammal genera in the Env_0313 and Env_0354 samples were related to species in the general food market, such as *Homo*, *Ovis*, *Bos*, *Canis*, *Sus*, and *Felis*. Many mammalian genera were observed in the Env_0126 sample, but the most abundant mammalian genera were also related to the general food market, including *Bos* (77.30%), *Ovis* (19.91%), *Homo* (0.77%), and *Bubalus* (0.57%). *Pipistrellus* (0.002%) and *Lutra* (0.001%) were found also found in this sample, but at extremely low relative abundance, raising the possibility of false detection. Moreover, we also noted that only *Homo*, *Ovis*, *Bos*, and *Sus* reads but not species related to wildlife were found in the Env_0020 samples, the one that belongs to the A/S lineage.

We illustrated the top-ranked genera in four areas of the market, where multiple SARS-CoV-2 PCR-positive samples were detected. As shown in Fig. 4F, the top-ranked genera in these areas were *homo* or other genera that generally exist in food markets. We also noted that *Nyctereutes* could be found in the shop 25 of street 8, while *Atelerix* and *Erinaceus* could be found in shops 15-17 of street 7 (Fig. 4F). These genera were detected in both SARS-CoV-2 positive and SARS-CoV-2 negative samples, and actually more often so in negative ones (Supplementary Table 6-9), and furthermore, this does not allow conclusions about whether these animals were infected with SARS-CoV-2.

We checked samples that might relate to wildlife, such as samples collected in the defeathering machine and areas with the visible blood spots. The most abundant mammal genera of the defeathering machine sample (Env_0584) was *Canis* (Extended data Fig 3). The most abundant mammal species of the visible blood spot sample (Env_0262) were *Bos*, *Sus*, *Ovis* and *Bison*, accordingly (Extended data Fig 3). Additionally, we plotted the distribution of some genera of concern, including *Myotis*, *Erinaceus*, *Mustela*, *Nyctereutes*, *Rhizomys*, *Meles*, and *Melogale*. Most of these samples were distributed in the western district of the market (Extended data Fig 4), where wildlife products were sold, though this also reflects the zone much more intensively sampled and analyzed by RNA-seq. The distribution locations of *Homo*, *Sus*, *Bos*, *Gallus* and *Anas* were also dominant in this area, where the enriched areas of SARS-CoV-2 PCR-positive samples were nearby. The repeated sampling of the locations with PCR-positive results may contribute some bias to the distribution analyses of enriched areas of SARS-CoV-2 PCR-positive samples. Additionally, We plotted the proportions of mammal genera in those SARS-CoV-2 positive samples with high abundance of genera related to wildlife, such as Env_0576 (*Nyctereutes* enriched), Env_0807 (*Lariscus* enriched), Env_0809 (*Erinaceus* enriched), and Env_0585 (*Erinaceus* enriched) (Extended Data Fig. 3).

Of particular note was the difference in the results from PCR and NGS. Among the 60 SARS-CoV-2 PCR-positive samples for RNA-seq analysis, 39 samples tested negative by NGS (no SARS-CoV-2 reads at all) (65.0%), including sample Env_0262. For these NGS-negative samples, the CT values ranged from 31.80 to 37.44. Since the RT-PCR detection assay employed in the very early stage of the pandemic was not formally verified, we believe that there may be some false positives in the PCR detection results in this study. Meanwhile, we also found that SARS-CoV-2 reads could also be detected by NGS in a portion of SARS-CoV-2 PCR negative samples (15.2%), which might be caused by the degradation of SARS-CoV-2 within the PCR target region or

contamination during library building.

In summary, we report the detection of SARS-CoV-2 RNA and live virus in environmental samples from the West Zone of the HSM. We should note that the selection of shops for sampling was biased because shops selling wildlife as well as shops linked to early cases were prioritized for sampling. The origin of the virus cannot be determined from all the analyses available so far. Although gene barcode analysis of animal species in the study suggested that *Myotis*, *Nyctereutes* and *Melogale* – species that have been recognized as potential host species of sarbecoviruses – were present at the market, these barcodes were mostly detected within the SARS-CoV-2 PCR negative environment samples. It remains possible that the market may acted as an amplifier of transmission due to the high number of visitors every day, causing many of the initially identified infection clusters in the early stages of the outbreak [24].

Recent reports traced the outbreak back to the HSM and proposed, after compiling information reported by various sources, including the WHO-China Joint Report and social media, etc. that the market sold live wild animals as recently as 2019 [28]. Another report hypothesized that SARS-CoV-2 spilled over from animals to humans at least twice in November or December 2019, and the raccoon dog was hypothesized to be the intermediate host animal [27]. The evidence provided in this study is not sufficient to support such a hypothesis [29]. Our study confirmed the existence of raccoon dogs, and other hypothesized/potential SARS-CoV-2 susceptible animals, at the market, prior to its closure. However, these environmental samples cannot prove that the animals were infected. Furthermore, even if the animals were infected, our study does not rule out that human-to-animal transmission occurred, considering the sampling time was after the human infection within the market as reported retrospectively[6]. Thus, the possibility of potential introduction of the virus to the market through infected humans, or cold chain products, cannot be ruled out yet.

More work, involving internationally coordinated efforts, is needed to investigate the potential origins of SARS-CoV-2 [24]. Surveillance of wild animals should be enhanced to explore the potential natural and intermediate hosts for SARS-CoV-2 [7,30], if any, which would help to prevent future pandemics caused by animal-origin coronaviruses.

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Figure legends

Fig. 1. The distribution of the positive environmental samples in the Huanan Seafood Market.

A. As the place of the early cluster of COVID-19 patients, the Huanan Seafood Market is separated into East and West Zones with the Xinhua Road between them. To detect for the presence of SARS-CoV-2 RNA, reverse transcription, quantitative polymerase chain reaction (RT-qPCR) was performed. The locations of the positive samples were marked in the map of the market within orange, while the location of the samples that the live viruses were isolated from were labeled with red. The map also shows locations of stalls where domesticated wildlife products were sold. B. Timeline of environmental and animal samples collected within and around the Huanan Seafood Market. The information of confirmed patients up to December 31st 2019 was referenced from the Report of WHO-convened global study of origins of SARS-CoV-2.

Fig. 2. The SARS-CoV-2 virus isolation from environmental samples of the Huanan Seafood Market.

The electron micrographs of the SARS-CoV-2 viruses isolated from the environmental samples in the Huanan Seafood Market. To determine whether SARS-CoV-2 particles could be visualized from the cell supernatant and lysate, we used transmission electron microscopy (EM) to observe the culture supernatant and ultra-thin section cells based from both VeroE6 and Huh7.5 cells. The electron micrographs showed that virus particles were present in both the supernatant (A, B) and the cells (C, D). Negative-stained virus particles were generally spherical, pleomorphic and 60-140 nm in diameter. Spike protrusions were observed around the particles in a crown (corona) shape (A, B). In ultra-thin cultured cell sections, a group of virus particles can be seen outside the cell (C), and sheets of virus particles can also be observed inside the cells (D). The graphs were the representatives of repeated experiments of electron micrographs.

Fig. 3. Genomic and phylogenetic analyses of SARS-CoV-2 virus genomes from the Huanan Seafood Market.

A. Sequence comparison of the full-length SARS-CoV-2 genomes in the environmental samples. B. Phylogenetic analysis of full-length SARS-CoV-2 genomes from the Huanan Seafood Market and representative strains from the early stage of the COVID-19 pandemic, showing that most environmental strains cluster together with the human strains in the B/L lineage, with Env_0020 in A/S lineage.

Fig. 4. Analysis of environmental samples in the Huanan Seafood Market.

A. Schematic illustration of the experimental design. All 73 SARS-CoV-2 positive samples were included for RNA-seq. A total of 60 RNA-seq libraries were successfully constructed. Additionally, RNA-seq libraries of 112 SARS-CoV-2 negative samples passed library quality control. The kranken2 was used for genus classification. The bowtie2 and sequences in the barcode of life data system was used for the classification of genus in the Mammalia class. B. Heatmap showing the reads distribution of the four domains (Bacteria, Eukaryota, Viruses and Archaea), the *Homo* genus, the *Mammalia* class and the SARS-CoV-2 species. SARS-CoV-2 PCR-positive or -negative were shown in the left panel. C. Positive ratio of illustrated genus in all tested samples. Top ranked genus within the *Mammalia* class were shown. D. Illustration of mammal genera in market using the threshold of 100 reads per millions. The samples were group by SARS-CoV-2 PCR results. The blue bar indicates the positive detected genera. E. Illustration of mammal genera distribution in samples with high viral load. The Env_0020, Env_0313, Env_0354 and Env_0126 were shown.

F. Distribution of the positively detected *Mammal* genera in the market. Samples in four areas where multiple SARS-CoV-2 PCR-positive samples were plotted. The distribution of top mammal genera in each area was shown.

Methods

Sample collection

The Huanan Seafood Market (HSM) was closed in the early morning of January 1st 2020, and at the same time, China CDC began collecting environmental and animal samples. Staff from China CDC entered the market about 30 times before the market's final clean-up on March 2nd 2020, with some stray animals sampled outside the market until March 30th. Environmental samples in the HSM were collected to represent exhaustively as possible, from a wide diversity of surfaces, animals and products (Supplementary Table 2 and Extended Data Table 6) according to different sampling principles, as described in detail in the Joint Report of WHO-convened Global Study of Origins of SARS-CoV-2: China Part [24].

The principles and ranges of in-market sampling covered: (1) environmental samples from stalls related to early cases; (2) environmental samples from doors and floors of all stalls in the blocks where the early cases were located; (3) environmental samples in the East Zone of the market were collected according to blocks; (4) transport carts, trash cans and similar objects; (5) environmental samples from stalls that sold livestock, poultry, farmed wildlife (also called "domesticated wildlife" or "domesticated wildlife products" in this report); (6) samples of sewage and silt from drainage channels and sewerage wells; (7) stray cats, rats and other stray animals in the market; (8) animal products and other commodity samples kept in the cold storages and refrigerators in the market; (9) the market's ventilation and air-conditioning system; and (10) public toilets, public activity rooms and other places where people gathered in the market.

The investigators used full personal protective equipment during the sampling in the market. Commercial products of swabs and virus preservation solution were used for the sampling (Disposable Virus Sampling Tube, V5-S-25, Shen Zhen Zi Jian Biotechnology Co., Ltd., Shenzhen, China). For environmental samples, sampling swabs were applied to smear the floors, walls or surfaces of objects and then preserved them in virus preservation solution.

For animal samples, depending on the type of animal and whether it was alive or frozen, pharyngeal, anal, body surface and body cavity swabs or tissue samples were

collected for nucleic acid testing (NAT). Generally, for alive animal and frozen full bodies, three samples, including pharyngeal, anal and body surface swabs were collected for each animal individuals. And for animal bodies after "bai tiao" disposing (remaining parts of poultry or livestock after removal of hair and viscera), the body cavity swabs were collected.

Drain samples were collected by the use of virus sampling swabs to probe into the silt at the bottom of drainage channels in the market. Wastewater and silt samples were preserved in virus preservation solution. For the sewage well (for the drain water), a container was used to take a silt-water mixture from a location near the bottom of the well, and an appropriate amount of sample was collected by using virus sampling swabs and then preserved in virus preservation solution.

Nucleic acid extraction and SARS-CoV-2 real-time PCR assay

A virus nucleic acid extraction kit (Xi'an Tianlong) was used to extract viral nucleic acid from samples using an automated nucleic acid extraction instrument according to the manufacturer's instructions. Real-time (RT) PCR was performed on extracted nucleic acid samples with a SARS-CoV-2 nucleic acid assay kit. The reagent brands include BioGerm (40/38, cycle number/cut-off value, the same as below), DAAN (45/40) and BGI (40/38).

Virus isolations

Virus isolations were performed in biosafety level (BSL)-3 laboratory in National Institute for Viral Diseases Control and Prevention, China CDC. Samples positive for SARS-CoV-2 were cultured in Vero E6 and Huh7.5 cells on January 11th, 2020. The cell lines were inoculated with positive samples and three blind passages were performed for each sample. The culture supernatant and cell pellet of each passage were harvested for RT PCR. The morphology of viral particles in the cell sections and the supernatant were firstly observed by transmission electron microscope (TEM) on January 22nd, 2020.

Metagenomic sequencing

Metagenomic sequencing was conducted at National Institute for Viral Disease Control

and Prevention, China CDC and Wuhan BGI. Nucleic acid was extracted using Qiagen's viral RNA microextraction kit and human nucleic acid was removed using an enrichment kit to improve the sensitivity of viral RNA detection. Extracted RNA was reverse transcribed into cDNA and segmented into 150-200 bp by enzyme digestion. After repair, fitting, purification, PCR amplification and purification, sample concentration was assayed by DNBSEQ-T7, and an average output of more than 200 million reads was obtained. Sequencing data were compared with those in a SARS-CoV-2 database to determine whether the samples contained coronavirus sequences. For the seven complete SARS-CoV-2 genome sequences, three sequences from environmental samples (Env_0020_seq01, Env_0313_seq02 and Env_0354_seq03) were obtained from DNBSEQ-T7, and four sequences from cell supernatants of Env_0313, Env_0354 and Env_0126 (Fig. 3) were obtained from NextSeq 550 platform. A few samples were re-sequenced using a multiplex PCR approach, including Env_0020_seq01, Env_0313_seq04, Env_0313_seq05, Env_0126_seq06, and Env_0354_seq07 (Supplementary Table 3 and 4). All raw data related to the genomes, including any partial genomes that were sequenced were fully reported and deposited to the public database (Supplementary Table 3 and 4).

Virus genome assembly and phylogenetic analysis

Raw reads were adaptor- and quality- trimmed with the Fastp (version 0.20.0) program. The clean reads were mapped to the SARS-CoV-2 reference genome (GenBank: NC_045512) using Bowtie2. The assembled genomes were merged and checked using Geneious (version 11.1.5) (https://www.geneious.com). The coverage and depth of genomes were calculated with SAMtools v1.10 based on SAM files from Bowtie2.

Reference genomes, IVDC-HB-01 (GISAID: EPI_ISL_402119) and Wuhan-Hu-1 (GenBank: NC_045512), were employed as a query. Multiple sequence alignment of the seven SARS-CoV-2 sequences obtained from this study and reference sequences were performed with Mafft (v7.450). Phylogenetic analyses were performed using RAxML v8.2.9 with 1000 bootstrap replicates, employing the GTR nucleotide substitution model and the Gamma distribution.

Bioinformatic analysis of the species abundances

The Kraken2 (version 2.1.2)[31] was used for species classification with the option '--confidence 0.1'. Sequences of all species in the Nucleotide (nt) database were used for generating the index. The bracken (version 2.5) was used for re-evaluating species abundance. The matrix of species was obtained by using the pavian algorithm[32]. ggplot2 package in R was used for plotting. Read counts of each genus were used for further analysis and plotting. Raw counts of four domains (Archaea, Viruses, Eukaryota, and Bacteria), SARS-CoV-2, *Homo* genus, and *Mammalia* class were shown by heatmap (4B). Two tail unpaired t-test was used for identification of differential genus between SARS-CoV-2 PCR-positive and -negative samples.

For the analysis of the Chordata genera characterization, the reference was generated using the sequence of mitochondrial cytochrome c oxidase subunit I (COI) in the barcode of life data (BOLD) system[33-35]. RNA-seq samples were mapped to the reference sequences by the bowtie2[36] algorithm with the default settings. Read counts of each genus were calculated by the samtools[37]. Read counts over 20 were used as cut-off for the identification of positively enriched genus. Fisher's exact test was used for comparing the differential genus in the Mammalia class between SARS-CoV-2 PCR-positive and -negative samples.

Ethics

The sample collection was determined by China CDC to be part of the emergency responses to the pneumonia of unknown etiology (PUE) and therefore exempt from institutional review board assessment.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

All the raw sequencing data and genomes have been uploaded onto the GISAID (China CDC Weekly, 2021, DOI: 10.46234/ccdcw2021.255). The list of accession codes in Supplementary Table 3 and 4. The raw sequence data reported in this paper have also been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics, 2021, DOI: 10.1016/j.gpb.2021.08.001) in National Genomics Data Center (Nucleic Acids Res, 2022, DOI: 10.1093/nar/gkab951), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences that are publicly accessible at https://ngdc.cncb.ac.cn/gsa (GSA: CRA010170). The viral genomes reported in this paper have been deposited in the GenBase in National Genomics Data Center (Beijing Institute of Genomics, Chinese Academy of Sciences/China National Center for Bioinformation under accession numbers C_AA002295.1 to C_AA002301.1 that are publicly accessible at https://ngdc.cncb.ac.cn/genbase/. Raw sequence data were also deposited into NCBI BioProject under accession number PRJNA948658 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA948658) and in China National Microbiology Data Center (NMDC) with accession numbers NMDC10018366 (https://nmdc.cn/resource/genomics/sample/detail/NMDC10018366).

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Acknowledgements

We gratefully acknowledge experts from Wuhan City, Hubei Province and across China who contributed to the study. We gratefully acknowledge the following experts for their invaluable contributions during this study: Prof. Naiying Mao and Prof. Yu Lan from National Institute for Viral Disease Control and Prevention, China CDC; Prof. Huaiqi Jing, and Prof. Qiyong Liu from National Institute for Communicable Disease Control and Prevention, China CDC; Prof. Lei Xu from Tsinghua University; Prof. Yongzhong Jiang, Junqiang Xu, Prof. Xixiang Huo and Dr. Bo Yu from Hubei Provincial CDC; Prof. Yan Xiong from Wuhan Municipal CDC; Prof. Juan Li from Shandong First Medical University; Prof. Weijun Chen and Dr. Honglong Wu from BGI PathoGenesis Pharmaceutical Technology. In addition, we also thank the work and suggestions of the joint team scientists of WHO-convened Global Study of Origins of SARS-CoV-2: China Part. Prof. William J. Liu is supported by the Excellent Young Scientist Program of the National Natural Science Foundation of China (NSFC, 81822040). Dr. Yun Tan's bioinformatics analyses in the study were supported by the ASTRA computing platform in the National Research Center for Translational Medicine (Shanghai) and the Pi computing platform in the Center for High Performance Computing at Shanghai Jiao Tong University.

Author contributions

The study was designed by G-Z.W., W.J.L and G.F.G. The onsite epidemiological survey and sample collection by W.J.L., W.L, Z.J., X.H., J.W., F.W., G.W., K.Q., R.G., J.Z., M.L. W.X. and G.F.G. The nucleic acid extraction and RT-PCR were performed by W.J.L., P.L., W.L, Z.J., X.H., J.W., F.W., K.C. and G.W. Next generation sequencing was performed by W.J.L., P.L., W.L, Z.J., X.H., J.W., F.W., G.W., and W.Z. Complete genome sequencing and analyses were performed by P.L., W.Z., W.S. and W.J.L. The virus isolation was performed by P.L., S.Z., W.Z., W.L., J.S. and Z.X. Data analyses were performed by W.J.L., P.L., Z.J., X.H., W.S., Y.T., S.Z., J.W., F.W., G.W., Y.G., Z.X., Y.Z., J.S., Jing Z., W.Z., W-T.Z., B.Y., J.S., M.Y., W-M.Z., Y.D., G.L., Y.B., W.T.,

and J.H. The manuscript was written by W.J.L., P.L., W.S., Y.T., Gary W., G.F.G. and G-Z.W.

Competing interest declaration

No competing interest exists.

Extended Data Figure Legends

Extended Data Fig. 1. The overground drainage pathway in the Huanan Seafood Market and environmental sample collection.

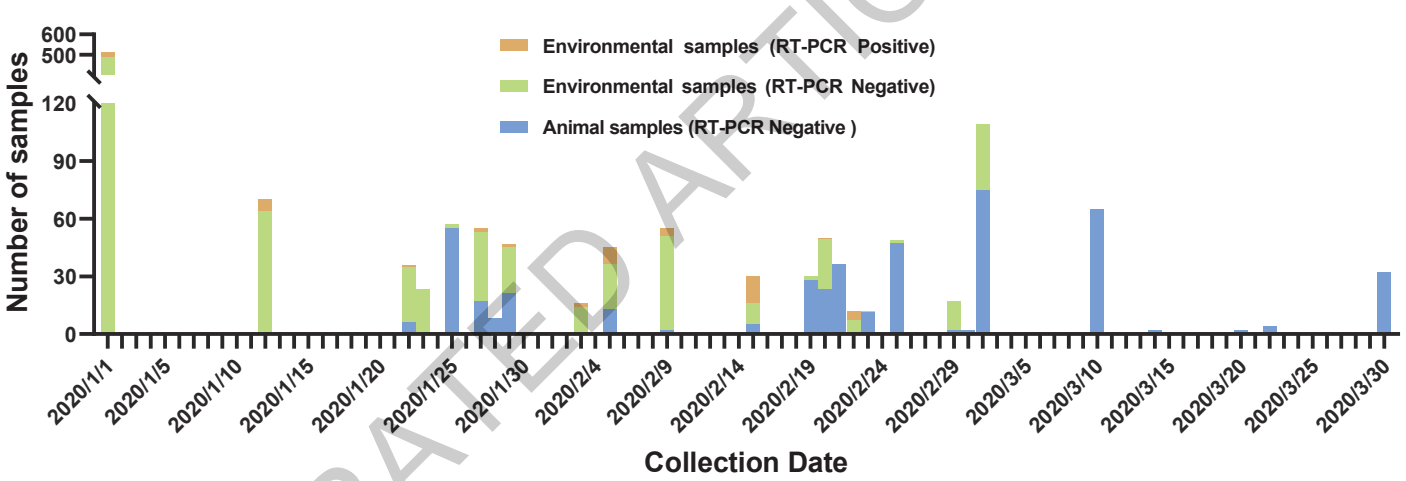
The wastewater in the overground drainage was lead into the underground drainage inside the market and then flow into the wells on the edge of the market. And we did a spot-check sampling across all the overground drainages. To detect for the presence of SARS-CoV-2 RNA, reverse transcription and quantitative polymerase chain reaction (RT-qPCR) were performed. The locations of the positive samples were marked in the map of the market within yellow.

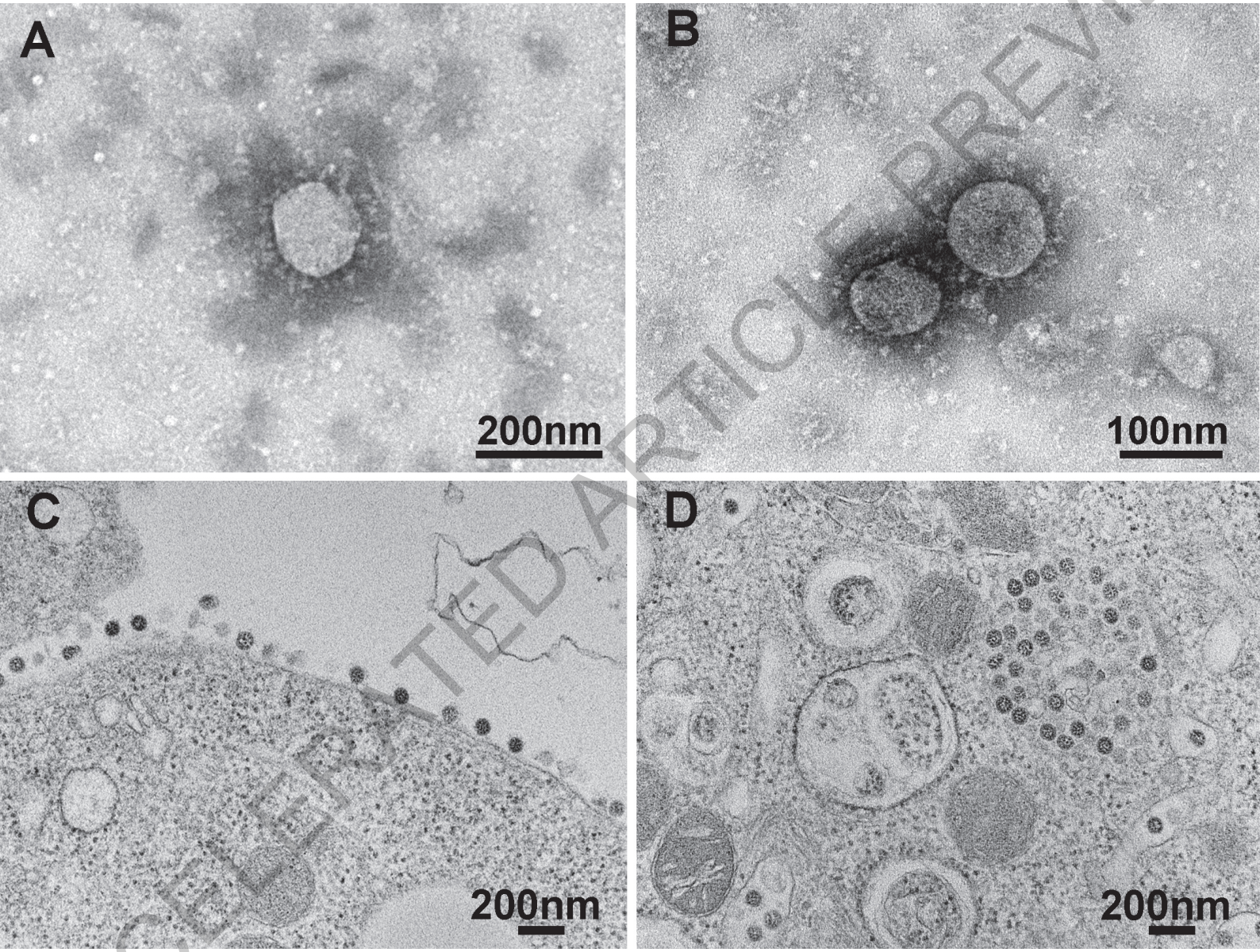
Extended Data Fig. 2. Positive environmental samples associated with different products in the Huanan Seafood Market.

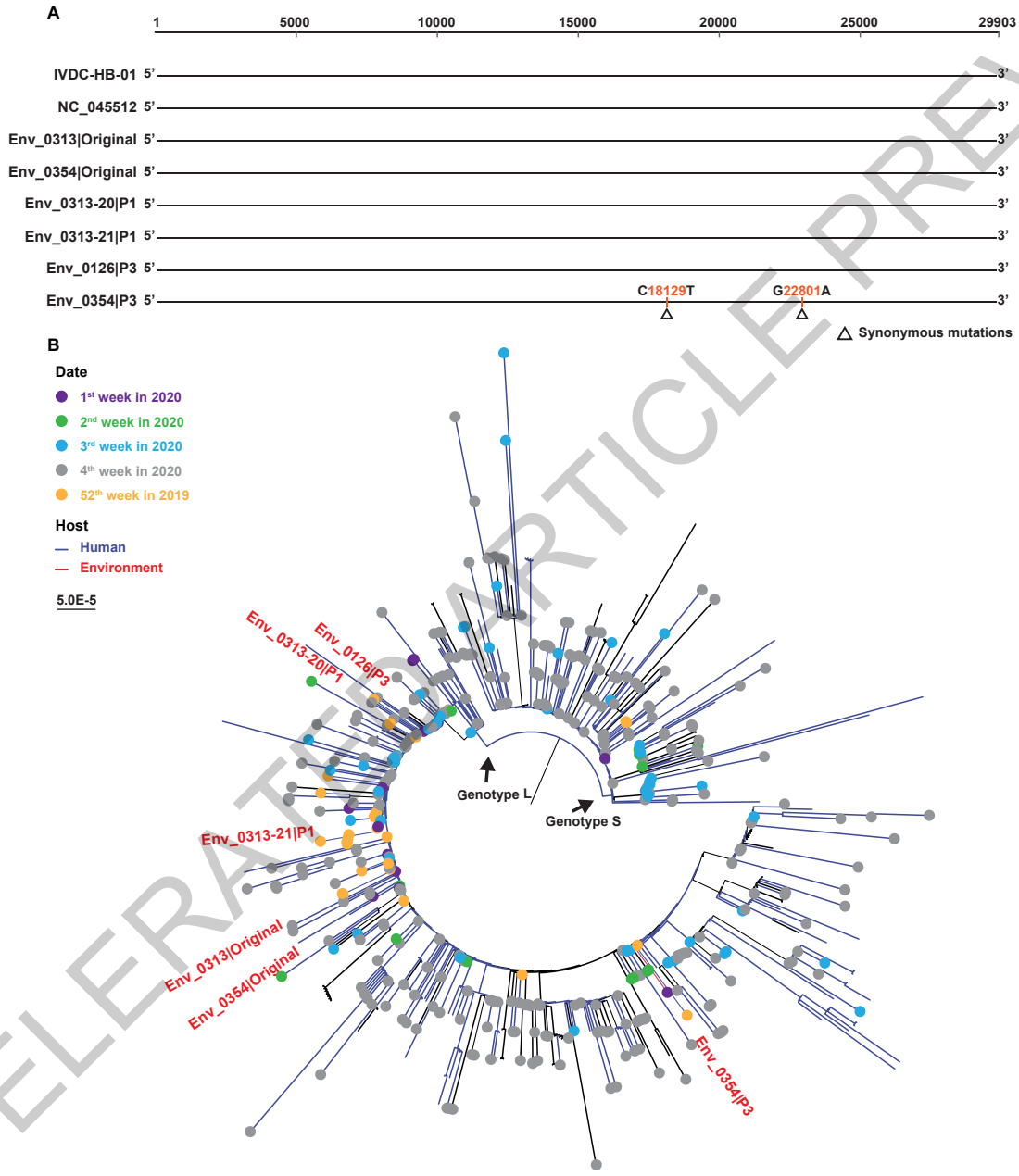
Dots represent the percentage of positive environmental samples associated with each product. Bars represent 95% confidence intervals for the binomials in the text above. Note that the confidence interval (CI) for some products (e.g. vegetables, farmed wildlife) have broad error bars that are likely due to the low number of vendors for these categories in the market. Nine of the 10 vendors selling farmed wildlife have been sampled. Data are represented as percentage in this figure.

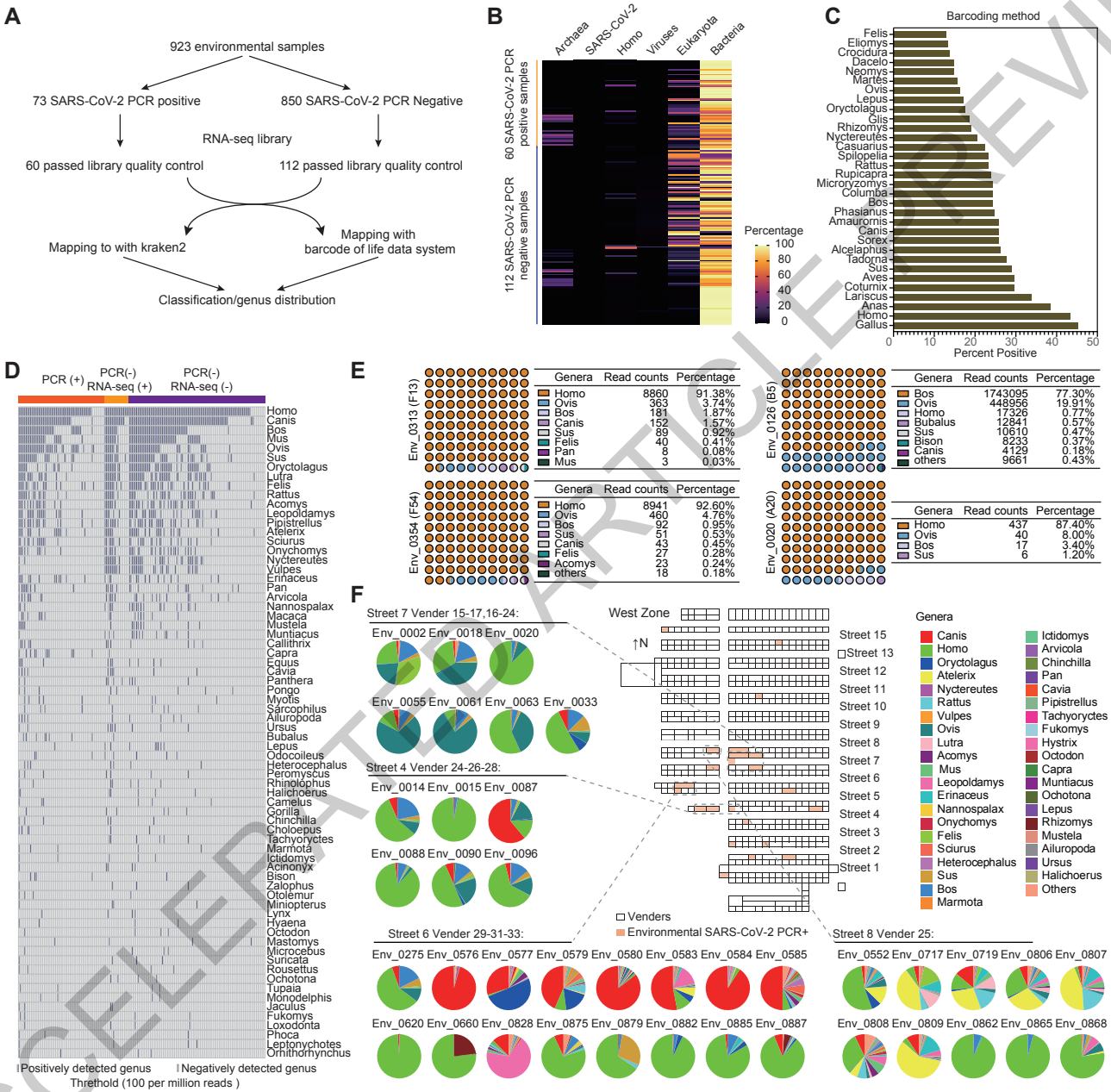
Extended Data Fig. 3. Illustration of mammal genera distribution in samples of concerns. Illustration of mammal genera distribution in samples of concerns. Samples related to the blood spot and the de-feather machine (Env_0262 and Env_0584) and samples enriched with genera related to wildlife (Env_0576, Env_0807, Env_0809, and Env_0585) were plotted. Animal genera identified by the BOLD method were shown

698 in the left panel, while mammal genera identified by the kraken2 method were shown
699 in the right panel.
700 **Extended Data Fig. 4. Distribution of the positively detected Mammal genera in**
701 **the market.** The distribution of SARS-CoV-2 and potential host were plotted by yellow
702 and blue dots, respectively. The density of the distribution of potential host was shown
703 in red, while the SARS-CoV-2 by green.
704
705 **Extended Data Tables**
706 **Extended Data Table 1. Overview of environmental sample sampling and testing**
707 **in the Huanan Seafood Market.**
708 **Extended Data Table 2. The collection logic of the environment samples.**
709 **Extended Data Table 3. The collection logic of the animal samples.**
710 **Extended Data Table 4. The information of the sampling in other markets.**
711 **Extended Data Table 5. Twenty-one shops of RT-PCR positive in the Huanan**
712 **Seafood Market.**
713 **Extended Data Table 6. The animal samples collected in the Huanan Seafood**
714 **Market.**
715

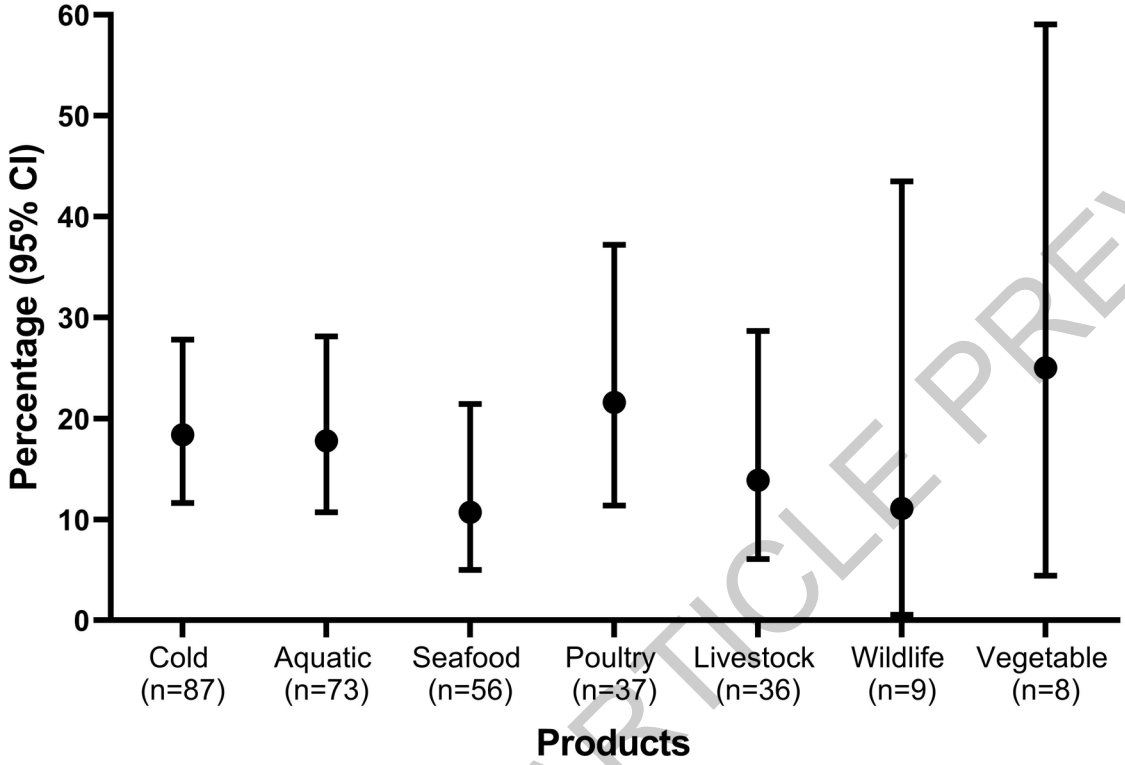




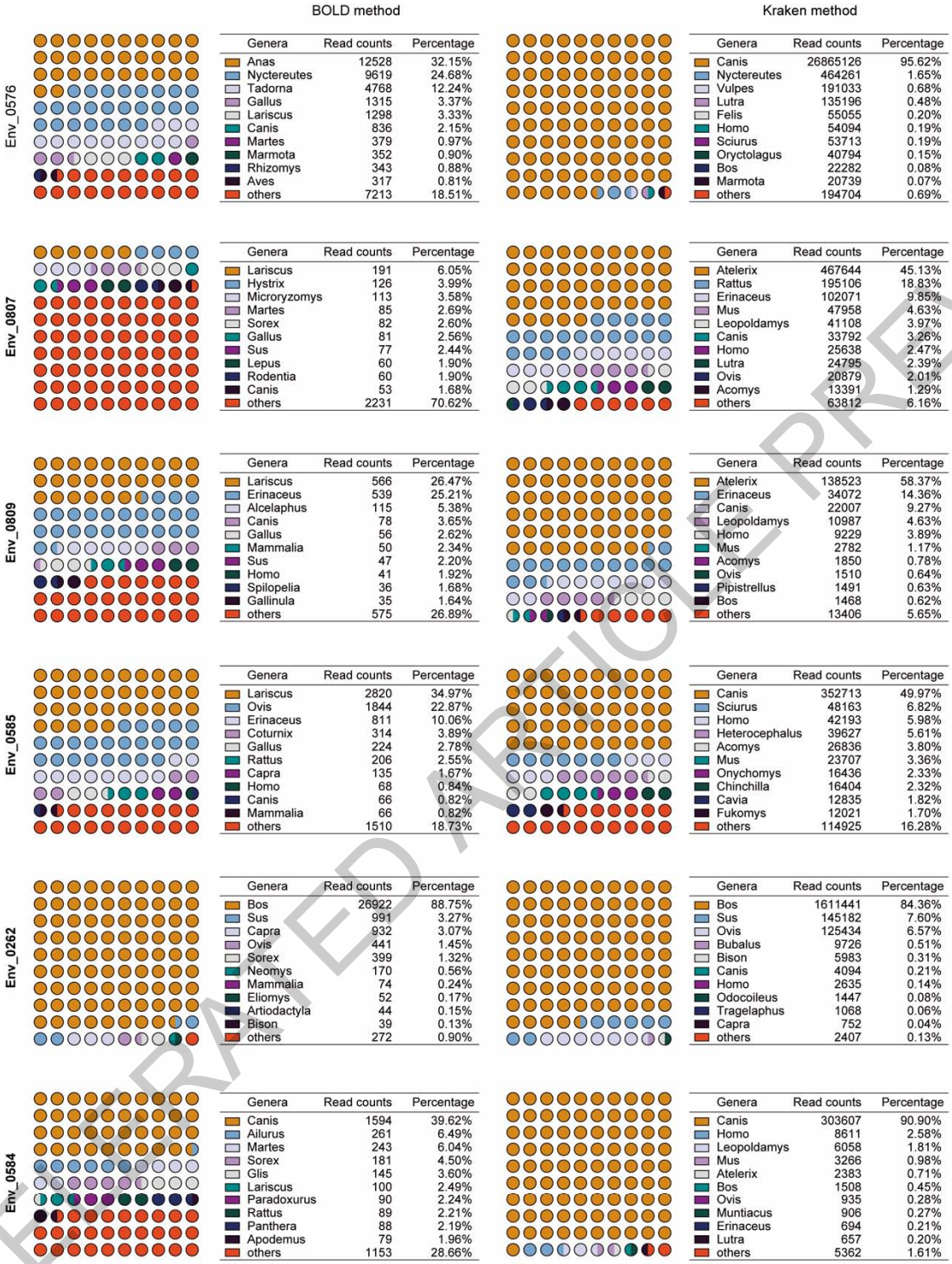




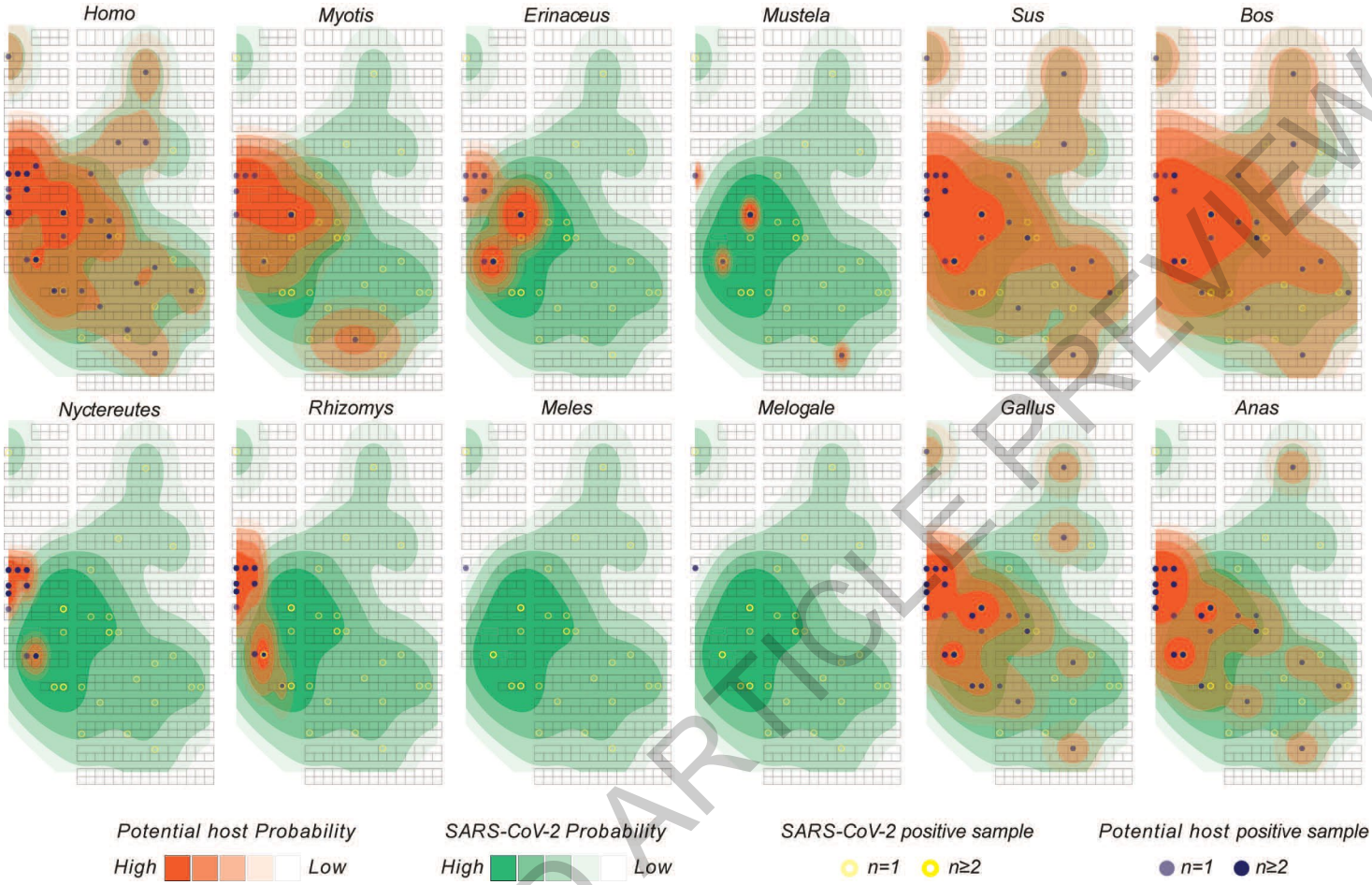
Extended Data Fig. 1



Extended Data Fig. 2



Extended Data Fig. 3



Extended Data Fig. 4

Extended Data Table 1. Overview of environmental sample sampling and testing in the Huanan Seafood Market.

	Number of samples	Number of positive samples by RT-PCR	Number of isolated viruses
Huanan Seafood Market	718	40	3
Warehouses related to the Huanan Seafood Market[a]	14	5	
Other markets in Wuhan and Huanggang[b]	30	1	
Drainage system in the Huanan Seafood Market	110	24	
Sewerage wells in surrounding areas	51	3	
Total	923	73	3

[a] The warehouses related to the Huanan Seafood Market were located out of the market.

[b] The one positive sample outside HSM was collected from Dongxihu Market in Wuhan. More information was provided in Extended Data Table 4.

Extended Data Table 2. The collection logic of the environment samples.

No.	Time	Objective	Sample time	Amount	Sum
1	1,Jan	(1) Environmental samples from stalls related to early cases; (2) Environmental samples from doors and floors of all stalls in the blocks where the early cases were located; (3) Environmental samples in the east wing of the market were collected according to blocks; (4) Transport carts, trash cans and similar objects.	1,Jan	515	515
2	12,Jan	Environmental samples from stalls that sold livestock, poultry, farmed wildlife (also called domesticated wildlife).	12,Jan	70	70
3	22,Jan	Environmental samples from other markets in Wuhan	22,Jan	30	30
4	23,Jan-19,Feb	The outdoor environmental samples from stalls that sold livestock, poultry, farmed wildlife.	23,Jan	23	52
			25,Jan	2	
			3,Feb	16	
			9,Feb	5	
			15,Feb	4	
			19,Feb	2	
5	27,Jan-15,Feb	Samples of sewage and silt from drainage channels and sewerage wells in the market.	27,Jan	38	94
			29,Jan	26	
			9,Feb	9	
			15,Feb	21	
6	5,Feb-9,Feb	Samples of sewage and silt from city sewerage wells around the market.	5,Feb	32	71
			9,Feb	39	
7	20,Feb-2,Mar	(1) Cold storages and refrigerators from stalls that sold livestock, poultry, farmed wildlife in the market; (2) The market's ventilation and air-conditioning system; (3) Public toilets, public activity rooms and other places where people gathered in the market.	20,Feb	27	91
			22,Feb	12	
			23,Feb	1	
			25,Feb	2	
			29,Feb	15	
			2,Mar	34	
Total				923	

Extended Data Table 3. The collection logic of the animal samples.

No.[a]	Time	Objectives	Sample time	Amount	Sum
8	22,Jan	Animal products in other markets.	22,Jan	6	6
9	25,Jan-10,Mar	Animal products and other commodity samples kept in the cold storages and refrigerators in the market.	25,Jan	55	306
			20,Feb	23	
			21,Feb	36	
			23,Feb	5	
			25,Feb	47	
			2,Mar	75	
			10,Mar	65	
10	27,Jan-1,Mar	Live animals captured around the market.	27,Jan	5	17
			5,Feb	3	
			9,Feb	2	
			15,Feb	3	
			29,Feb	2	
			1,Mar	2	
11	18,Jan-30,Mar	Stray cats, mice, cat feces and other stray animals (one dog and one weasel in the market).	18,Jan	1	96
			27,Jan	12	
			28,Jan	8	
			29,Jan	21	
			5,Feb	10	
			15,Feb	2	
			23,Feb	2	
			14,Mar	2	
			20,Mar	2	
			22,Mar	4	
			30,Mar	32	
12	19,Feb-23,Feb	Animal products and other commodity samples kept in the cold storages.	19,Feb	28	32
			23,Feb	4	
Total				457	

[a] The number follows the upper Table for environment samples.

Extended Data Table 4. The information of the sampling in other markets.

District	Number of environment samples [a]	Number of positive environment samples by RT-PCR	Number of animal samples [b]	Number of positive animal samples by RT-PCR
Jiang'han district	7	0	2	0
Jiang'an district	8	0	2	0
Donxihu district	7	1	1	0
Huanggang city	8	0	1	0
Total	30	1	6	0

[a] Swab sample collected from the floor, wall or chopping board.

[b] The heart, liver and large intestine tissues from pigs.

Extended Data Table 5. Twenty-one shops of RT-PCR positive in the Huanan Seafood Market.

Vendors No.	Location	Product types [a]						
		Cold-chain products	Aquatic products	Seafood products	Poultry	Livestock	Wildlife products	Vegetables
1	West	no	no	no	yes	no	no	no
2	West	yes	yes	yes	no	no	no	no
3	West	yes	yes	no	yes	yes	yes	no
4	East	yes	no	no	yes	yes	no	no
5	West	no	no	no	no	no	no	no
6	West	no	yes	no	yes	yes	no	no
7	West	yes	no	no	yes	no	no	no
8	West	yes	yes	yes	yes	no	no	no
9	West	yes	yes	yes	no	no	no	no
10	West	yes	yes	yes	yes	yes	no	no
11	West	yes	yes	no	no	no	no	no
12	West	yes	yes	yes	no	no	no	no
13	West	yes	yes	no	no	no	no	no
14	West	yes	yes	no	no	no	no	no
15	West	yes	yes	no	no	no	no	no
16	West	yes	yes	no	no	no	no	no
17	West	no	no	no	no	no	no	no
18	West	yes	no	no	yes	yes	no	no
19	West	no	no	no	no	no	no	yes
20	West	yes	no	no	no	no	no	yes
21	East	yes	yes	yes	no	no	no	no
Sum of NAT positive vendors		16	13	6	8	5	1	2
Vendors sampled in the study selling such products		87	73	56	37	36	9	8

[a] "yes" indicates product sold by vendors; "no" indicates product not sold by vendors.

Extended Data Table 6. The animal samples collected in the Huanan Seafood Market.

Species	Animal number	Sample number	RT-PCR positive number
Rabbit/Hares	52	104	0
Stray cat	27	80[a]	0
Snake	40	80	0
Hedgehog	16	67	0
Muntjac	6	18	0
Dog	7[b]	17	0
Badger	6	16	0
Bamboo rat	6	15	0
Mouse	10	12	0
Pig	NA[c]	6[d]	0
Chicken	5	5	0
Chinese giant salamander	3	5	0
Crocodile	2	4	0
Wild boar	2	4	0
Soft-shelled turtle	2	3	0
Weasel[e]	1	2	0
Fish	2	2	0
Sheep	1	1	0
Others	NA[f]	16	0
Total	188	457	0

[a] Six of the cats were from the Huanan Seafood Market. And the samples included faeces.

[b] Including one stray dog in the Huanan Seafood Market.

[c] Not applicable due to the processed pork.

[d] Collected from other markets.

[e] The weasel was not sold in the market, but caught alive in the Market.

[f] Not applicable due to the unrecognized “bai tiao” product as described in the methods.

nature portfolio

Corresponding author(s): Guizhen wu

Last updated by author(s): Mar 29, 2023

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☒	☐	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	For RNA-seq, raw datasets were collected with the BGI's Sequencing Systems.
Data analysis	Virus genome assembly and phylogenetic analysis Raw reads were adaptor- and quality- trimmed with the Fastp (version 0.20.0) program. The clean reads were mapped to the SARS-CoV-2 reference genome (GenBank: NC_045512) using Bowtie2. The assembled genomes were merged and checked using Geneious (version 11.1.5) (https://www.geneious.com). The coverage and depth of genomes were calculated with SAMtools v1.10 based on SAM files from Bowtie2. Reference genomes, IVDC-HB-01 (GISAID: EPI_ISL_402119) and Wuhan-Hu-1 (GenBank: NC_045512), were employed as a query. Multiple sequence alignment of the seven SARS-CoV-2 sequences obtained from this study and reference sequences were performed with Mafft v7.450. Phylogenetic analyses were performed using RAxML v8.2.9 with 1000 bootstrap replicates, employing the GTR nucleotide substitution model and the Gamma distribution. Bioinformatic analysis of the species abundances The Kraken2 (version 2.1.2) was used for species classification with the option '--confidence 0.1'. Sequences of all species in the Nucleotide (nt) database were used for generating the index. The bracken (version 2.5) was used for re-evaluating species abundance. The matrix of species was obtained by using the pavian algorithm. ggplot2 package in R was used for plotting. Read counts of each genus were used for further analysis and plotting. Raw counts of four domains (Archaea, Viruses, Eukaryota, and Bacteria), SARS-CoV-2, Homo genus, and Mammalia class were shown by heatmap. Two tail unpaired t-test was used for identification of differential genus between SARS-CoV-2 PCRpositive and -negative samples. For the analysis of the mammalian genus characterization, the reference was generated using the sequence of mitochondrial cytochrome c oxidase subunit I (COI-5P) in the barcode of life data (BOLD) system. RNA-seq samples were mapped to the reference sequences by the bowtie2 algorithm with the default settings. Read counts of each genus were calculated by the samtools. Read counts over 20 were used as

cut-off for the identification of positively enriched genus. Fisher's exact test was used for comparing the differential genus in the Mammalia class between SARS-CoV-2 PCR-positive and -negative samples.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All the raw sequencing data have been uploaded onto the GISAID (China CDC Weekly, 2021, DOI: 10.46234/ccdcw2021.255). The list of accession codes in Extended Data Table 6 and 7. The raw sequence data reported in this paper have also been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics, 2021, DOI: 10.1016/j.gpb.2021.08.001) in National Genomics Data Center (Nucleic Acids Res, 2022, DOI: 10.1093/nar/gkab951), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA010170) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa. Raw sequence data was deposited into NCBI BioProject under accession number PRJNA948658 (http://www.ncbi.nlm.nih.gov/bioproject/948658) and in China National Microbiology Data Center (NMDC) with accession numbers NMDC10018366 (https://nmdc.cn/resource/genomics/sample/detail/NMDC10018366).

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	n/a

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	We presented the SARS-CoV-2 detection results of 1380 samples collected from the environment and the animals within the market in early 2020. We further conducted RNA-seq analysis.
Research sample	Environmental samples in the Huanan Seafood Market were collected to represent exhaustively as possible, from a wide diversity of surfaces, animals and products.
Sampling strategy	Please refer to the sample collection in the method section.
Data collection	Please refer to the sample collection in the method section.
Timing and spatial scale	Please refer to the sample collection in the method section and Table 1, Table 2 and Supplementary Table 1.
Data exclusions	For the RNA-Seq analysis. 73 SARS-CoV-2 positive environmental samples were used for RNA-seq library construction. Among these, 60 samples successfully passed the library quality control and were used for analysis. A total of 850 SARS-CoV-2 negative environmental samples were obtained. Among these, 112 samples with high RNA abundance were used for RNA-seq analysis. Finally, 172 samples were used for analysis, and no samples were excluded.
Reproducibility	All samples used in the current study were unique, thus it would not be able to repeat the experiments.

Randomization	Not related.
Blinding	Not related.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions	Please refer to the sample collection in the method section, Extended Data Table S1 to Table S5, and also Supplementary Table 1.
Location	Please refer to the sample collection in the method section, Extended Data Table S1 to Table S5, and also Supplementary Table 1.
Access & import/export	The sample collection was guided and conducted by China CDC.
Disturbance	Huanan Seafood Market was a large market with more than 600 stalls inside. Thus, it was a tough job to finish the sampling of all the stalls in a short time during the emergency response to COVID-19. Thus, we performed the sample collection according to the ten different sampling principles we summarized in the methods. Especially, early case-related stores and wildlife-related stores were prioritized for sample collection and repeated sampling were also performed in these locations. Thus, it should be noted that these factors may lead to a biased sampling in the market.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	ATCC.
Authentication	None of the cell lines used were authenticated.
Mycoplasma contamination	Tested negative.
Commonly misidentified lines (See ICLAC register)	None.

Biosafety and Health 2 (2020) 199–201

Contents lists available at ScienceDirect

Biosafety and Health

journal homepage: www.elsevier.com/locate/bsheal

Cold-chain transportation in the frozen food industry may have caused a recurrence of COVID-19 cases in destination: Successful isolation of SARS-CoV-2 virus from the imported frozen cod package surface

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ARTICLE INFO

Article history:
Received 2 November 2020
Received in revised form 17 November 2020
Accepted 17 November 2020
Available online 19 November 2020

Keywords:
COVID-19
SARS-CoV-2
Cod outer package
Frozen food

ABSTRACT

Coronavirus disease 2019 (COVID-19) pandemic has spread in 220 countries/regions to wreak havoc to human beings around the world. At present, the second wave of COVID-19 has begun in many European countries. The complete control of COVID-19 is very urgent. Although China quickly brought the virus under control, there have been eight sporadic outbreaks in China since then. Both in Xinfadi of Beijing and Dalian outbreak of COVID-19, environmental swab samples related to imported cold chain food were tested nucleic acid positive for SARS-CoV-2. In this outbreak in Qingdao, we directly isolated SARS-CoV-2 from the cod outer package's surface swab samples. This is the first time worldwide, SARS-CoV-2 were isolated from the imported frozen cod outer package's surface, which showed that imported frozen food industry could import SARS-CoV-2 virus.

Since the outbreak in December 2019, the coronavirus disease 2019 (COVID-19) has spread in 219 countries (regions) worldwide. 45,678,440 confirmed cases and 1,189,945 deaths caused by the COVID-19 global pandemic have brought a massive disaster to human beings. At present, as the northern hemisphere enters autumn and winter, which may indicate a high incidence of respiratory infectious diseases, many European countries have started a second wave of the COVID epidemic. After the COVID-19 outbreak, China quickly took adequate measures to bring the outbreak under control in Wuhan and across the country. Since then, there have been eight sporadic and localized outbreaks in Suifenhe, Harbin [1], Shulan [2], Beijing, Urumqi [3], Dalian [4], and most recently Qingdao and Kashgar, respectively.

On September 24, 2020, during the routine nucleic acid inspection of the personnel in Qingdao Port, two stevedores were found to be SARS-CoV-2 positive and were identified as asymptomatic infection. The Qingdao Center for Disease Control and Prevention responded quickly by conducting an extensive epidemiological investigation to identify the source of infection. It was revealed that both cases had no COVID-19 case contact history and no foreign personnel contact history. However, both carried out loading and unloading of frozen cod in bulk on September 19, 2020.

Subsequently, the surface swab samples of the frozen cod outer package were collected and then tested. Out of 421 surface samples, 50 were tested SARS-CoV-2 nucleic acid positive. After that, the whole-genome sequencing and virus isolation were performed on the throat swab samples taken from the two workers and the frozen cod outer package's surface swab samples. Full-length genome sequences were successfully achieved for seven surface swab samples and two nasopharyngeal swab samples. After inoculation on Vero-E6 cells, cytopathic effects (CPE) were not observed during six days of culture; however, the supernatants of one surface sample and one nasopharyngeal swab sample were tested positive for SARS-CoV-2. Then the two supernatants were inoculated on the new surface layers of Vero-E6 cells for continuing virus isolation. Six days after the passage, two infections, CPE were observed, and the supernatants were tested positive for SARS-CoV-2 nucleic acid (Fig. 1). Meanwhile, we got the whole viral genome sequence using the Illumina MiSeq platform successfully. For the two cell-cultured samples, genome sequence alignments also confirmed the positive results of SARS-CoV-2 isolation.

A total of 16 nucleotide variations for these viruses were observed compared with the reference sequence (NC 045512), isolated from Wuhan City in Hubei Province on January 7, 2020. Phylogenetic analysis indicated that the SARS-CoV-2 (hCoV-19/Qingdao/IVDC-QD-11-2P2/

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[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bsheal.2020.11.003

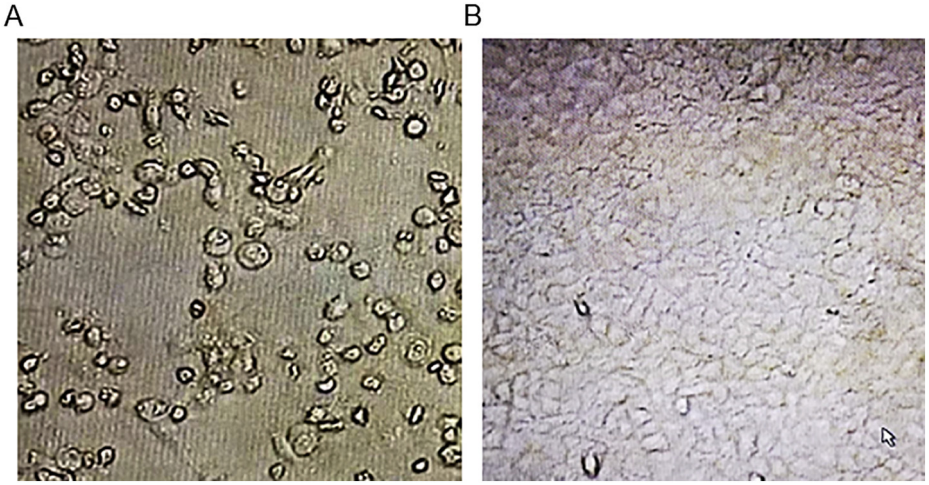

Fig. 1. Cytopathic effects in Vero-E6 cell cultures after inoculation with cod outer package surface swab samples. A. Six days after the passage 2 in Vero-E6 cell, cytopathic effects were observed with a 10 × objective lens; B. negative control were observed with a 10 × objective lens.

2020 and hCoV-19/Qingdao/IVDC-QD-G2020QD80P2/2020 isolated from cod package surface and a worder' throat swab sample, respectively) resulted in the outbreak in Qingdao City fell in a European branch (L lineage B1.1), which originated in Europe (Fig. 2). The virus' full genome sequence isolated from the patient's nasopharyngeal swab has a 100% similarity to that of the original sample. In contrast, the virus' genome sequence is highly homologous to the original piece from the frozen cod outer package, with two distinct nucleotides (Table 1).

The first time worldwide, we isolated SARS-CoV-2 from the imported frozen cod outer package's surface. Together with the epidemiological data, we concluded that the COVID-19 outbreak of Qingdao was probably caused by SARS-CoV-2 contamination of cod outer package during production or cold-chain transportation. To our knowledge, when the temperature reaches −18 °C during cold-chain transportation, viruses can survive for more than 21 days under the low-temperature condition [5]. Once the destination is reached, the SARS-CoV-2 virus may infect stevedores or other staff working in the port through direct contact with the goods (Fig. 3). The salmon surface was nucleic acid positive for SARS-CoV-2 in the COVID-19 epidemic of Beijing's Xinfadi market. However, the live virus has not been isolated due to the low nucleic acid concentration of the

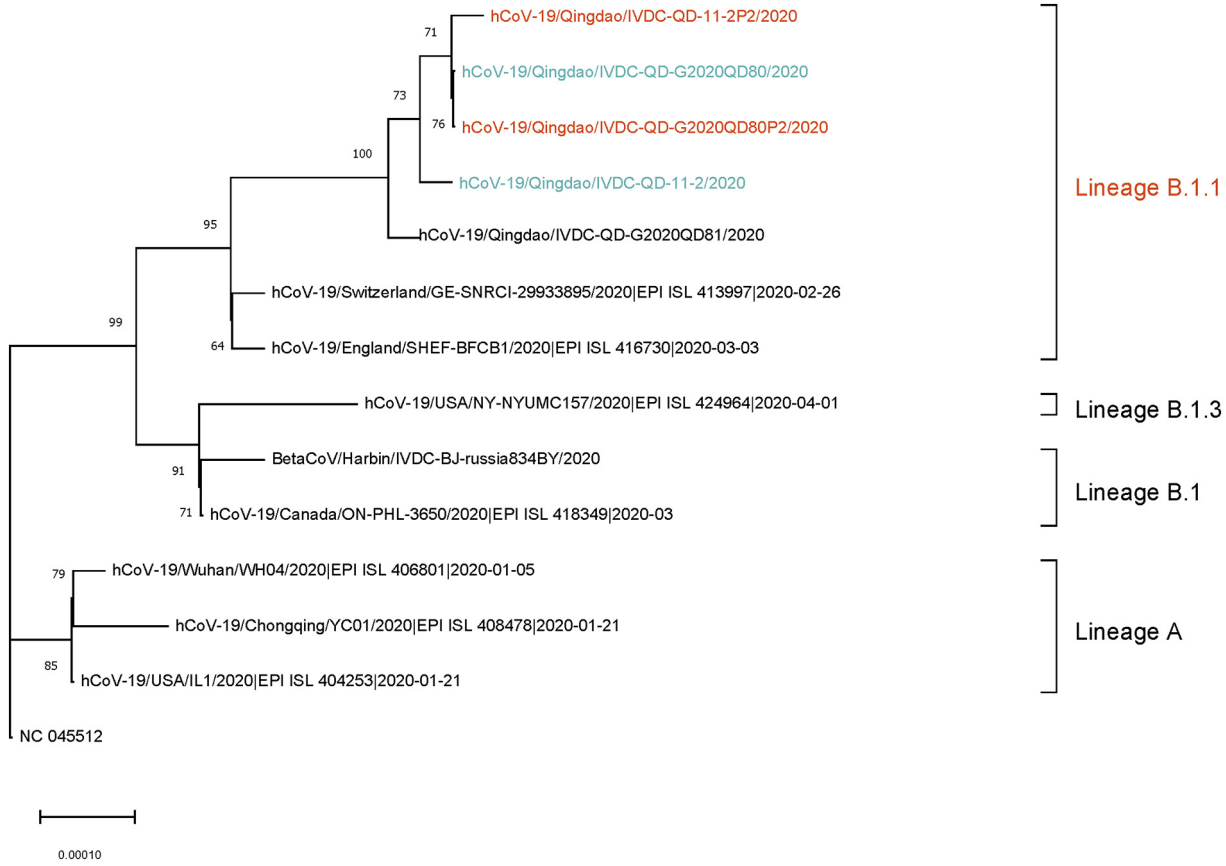

Fig. 2. Phylogeny of isolated SARS-CoV-2 and corresponding original samples from Qingdao based on the full-length genome sequences of the COVID-19 virus. The virus isolated from workers and cod package surface along with the corresponding original samples are indicated with red and cyan characters, respectively.

Table 1
Nucleotide variations for distinct SARS-CoV derived from Qingdao samples.

Position	Ref[a]	Alt[b]	11–2	11-2P2	G2020QD80	G2020QD81	QD-80P2
241	C	T	1	1	1	1	1
1,282	C	T	1	1	1	0	1
2,523	C	T	0	0	0	1	0
3,037	C	T	1	1	1	1	1
3,743	C	T	1	1	1	1	1
3,773	C	T	1	1	1	1	1
5,144	C	T	0	1	1	0	1
5,170	C	T	1	1	1	1	1
14,408	C	T	1	1	1	1	1
23,299	A	G	1	1	1	1	1
23,403	A	G	1	1	1	1	1
23,755	G	T	1	1	1	1	1
28,881	G	A	1	1	1	1	1
28,882	G	A	1	1	1	1	1
28,883	G	C	1	1	1	1	1
29,868	G	T,*,C	3	1	0	2	0

[a] Ref means reference sequence.
[b] Alt means alteration compared with the reference sequence.

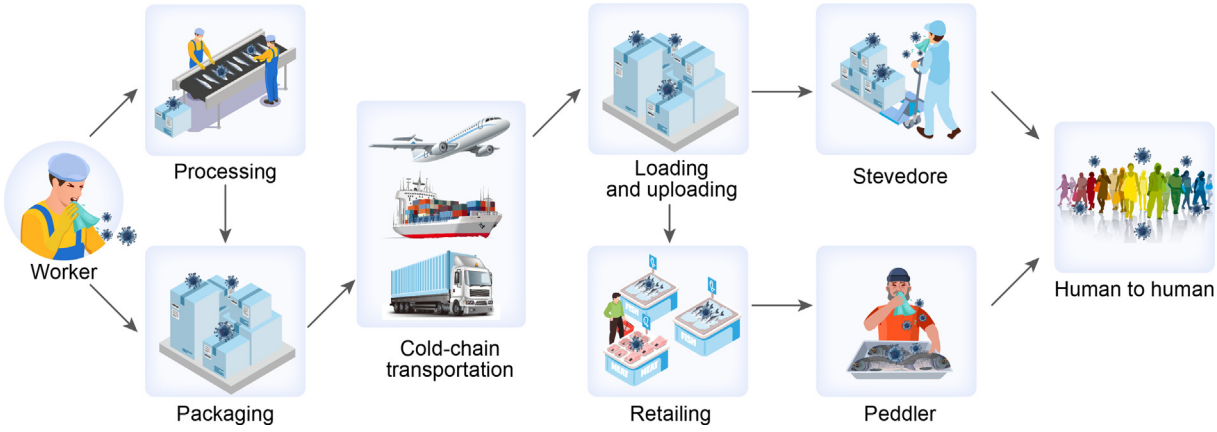

Fig. 3. Probable route of transmission for SARS-CoV-2 through cold-chain transportation in the frozen food industry. Frozen food and outer package were contaminated with SARS-CoV-2 during processing and packaging. Through cold-chain transportation with low temperature (such as -20°C), SARS-CoV-2 survived and then infected stevedores or peddlers after reaching the destination.

samples tested. The successful SARS-CoV-2 virus isolation from the seafood packaging surface proves that the imported virus that re-infected humans and caused the outbreaks through cold-chain transportation is all possible. In the future, we should strengthen the inspection and quarantine of imported cold-chain food and pay more attention to the personal protection of relevant workers to better prevent and control COVID-19.

Acknowledgements

This work was supported by the National Key Research and Development Project (2020YFC0846200 and 2020YFC0840800). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of interest statement

The authors declare that there are no conflicts of interest. Given their roles as Editorial Board Member, William J. Liu and Guizhen Wu had no involvement in the peer-review of this article and had no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to the editor Jianwei Wang.

Author contributions

Peipei Liu: Investigation, Methodology, Writing - original draft. **Mengjie Yang:** Investigation, Data curation. **Xiang Zhao:** Investigation, Software. **Yuanyuan Guo:** Investigation. **Liang Wang:** Software, Visualization. **Jing Zhang:** Investigation. **Wenwen Lei:** Investigation. **Weifang Han:** Resources. **Fachun Wang:** Resources. **William J. Liu:** Writing - review & editing, Validation. **George F. Gao:** Supervision. **Guizhen Wu:** Conceptualization, Funding acquisition, Supervision, Project administration, Writing - review & editing.

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引用格式：刘培培, 江佳富, 路浩, 等. 加快推进生物安全能力建设，全力保障国家生物安全. 中国科学院院刊, 2023, 38(3): 414-423, doi: 10.16418/j.issn.1000-3045.20221105002.
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加快推进生物安全能力建设，全力保障国家生物安全

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摘要 生物安全是国家安全体系的重要组成部分，关乎人民生命健康、国家长治久安、民族永续发展，是必争必保的底线。当前，国际生物安全形势严峻复杂，国内生物安全持续面临挑战。生物安全能力建设成为国际的热点，其中科技创新、人才培养、基础平台建设更是重中之重。在抗击新型冠状病毒感染中，我国虽然依靠科技支撑在病原微生物的快速鉴定、疫苗和药物研发等方面取得了系列重大成果，但也暴露了我国在生物安全科技创新领域的短板弱项。根据党的二十大作出的“推进国家安全体系和能力现代化，坚决维护国家安全和社会稳定”战略部署，我国应通过加快完善科技前沿布局、推进学科建设、加大专项人才培养、完善基础平台和生物安全领域全国重点实验室建设，进一步提升生物安全能力建设，完善我国生物安全体系，筑牢国家生物安全屏障，实现保护人民健康，保障国家安全，维护国家长治久安的重大目标。

关键词 生物安全，能力建设，生物安全学，科技布局，人才培养

DOI 10.16418/j.issn.1000-3045.20221105002

党的二十大报告中提出“推进国家安全体系和能力现代化，坚决维护国家安全和社会稳定”的战略部署，并单独使用一个章节的篇幅加以阐述，凸显了国家安全能力建设的重要性[1]。近 20 年来，生物安全问题日益成为全人类面临的重大生存和发展威胁之一。新型冠状病毒感染（COVID-19）在全球大流行后，更加凸显了生物安全的重要影响。我国将生物安全纳入国家安全体系，彰显了保障国家生物安全的重大意

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资助项目：国家重点研发计划项目（2022YFC2603500、2022YFC2603505）
修改稿收到日期：2022年12月26日

义。2021 年开始实施的《中华人民共和国生物安全法》明确提出生物安全是指国家有效防范和应对危险生物因子及相关因素威胁，生物技术能够稳定健康发展，人民生命健康和生态系统相对处于没有危险和不受威胁的状态，生物领域具备维护国家安全和持续发展的能力。生物安全主要包括防控重大新发突发传染病、动植物疫情；生物技术研究、开发与应用；病原微生物实验室生物安全管理；人类遗传资源与生物资源安全管理；防范外来物种入侵与保护生物多样性；应对微生物耐药；防范生物恐怖袭击与防御生物武器威胁；其他与生物安全相关的活动等。2003 年非典型肺炎（严重急性呼吸道综合症，SARS）以来，我国在以传染病防控为代表的生物安全的各个领域取得了一系列战略性成果，但也暴露出一些短板、弱项。党的二十大高瞻远瞩，对国家安全体系和能力建设提出了新的要求，在生物安全领域，我们亟须通过加快推进科技创新的力度，进一步推进能力和平台建设，提升我国的生物安全领域综合实力，保障国家安全。

1 全球生物安全形势及我国生物安全面临的主要挑战

1.1 生物安全领域已成为大国博弈制高点

当前，国际生物安全形势动荡，生物威胁日益复杂化、多样化，风险加剧，生物安全问题日益突出，生物安全领域已成为大国博弈制高点。美国、英国等已将生物安全置于国家战略的高度，分别发布了美国《国家生物防御战略》和《英国生物安全战略》，进一步完善了各自的生物安全治理架构，步步走实、走深。在传染病防控领域，经济全球化、工业化及城镇化导致病原微生物传播速度加快、传播途径增多；全球气候变暖引起的冰川融化及两极冰盖融化，导致极端环境中一些新的未知甚至古老病原微生物释放[2]；人类活动范围扩大不断侵占野生动物活动范围；野生动物贸易与消费使得人类与病原微生物接触的几率增加[3]。系列因素致使近 20 年来全球新发突发传染病频发，仅进入 21 世纪以来，全球出现数十种新发传染病。此外，随着合成生物学、基因编辑和微生物组等技术的迅猛发展，应用手段逐渐成熟化、技术门槛大幅降低，导致生物技术误用、谬用、滥用风险剧增[4]。人工合成或改造出新型病原体成为可能，且这些新型病原体甚至能显示出毒性更高、传播更快、溯源更难等特点。国外高等级生物安全实验室泄露事件仍时有发生，并造成人与动物传染病的流行和生态环境的破坏①。全球生物资源及其风险调查不断深入，如美国的“国家微生物组计划”“全球病毒组项目”均在持续推进。随着 COVID-19 的持续，国际生物安全秩序和治理体系进入深度变革和加速调整期，我国生物安全面临的风险和挑战急剧增加[5]。

1.2 我国生物安全面临的系列挑战

目前，以重大新发突发传染病为代表的国家生物安全在多方面持续面临挑战，突发性公共卫生事件的频发对人民生命健康和社会经济发展造成难以估量的损失。2003 年至今，我国经历过多种传染病疫情暴发，例如，2003 年 SARS、2009 年甲型 H1N1 流感与 2019 年底开始的 COVID-19 等与人相关的传染病。农业领域的非洲猪瘟（ASF）、口蹄疫（FMDV）等与动物相关的传染病，给我国农业生产造成严重破坏[6,7]。随着我国“一带一路”倡议的深入推进，国际交往不断增加，埃博拉出血热（EBHF）、尼帕病毒病（NVD）、寨卡病毒病（Zika）、裂谷热（RVF）等重大传染病输入我国并导致局部暴发的风险持续存在，我们与传染病之间只隔着“一个航班”的距离[8]。高等级生物安全实验室和菌（毒）种保藏库是生物安全领域的国之重器[9]，我国近年也有生物安全

① Army germ lab shut down by CDC in 2019 had several ‘serious’ protocol violations that year. (2020-01-23)[2023-02-15]. https://wjla.com/news/local/cdc-shut-down-army-germ-lab-health-concerns.

实验室泄露感染事件[10]。COVID-19 以来，国家出台了提升公共卫生实验室检测能力建设方案，一大批生物安全二级、三级实验室开始建设并投入使用，实验室生物安全监管的压力骤增，监管的及时性和覆盖面存在不足的风险。据中华人民共和国生态环境部统计，我国已有超过 660 余种入侵物种，其中重大入侵物种 120 余种，每年造成至少 2 000 亿元经济损失[11]。随着跨境电商和国际快递等新行业的发展，外来生物入侵渠道更趋多样化，风险逐步增加。另外，生物技术误用、滥用和谬用风险也在持续增大[4]，基因编辑和相关的人工智能还面临伦理问题[12]，生物恐怖和生物战的风险也在增加，人类遗传资源流失等问题日渐显现[13]。近些年，涉及人类遗传资源样本收集的个性化商业检测增多，基因组数据采集难度逐渐降低，人类遗传资源监管难度不断增加，数据流失风险较为突出。

2 我国生物安全能力建设的主要短板

面对生物安全领域的各种挑战，按照二十大报告中推进其现代化的部署，我们需要通过持续加强自身能力建设来加以应对。能力建设涉及面广，但核心在于科研基础和科技创新、人才培养和学科建设、平台设施（如高等级生物实验室）构建等。2020 年 3 月 16 日出版的《求是》杂志发表习近平总书记的重要文章《为打赢疫情防控阻击战提供强大科技支撑》，文章强调“人类同疾病较量最有力的武器就是科学技术，人类战胜大灾大疫离不开科学发展和技术创新”。我国生物安全能力建设起步晚，相较于先行发达国家，还有明显差距。

2.1 我国生物安全科技布局和顶层设计仍需进一步优化完善

*我国在生物安全领域的科技布局起步晚，系统化不足，布局的项目数量和投资的额度都严重偏低。*2018 年，美国发布的《国家生物防御战略》中将自然发生、意外事故或人为故意造成的生物威胁并重，突出传染病和生物武器威胁，确定了感知、预防、准备、响应和恢复等五大重点建设和管理目标[14]；部署了一系列生物安全科技计划，主要包括“生物盾牌”“生物监测”“生物感知”三大计划[15]。“生物盾牌”针对可用于生物恐怖袭击的病原体，研发疫苗、药物、诊断与治疗方法[16]；“生物监测”重点资助生物监测预警关键技术[17]；“生物感知”旨在缩短从发现危险病原体到快速反应的时间[18]。截至 2013 年，“生物盾牌”计划已经累计投入约 56 亿美元，美国已经能够供应炭疽疫苗、天花疫苗等重要生防药品，形成有效的“国家战略储备”。2021 年 9 月 3 日，美国白宫发布《美国大流行病防范：转变我们的能力》报告，指出科学技术是保障生物安全的根本手段，彻底转变美国应对生物威胁的能力，标志着美国“阿波罗生物防御计划”的正式落地。该计划覆盖疫苗、诊断、治疗 3 个方面，提出 9 个方向 21 项关键技术，预计在未来 7—10 年内投资 653 亿美元[19]。

2.2 我国生物安全研究基础资源等仍受制于人

*我国高致病病原微生物菌（毒）种资源和相应的技术储备缺乏。*我国在 2003 年 SARS 之后，虽然针对重大新发突发传染病疫情的处置和相应的生物安全支撑能力取得了长足的进步[20-23]，但与发达国家相比，我国针对重大新发突发传染病的监测预警网络还不够健全，尚未建立能够互联互通的全息监测体系；尤其是针对高致病性病原微生物检测技术，我国的短板十分明显，战略储备不足。我国《人间传染的病原微生物名录》里 82.14% 的一类病毒和 86.27% 的二类病毒尚未研发出抗原检测试剂，67.86% 的一类病毒和 74.51% 的二类病毒尚未研发出抗体检测试剂；更为严重的是，89.29% 的一类病毒和 88.24% 的二类病毒尚未研发出疫苗；100.00% 的一类病毒和 94.12% 的二类病毒尚未研发出预防用药；100.00% 的一类病毒和 92.16% 的二类病毒尚未研发出治疗用药；我国面临

的更为严峻的问题是毒种资源严重不足，78.57% 的一类病毒和 66.67% 的二类病毒缺乏毒种储备（图 1），无法开展活病毒相关原创研究，导致在相应病原微生物的防控技术储备上我国必然落后于人。对于疫苗和药物研发必需的病原体感染类人化动物模型，同样存在储备不足的问题[24]。这意味着一旦我国出现上述相关高致病性病原微生物引发的传染病，将面临极大的风险和挑战。

2.3 我国生物安全大数据的本底和积累及数据中心建设滞后

我国生物安全领域信息化建设落后于先行国家，大数据的底层技术研发及相关数据库建设仍需加强。近年来，国家已部署建设一系列国家科学数据中心，形成多个具有一定国际竞争力的支撑公益性科学研究的国家级中心和平台。但我国生物资源库的信息化、智能化建设依然落后，生物资源流失预警识别手段和体系缺乏[25]；在与国家生物安全八大领域都息息相关的生物安全大数据和信息方向，国家级生物安全大数据分析核心算法匮乏，也是我国生物安全面临的重要问题[26]。而且我国国家级的生物数据库资源建设仍然相对年轻，国内零散发展的模式未能形成合力，缺乏涵盖数据、信息、知识和文献的完备的多维资源体系，与美国国家生物技术信息中心（NCBI）建立的国际知名的基因序列数据库 GenBank 和生物医药领域的文献摘要检索数据库 PubMed 还有相当大的差距。

2.4 我国生物安全领域的专业队伍人才仍显匮乏

我国生物安全较之于发达国家起步晚，生物安全专业人才紧缺、战略型领军人才匮乏，亟待建立学术交流平台。后疫情时代，在对重大新发突发传染病的深入认识的背景下，我国规划建设了一批高等级生物安全实验室，而管理和运维相关的生物安全专业人才，尤其是高级人才愈发匮乏。当前，生物安全领域的专业人才培养仍然以“边工作、边学习、边训练”的培养模式为主，我国的学科体系里尚没有生物安全学科，更没有启动从本科到研究生的贯通式专业教育[27]，提示我们需要加强多元化培养体系的建设。此外，我国也缺少生物安全学术交流平台，例如，美国在 1984 年成立了美国生物安全

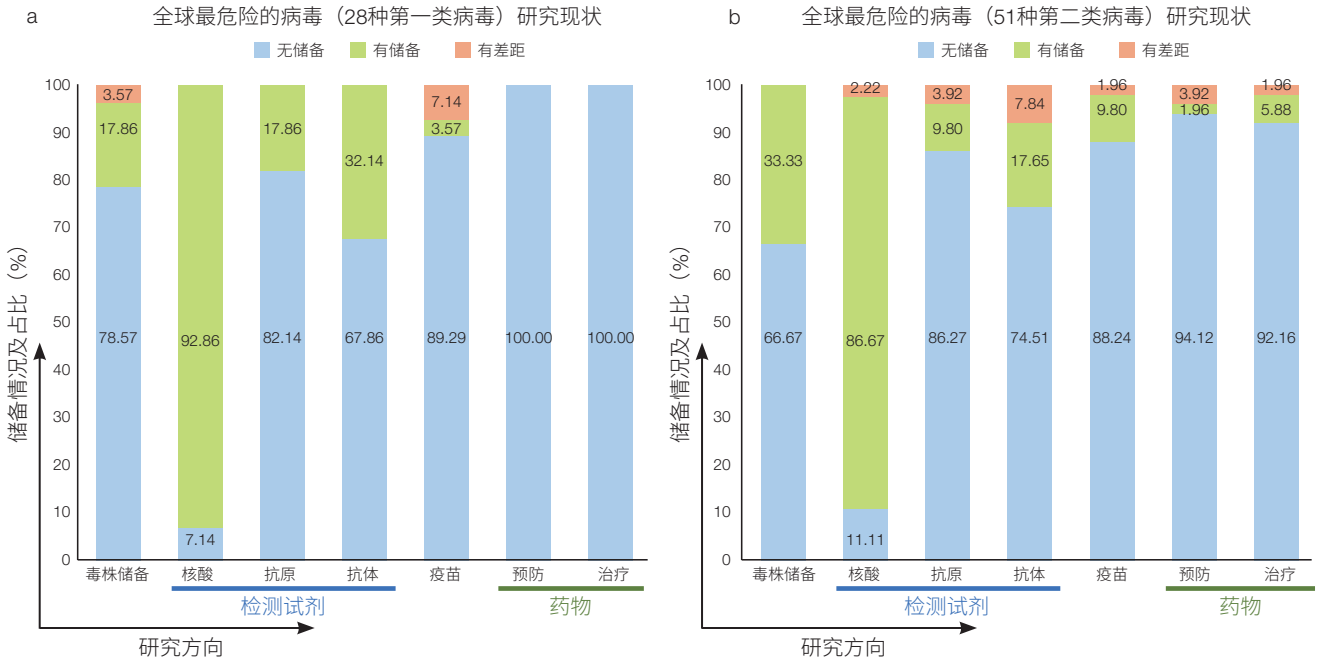

图 1　我国针对全球最危险的一类病毒（a）和二类病毒（b）在检测试剂、疫苗和药物方面的研究进展

Figure 1　Research progress of most dangerous Class 1 (a) and Class 2 (b) viruses on detection reagents, vaccines, and medicine

协会（ABSA），现已发展成为一个国际性的协会组织，会员遍布全球多个国家，定期开展学术交流和专业培训，经常举办各种学术活动，影响力遍布全世界。美国生物安全协会培养招揽生物安全领域人才的同时，其实也为美国在国际生物安全领域议题赢得了一定的话语权。

2.5 我国高等级生物安全实验室数量不足且分布不均

*我国的高等级生物安全实验室体系建设仍不健全，尚没有形成能够高效协同运行的实验室网络。*高等级生物安全实验室是传染病防控领域的国之重器。美国几乎所有高水平大学医学院、医院都配备了生物安全三级实验室，已建成1 500多个生物安全三级实验室，至少15个生物安全四级实验室。截至2021年，全球23个国家已建成和在建的生物安全四级实验室共有54个。另据俄罗斯国防部报告，美国加速其全球部署，在包括我国邻国哈萨克斯坦、乌兹别克斯坦、阿富汗等国在内的全球30多个国家建立了300余个生物实验室[28,29]。相比之下，我国的生物安全实验室数量明显偏少，生物安全三级实验室不足100家，建成并投入使用的生物安全四级实验室只有2家；实验室地域分布不平衡，东部沿海发达省份多，中西部省份少；实验室协同运行少，没有形成合力。同时，实验室核心关键装备仍然受制于人，如气密型正压防护头罩、防护面具、正压防护服、高性能防护手套/靴、实验动物独立通风笼具（IVC笼具）等均依赖进口[30]。

3 推进生物安全能力建设的建议

“生命安全和生物安全领域的重大科技成果是国之重器，一定要掌握在自己手中”，“科技创新是核心，抓住了科技创新就抓住了牵动我国发展全局的牛鼻子”，习近平总书记的讲话应该成为我们推进科技创新、加强能力建设以保障生物安全的根本遵循。我国生物安全能力建设涉及多个方面，应重点加快以科技创新和人才培养等为核心的能力建设。

3.1 进一步优化完善中国生物安全战略规划，系统解决重大问题

*制定并启动我国生物安全科技战略规划。*立足生物安全国情，深入贯彻落实好习近平新时代中国特色社会主义思想和总体国家安全观，以维护人民健康安全为核心，强化需求牵引，借鉴国际经验。制定中国的生物安全科技战略规划，加强能力建设，全面提升维护和塑造国家生物安全的能力，防范和化解重大风险，有效处置各类安全事件。根据我国实际情况，全链条梳理生物防御的所有环节，弄清存在的薄弱环节。从统筹安全和发展角度出发，从战略安全角度出发，从维护和塑造国家生物安全、国家生物科技体系安全角度进行前瞻总体设计、战略谋划、战略运筹，拿出代表生物安全发展趋势、体现国家战略需求的战略规划方案。利用全国重点实验室改革的契机，加强生物安全领域整体规划布局和实施。

3.2 持续实施并完善现有的科技攻关计划，夯实研究基础

*尽快建立并启动我国的生物安全防御科技计划。*组织优势力量在监测预警、溯源追踪、应急处置、事后重建、生物伦理、国门动植物检验检疫设施设备和野外生物安全屏障等领域开展关键技术或者前沿技术的科研攻关，尤其是原创性颠覆性技术的研究。要将科研成果努力转化成产品，尽快为生物防御服务，并为未来应对可能发生的生物安全威胁做好技术储备。进一步加大资金支持力度，组织优势力量全力攻关，解决目前面临的高等级生物安全实验室核心零部件及生物医学研究中所用的原材料、试剂、装备、实验动物严重依赖进口，生物信息数据库几乎完全被国外垄断等一系列问题[31,32]。

3.3 不断完善生物安全协调机制，加强资源共享

*加强生物安全相关资源的共享和管理。*利用和完善目前已有的协调机制，打破各部门的界限，突破各学科

之间的壁垒，有机整合国家主要力量和资源，充分发挥我国举国体制，统一部署、协同攻关。“平时”根据整体规划，依托已有体系全面布局、系统开展工作究，“战时”依托协调决策机制快速反应、统一决策、有力执行。快速提升生物信息数据汇交管理能力，加速国际核心数据本地化集成整合，形成生物信息数据共享服务核心平台，打破我国生物大数据研究领域长期存在的“数据流失”“数据孤岛”“共享匮乏”等现象，切实保障我国人类遗传资源信息和重要战略生物资源信息的数据安全与共享利用；快速提升生物信息数据分析挖掘能力，开发有自主知识产权的数据分析软件和平台，支撑数据的快速解析；加强数据共享的国际合作，扩大我国在生物信息领域的国际话语权。

3.4 加快推动人才队伍建设，系统培养高级生物安全专业人才

*着力培养战略型领军人才，加强生物安全智库建设。*习近平总书记在二十大报告中提出了“实施科教兴国战略，强化现代化建设人才支撑”战略部署，强调人才和科技的重要性。强化人才考核与流动机制，确保各层次研究人才规模适度，整体科研素质和能力显著提高，层次结构均衡合理，体制机制充满活力，各类人才协调发展。积极推动生物安全二级学科的建立[33]，努力打造一批高水平的优秀领军人才和学科团队，引领并推动生物安全领域的科技创新和快速发展。应尽快筹建中国生物安全协会/学会（一级），促进生物安全领域的学术交流，进一步推进专业人才的培养。协会的建立也可为国家战略和政策制定提供智库支持；开展战略研究，促进我国生物安全战略和政策的迭代升级；协调科学研究和技术转化，提高我国生物科技创新能力；积极开展国际国内学术交流，提升生物安全学术水平，在国际上代表中国发出声音。

3.5 加强我国高等级生物安全实验室体系建设，设立全国重点实验室和国家实验室

*合理规划和布局新的高等级生物安全实验室和研究平台。*根据国际的先进经验和我国的实际情况，综合人口密度、经济发展、传染病流行风险及地理区域等因素，充分考虑我国经济和产业的发展特点，按照按需设置、合理布局、同步建设的思路，在现有的高等级生物安全实验室基础上，加大应急响应方面的高等级生物安全实验室的建设力度，实现科学合理布局。生物安全实验室网络实现主要地域和功能的有效覆盖，完善区域和功能布局，促进已有实验室的扩建和改建及新实验室的建设，规划建成涵盖全国的高等级生物安全实验室平台体系、安全运行和资源共享的管理体系，建立严格管理制度和协调机制，加强实验室之间的交流沟通和信息共享机制。适应形势的需要，积极创造条件，加强对高级别生物安全实验室建设的投入，多渠道、多层次、多形式筹集资金，形成多元化投入格局和多方联合建设机制。支持和引导有能力的企业自主建设高等级生物安全实验室。鼓励企业开展生物安全实验室关键技术和设备的研制[34]。同时，围绕高等级实验室设施群和优势单位、布局有研究功能的全国重点实验室、进一步谋划生物安全国家实验室，对于学科发展与人才培养尤为重要。应瞄准未来生物安全科技的可持续发展，形成一批国内领先、国际一流的研究技术平台，打造国家级生物安全科研创新基地、建设生物安全研究领域的引领力量、重大传染病防控研究的支撑力量，提升主动应对能力，实现生物科技创新和全球生物安全治理的整体跨越。

3.6 深入开展国际对话合作，积极参与生物安全国际治理

*广泛参与生物安全国际治理，与相关国家开展深入合作。*应加快推进援建非洲疾控中心、联合实验室等重点项目建设，建立健全外派公共卫生专家的体制机制。建设性参与全球卫生治理及相关国际规则制定，推动《国际卫生条例（2005）》能力建设，大力推动重启《禁止生物武器公约》核查议定书谈判，

尽快建立多边生物核查机制。深入参与世界卫生组织《大流行病条约》的谈判进程，与各国建立对话合作机制。加大向世界卫生组织等国际组织中国籍官员的推荐和选派，深度参与生物安全国际治理。在“一带一路”沿线、我国重点边境地区试点建设卫生应急示范哨点，开展疾病和流行病原体的监测，完善跨境卫生应急合作机制，逐步建立海外监测网络，实现生物安全关口前移。

4 总结

我们要不断优化生物安全领域的整体科技布局，积极鼓励支持原创性颠覆性科学研究，突破卡脖子技术；开展学科建设，系统性加强专业的人才培养；加快高等级生物安全实验室在全国的布局建设，编织实验室协同网络；不断完善生物安全协调机制，加强资源共享；建立对话合作机制，深度参与生物安全国际治理。通过上述方式不断强化我国的生物安全能力建设，保障国家生物安全，从而实现党的二十大提出的重大战略部署。

致谢 文章撰写过程中得到中国科学院张亚平的指导，中国疾病预防控制中心病毒病预防控制所刘军提供了修改意见，在此一并表示诚挚的谢意。

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Accelerating Biosafety Capacity Building to Ensure National Biosecurity

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Abstract Biosafety is an essential part of the national security system, which is related to people's lives and health, the country's long-term stability, and sustainable development, which is the bottom line that must be guaranteed. The international biosafety situation is grim and complex, while domestic biosafety faces challenges. Therefore, biosafety capacity building has become an international hot spot, among which scientific and technological innovation, talent training, and infrastructure platform construction are the top priorities. Although China

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has achieved strategic results in the rapid identification of pathogens, research, and development of specific vaccines and medicine in fighting against COVID-19 by relying on scientific research, it has shown the urgency for scientific and technological innovation in biosafety. Therefore, China has developed a strategic plan on “promoting the modernization of the national security system and capabilities, resolutely safeguarding national security and social stability” included in the 20th National Congress of the Communist Party of China. Hence, it is suggested to promote biosafety capacity building further to improve China’s biosecurity system, protect people’s health, ensure national security, and maintain long-term peace and stability by improving the layout of scientific and technological frontiers, promoting the construction of biosafety discipline, training of more special talents, and infrastructure platform construction.

Keywords biosafety, capacity building, biosafety discipline, scientific and technological innovation, talent training

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DOI:10.3784/j.issn.1003-9961.2008.10.001

·论　　坛·

中国疾病预防控制机构实验室信息管理系统的建设与管理

魏强，武桂珍

摘要： 中国疾病预防控制机构在全国公共卫生领域承担着越来越重要的任务和角色，在新时期、新形势下，国家对疾病预防控制机构也提出了更高要求。作为疾病预防控制工作的技术支撑和条件保障，实验室采用基于实验室质量和安全管理标准开发的信息化管理系统，是提高中国疾病预防控制机构实验室能力建设、提高实验室管理水平的重要环节和有效方式，也将是疾病预防控制机构业务进一步发展的必然趋势。实验室信息管理系统的应用对规范疾病预防控制机构实验室建设与管理工作必将起到积极推动作用。

关键词： 实验室信息管理系统；疾病控制；建设；管理

中图分类号： R184　　**文献标识码：** A　　**文章编号：** 1003-9961(2008)10-0599-03

Construction and management of the laboratory information management system for disease control centers in China

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Abstract: The centers for disease control and prevention are assuming an increasingly important role in the field of public health in China. Furthermore, higher requirements have been put forward for national disease control centers in the new era under the new situation of China. As the security and technical support and an inevitable trend for disease prevention and control, a digital management system complying with the current quality and safety standards has been developed for the laboratory to effectively improve the capacity of laboratories in disease control centers and laboratory management. The application of laboratory information management system for disease control and prevention will play an active role in promoting the development and management of CDC laboratories.

Key words: laboratory information management system; disease control; construction; management

实验室是疾病预防控制中心(CDC)开展疾病预防控制(疾控)和相关科研工作的重要场所，是做好疾控工作的技术支撑和条件保障。近年来卫生部相继出台了《关于疾病预防控制体系建设的若干规定》、《省、地、县级疾病预防控制中心实验室建设指导意见》等一系列规定和要求，进一步明确了疾控机构实验室建设规范，同时对实验室能力、安全、质量等管理工作提出了更高要求。作为现代化的实验室管理模式和手段，重视和加强实验室信息管理系统的建设与管理是提高中国疾控机构实验室能力建设、提高实验室管理水平的重要环节和有效方式。本文在介绍实验室信息管理系统基本情况和发展趋势的基础上，针对目前中国疾控机构现状，就中国疾控机构实验室信息管理系统建设与管理做一阐述。

1　实验室信息管理系统概述

实验室信息管理系统 (laboratory information management system，LIMS)是一个随着相关技术和管理理念不断丰富和发展而发展着的概念，最早于 20 世纪 60 年代末提出，它是集现代化管理思想与基于计算机的高速数据处理技术、海量数据存储技术、宽带传输网络技术、自动化仪器分析技术于一体，以实验室业务和管理工作为核心，遵循实验室管理国际规范，实现对实验室全方位的科学、统一、有序和高效管理的计算机信息管理系统[1,2]。

基金项目：国家高技术研究发展计划资助项目(2006AA02Z4F1)；
国家公益性行业科研专项建议项目(200802021)

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收稿日期：2008-04-18

到目前为止，实验室信息管理系统经历了从用户自行开发、商业化软件到完全面向实验室的客户化软件的 3 个发展阶段。早期的实验室信息管理系统大都是针对某一特定的、单一功能的分析实验室使用[3]。中国实验室信息管理系统的开发和应用开始于 20 世纪 90 年代中期[4]。随着计算机技术的不断发展、各行各业实验室工作内容和内涵的不断拓展，实验室信息管理系统遵循实验室管理相关规范的要求，在不断改进和升级过程中，日趋全面、系统和完善，广泛应用医药、石化、环保等各行业、各类型实验室[5,6]。

实验室信息管理系统是实现实验室规范化管理的有效工具，通过使用实验室信息管理系统，可保证实验室质量和安全体系在严格控制下运行，从而能使实验室检测或管理数据、信息均符合相关的质量标准或规范，在保障实验室检测数据质量的同时，实现统计查询、数据溯源、事故追踪调查，从而提高实验室安全监管工作能力和水平。另一方面，通过使用实验室信息管理系统，实验室可以达到自动化运行、信息化管理和无纸化办公的目的，对提高实验室工作效率、降低运行成本也将起到重要的作用[7]。

2 实验室信息管理系统的现状

疾控机构实验室涉及传染病、食品、环境、职业健康、辐射安全等与人体健康有关的各个方面检测和科研工作。根据《中华人民共和国计量法》、《中华人民共和国认证认可条例》、《实验室和检查机构资质认定管理办法》等法律法规的规定，目前中国各级疾控机构绝大多数成立了专门实验室管理或质量管理部门，实验室建立并运行了实验室质量管理体系，通过了遵循于 ISO/IEC 17025-2005 《检测和校准实验室能力的通用要求》的实验室资质认定。同时，根据 2004 年国务院《病原微生物实验室生物安全管理条例》关于高等级生物安全实验室必须通过国家实验室认可的规定，一部分单位的 BSL-3 实验室还通过了生物安全体系认可。这些工作的开展，为实验室信息管理系统的建设打下了一定基础、创造了一定条件。

随着实验室信息管理系统重要作用的日益显现，全国大部分疾控机构积极为系统建设做各项准备工作。目前，全国疾控机构已进入了实验室信息管理系统建设、使用、快速发展阶段。部分省级、市级疾控机构已经相继建立、使用实验室信息管理系统，广东、江苏、上海、深圳等东南沿海省市 CDC 在全国疾控机构中走在前列。这些单位在实际应用过程中，不断丰富内容，不断升级完善系统软件，为中国疾控机构实验室信息管理系统建设积累了一定经验[8]。作为国家级疾病预防控制单位，中国 CDC 承担大量疾病监测和产品检测工作，目前正在建设面向全中心所有实验室使用的实验室信息管理系统。通过建设实验室信息管理系统，将全面实现实验室检测业务流程自动化管理和实验室安全与资源的信息化管理，提升中国 CDC 的实验室管理水平和能力。

目前疾控机构实验室信息管理系统功能需求主要包括实验室资源与安全管理、实验室检测业务流程管理、质量管理、客户关系管理、报告查询与统计分析管理以及与其他系统和设备接口等需求。实验室资源与安全功能需求包括设备管理、人员管理、材料[特别是菌(毒)种、危险化学品、放射性物质]管理、恒温恒湿样品库管理、环境管理、事故管理等。检测流程功能需求基本包括样品受理、样品分派、样品测试、检验结果分析、结果审核、结果发布或检验报告自动生成、检验报告审核、报告书核发及发出、样品状态过程监控。

3 实验室信息管理系统的建设与管理

当前全国疾控机构工作重点之一是继续完善体系建设和运行机制建设，加强疾控体系实验室的科学建设与管理，加快全国传染病实验室网络信息平台的建设，推动国家级和省级传染病网络实验室间的信息交流与共享。实验室信息管理系统对于规范全国疾控机构实验室建设，提高全国传染病实验室网络信息平台数据质量、为管理和决策提供及时而准确的数据资源，推进疾病监测水平、突发公共卫生事件应急处置能力将起到重要作用。

3.1 实验室信息管理系统的建设 建设和使用实验室信息管理系统是对实验室人工管理模式一次重大的调整，它打破了实验室管理和工作人员已经熟悉的管理模式、工作流程乃至思维方式，需要工作人员重新学习新的知识，接受新的事物。因此，在建设、实施过程中各单位将面临大量问题和困难，工作难度大，相对复杂，这就需要各级领导的高度重视和坚定支持。

实验室信息管理系统建设是系统性工程，在实施之前，各单位要根据实验室相关管理规范和准则，认真分析本单位实际情况，科学筹划，理清工作思路和管理程序，制定工作流程，使实施工作程序化、规范化。同时，实验室信息管理系统建设过程中宜采取统一设计、分块实施、先易后难的原则开展工作。在

充分认识现实需要的基础上，采取循序渐进策略，让使用人员逐步了解、消化、掌握，推进系统建设工作的深入开展。

3.2 实验室信息管理系统的管理 2003 年卫生部出台的《全国卫生信息化建设发展规划纲要》中明确指出卫生信息化建设的基础工作是统一标准，它是进行信息交换与共享的基本前提。所谓信息标准，就是在信息的产生、传输、交换和处理时采用统一的规则、概念、名词、术语、传输格式、表达格式和代码[9]。目前全国疾控机构已进入实验室信息管理系统建设发展阶段。实验室信息管理系统信息标准化关系到各级疾控机构实验室信息管理系统建成后，全国疾控机构网络实验室之间数据能否交流，疾病监测信息能否共享，能否更好地为传染病监测和突发公共卫生事件处置提供决策服务。因此，疾控机构在实验室信息管理系统建设过程中应以《全国卫生信息化发展规划纲要》为指导，以实验室业务和管理相关法律法规为依据，加强操作平台建设规范、应用系统功能规范、基本数据集规范、数据代码规范、统计指标规范和数据传输规范等方面的管理工作，建立和制定全国疾控机构实验室信息系统功能规范和建设规范，强化实验室相关信息的分析、利用和共享，确保中国疾控机构实验室质量和安全管理工作的健康持续发展。

2007 年国务院发布实施的《卫生事业发展“十一五”规划纲要》中明确提出进一步完善疾控体系，推进各级疾控机构规范化建设，提高实验室检测检验能力，全面提高服务能力。随着中国经济社会的不断发展，在国际社会地位的不断提高，疾控机构实验室面临着前所未有的发展机遇与挑战，我们应以社会发展与需要为契机，积极推进实验室信息管理系统建设与管理工作，加快实验室规范化建设，不断提升疾控机构实验室能力和水平。

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编辑推荐

NATIONAL BIOSAFETY SCIENCE

国家生物安全学

武桂珍　主编

科学出版社

国家生物安全学

武桂珍　主编

科学出版社
北京

内 容 简 介

生物安全已经成为关乎国计民生的头等大事，深刻认识新形势下加强生物安全建设的重要性和紧迫性，贯彻落实生物安全法，加强国际生物安全风险防控和治理体系建设，提高国家生物安全治理能力，是全社会各行各业的需求。本书由中国疾病预防控制中心生物安全首席专家、亚太生物安全协会主席武桂珍研究员主持编写，国内生物安全领域的其他权威专家共同参与，分为八篇，第一篇为生物安全概述，第二～八篇围绕生物安全法的“四梁八柱”，从传染病与动植物疫情、两用生物技术威胁与安全、实验室生物安全、人类遗传资源与生物资源、生物入侵防控、微生物耐药、生物恐怖与生物武器等方面全面展现了国内外生物安全的总体概况，为读者了解、分析生物安全现状提供理论依据。

本书可供生物学、医学、农林、食品及其相关领域的师生、科研人员以及企业管理人员参考使用。

图书在版编目（CIP）数据

国家生物安全学 / 武桂珍主编 . — 北京：科学出版社，2023.3

ISBN 978-7-03-075197-3

Ⅰ . ①国… Ⅱ . ①武… Ⅲ . ①生物工程－安全科学－中国 Ⅳ . ① Q81

中国国家版本馆 CIP 数据核字（2023）第 047544 号

责任编辑：王 静 罗 静 刘 晶 / 责任校对：郑金红

责任印制：肖 兴 / 封面设计：无极书装

科学出版社出版

北京东黄城根北街 16 号

邮政编码：100717

http://www.sciencep.com

北京虎彩文化传播有限公司 印刷

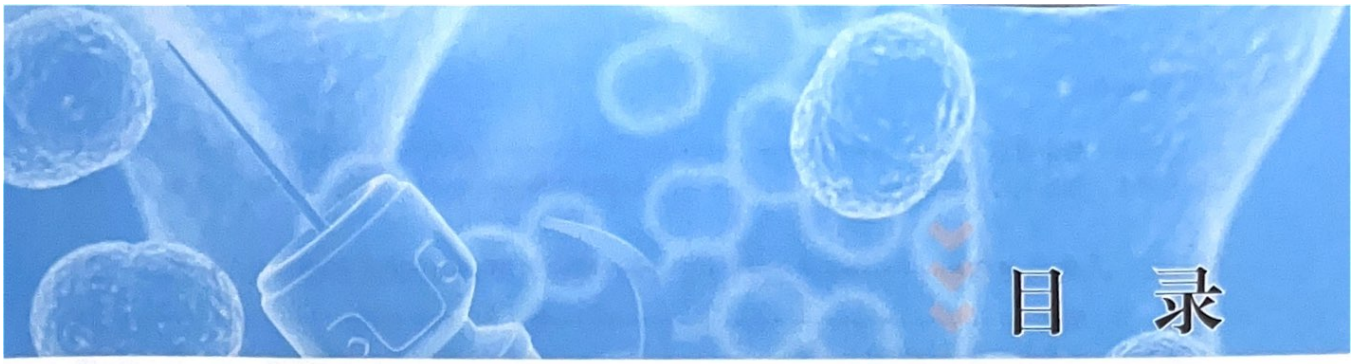
科学出版社发行 各地新华书店经销

*

开本：889×1194 1/16 印张：59.75 字数：1 935 000

定价：698.00 元

（如有印装质量问题，我社负责调换）



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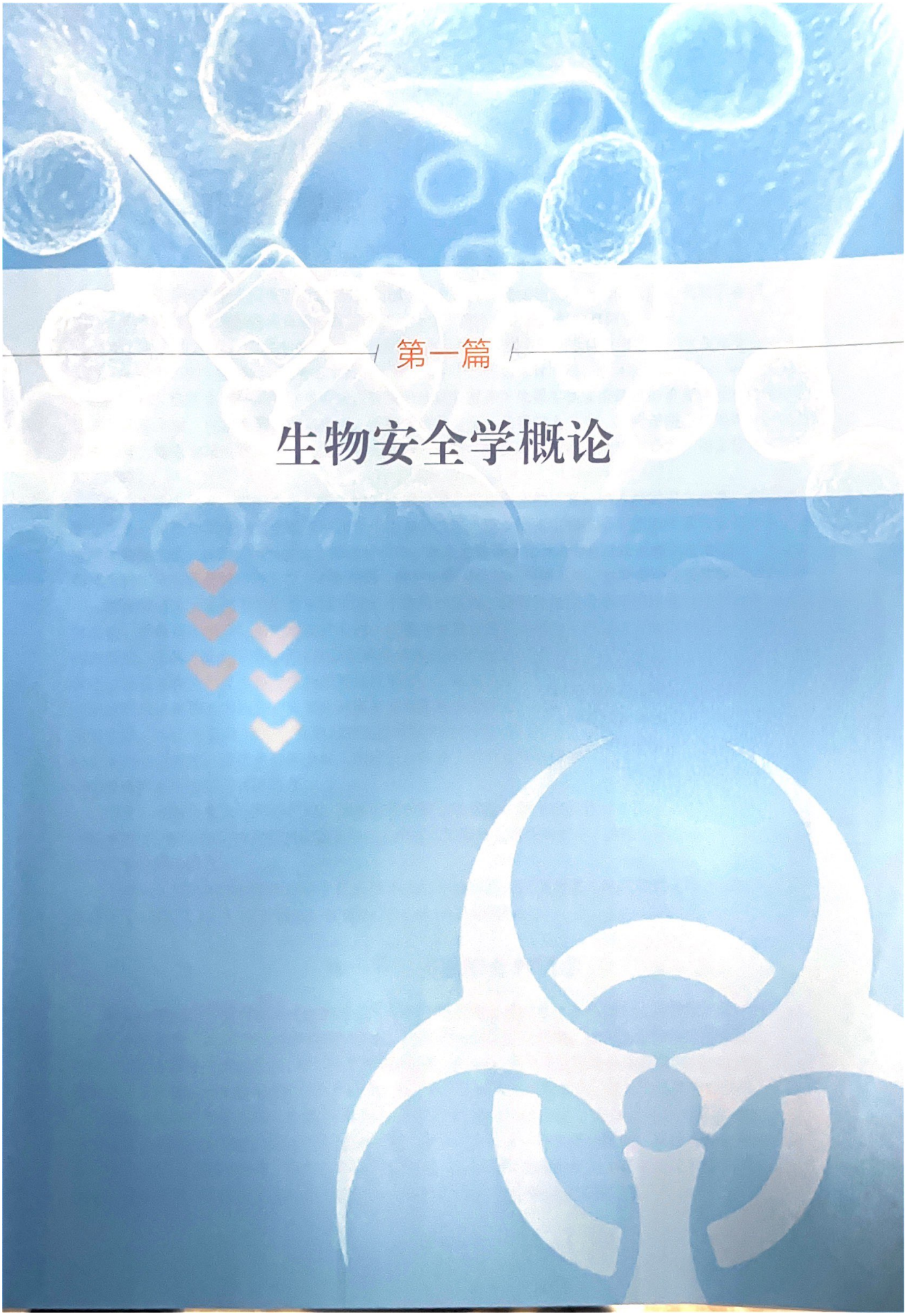

第一篇

生物安全学概论

第一章
生物安全学概论

生物安全是国家安全的重要组成部分，与国家安全的其他领域相互渗透，相互作用，相互影响，相互传导，对国家经济社会发展的影响具有战略性、全域性特点，没有生物安全就没有国家安全。

2021 年 9 月 29 日，中共中央总书记习近平在主持加强我国生物安全建设第三十三次集体学习时强调，生物安全关乎人民生命健康，关乎国家长治久安，关乎中华民族永续发展，是国家总体安全的重要组成部分，也是影响乃至重塑世界格局的重要力量。要深刻认识新形势下加强生物安全建设的重要性和紧迫性，贯彻总体国家安全观，贯彻落实生物安全法，统筹发展和安全，按照以人为本、风险预防、分类管理、协同配合的原则，加强国家生物安全风险防控和治理体系建设，提高国家生物安全治理能力，切实筑牢国家生物安全屏障。

党的十八大以来，党中央把加强生物安全建设摆上更加突出的位置，纳入国家安全战略，颁布施行生物安全法，出台国家生物安全政策和国家生物安全战略，健全国家生物安全工作组织领导体制机制，积极应对生物安全重大风险，加强生物资源保护利用，举全党全国全社会之力打好新冠肺炎疫情防控人民战争，我国生物安全防范意识和防护能力不断增强，维护生物安全基础不断巩固，生物安全建设取得历史性成就。

新冠疫情大大促进生物安全在国家安全中地位的认识。新冠肺炎疫情是新中国成立以来发生的传播速度最快、感染范围最广、防控难度最大的一次重大突发公共卫生事件。面对这一突发事件，习近平总书记高度重视，强调“重大传染病和生物安全风险是事关国家安全和发展、事关社会大局稳定的重大风险挑战。要把生物安全作为国家总体安全的重要组成部分。”这是总体国家安全观理论和实践的创新。总体国家安全观的形成，来源于国内外形势变化和国家安全事业发展需要，国家安全发展实践又丰富和完善了总体国家安全观。2022 年 2 月 14 日，在新冠肺炎疫情发生的特殊背景下召开的中央全面深化改革委员会会议上，习近平总书记指出，要把生物安全纳入国家安全体系，系统规划国家生物安全风险防控和治理体系建设，全面提高国家生物安全治理能力。

当下，全球生物安全形势严峻，世界面临多种生物威胁，新发突发烈性传染疾病、外来物种入侵、生物技术谬用、病原微生物实验室生物安全、人类遗传资源与生物资源安全、防范生物恐怖与生物武器威胁，成为人类共同的挑战。

《中华人民共和国生物安全法》已由中华人民共和国第十三届全国人民代表大会常务委员会第二十二次会议于 2020 年 10 月 17 日通过，自 2021 年 4 月 15 日起施行。

第一节　生物安全的概念

所谓生物安全一般指由现代生物技术开发和应用所能造成的对生态环境和人体健康产生的潜在威胁，及对其所采取的一系列有效预防和控制措施。生物安全攸关民众健康、社会安定和国家战略安全。国际生物安全形势发展正处于大动荡、大变革的重要转折期。短期内，生物安全风险总体可控，但面临生物袭击威胁、新发突发传染病、两用技术风险等棘手问题；长期看，战略安全风险加大，亟须加强战略引导和技术攻关。

国际生物安全形势基本走势是 2000~2014 年总体保持温和可控状态，但 2015 年以来形势转向相对严峻。生物威胁已经从偶发风险向现实持久威胁转变，威胁来源从单一向多样化转变，威胁边界从局限于少数区域向多区域甚至全球化转变，突发生物事件影响范围已经从民众健康拓展为影响国家安全和战略利益。传统生物安全问题与非传统生物安全问题交织，外来生物威胁与内部监管漏洞风险并存。

(Q-5023.01)

生物安全已经成为关乎国计民生的头等大事，深刻认识新形势下加强生物安全建设的重要性和紧迫性，贯彻落实生物安全法，加强国际生物安全风险防控和治理体系建设，提高国家生物安全治理能力，是全社会各行各业的需求。本书由中国疾病预防控制中心生物安全首席专家、亚太生物安全协会主席武桂珍研究员主持编写，国内生物安全领域的其他权威专家共同参与，分为八篇，第一篇为生物安全概述，第二～八篇围绕生物安全法的“四梁八柱”，从传染病与动植物疫情、两用生物技术威胁与安全、实验室生物安全、人类遗传资源与生物资源、生物入侵防控、微生物耐药、生物恐怖与生物武器等方面全面展现了国内外生物安全的总体概况，为读者了解、分析生物安全现状提供理论依据。

科学出版社 生物分社
联系电话：010-64012501
E-mail：lifescience@mail.sciencep.com
网址：https://life.sciencereading.cn

销售分类建议：生物科学

科学出版社
互联网入口

生命科学订阅号
科拉艾文

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www.sciencep.com

ISBN 978-7-03-075197-3

定价：60.00元

实验室生物安全手册

组织编写　中国疾病预防控制中心病毒病预防控制所
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人民卫生出版社
PEOPLE'S MEDICAL PUBLISHING HOUSE

图书在版编目（CIP）数据

实验室生物安全手册 / 中国疾病预防控制中心病毒病预防控制所组织编写 . —北京：人民卫生出版社，2020

ISBN 978-7-117-29208-5

Ⅰ. ①实… Ⅱ. ①中… Ⅲ. ①生物学-实验室管理-手册 Ⅳ. ①Q-338

中国版本图书馆 CIP 数据核字（2019）第 240856 号

实验室生物安全手册

组织编写：中国疾病预防控制中心病毒病预防控制所
出版发行：人民卫生出版社（中继线 010-59780011）
地　　址：北京市朝阳区潘家园南里 19 号
邮　　编：100021
E - mail：pmph @ pmph.com
购书热线：010-59787592　010-59787584　010-65264830
印　　刷：北京铭成印刷有限公司
经　　销：新华书店
开　　本：787 × 1092　1/16　　印张：17
字　　数：382 千字
版　　次：2020 年 6 月第 1 版　2020 年 6 月第 1 版第 1 次印刷
标准书号：ISBN 978-7-117-29208-5
定　　价：59.00 元
打击盗版举报电话：010-59787491　E-mail：WQ @ pmph.com
质量问题联系电话：010-59787234　E-mail：zhiliang @ pmph.com

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序 言

实验室生物安全是国家生物安全的重点领域之一，一方面是传染病防控的基础支撑，是我国重大传染性疾病防控工作的重中之重；另一方面又与环境安全和社会安全等重点领域相互关联，是国家安全必须保障的底线。党的十八大以来，以习近平同志为核心的党中央高度重视生物安全工作，把生物安全纳入国家安全体系。在即将出台的《中华人民共和国生物安全法》中，实验室生物安全管理作为单独的章节，足可见实验室生物安全的重要性。

确保实验室生物安全，就是在确保病原体实验活动有效实施的同时，确保从事病原微生物实验活动工作人员的人身安全，确保实验室涉及的有害因子不会泄漏至实验室外，给社会和环境造成危害，保护环境、保护社会、保护人类。自2004年国务院颁布实施《病原微生物实验室生物安全管理条例》（简称“条例”）后，为规范和指导我国实验室生物安全管理工作，我国又陆续颁布了条例配套法规制度、出版了实验室生物安全相关教材、编制了实验室生物安全管理体系要求，为我国实验室生物安全工作健康发展奠定了坚实的基础。经过十多年的快速发展，我国实验室生物安全工作面临的形势和任务发生了重大变化。因此，调整实验室生物安全发展思路，从宏观管理向具体化、规范化、精细化方向转变，才能更好地适应工作需求、形势变化和发展要求，更加贴近实际需求，解决实际问题，从而将实验室生物安全风险控制在萌芽之中。

在包括传染病防控在内的生物医学领域的科研教学、药品和生物制品的生产、出入境检验检疫等工作中，实验室生物安全是保障上述工作顺利开展的基础，尤其是传染病防控方面，生物安全实验室是必备的基础平台。近年来，由于气候与生态环境的变化、媒介生物种群密度变化、人员流动和活动范围扩大以及贸易全球化等多种因素影响，病原体跨物种、跨地域传播加速，新发和再发传染病威胁持续存在，传染病疫情的发生和流行呈不断加剧态势。生物安全实验室作为病原研究、监测和防控应用研究的基本平台，必须得到保障与加强。

如何确保实验室生物安全？自从1983年世界卫生组织出版发行第一版 *Laboratory Biosafety Manual* 以来，几经修订，其第四版预计于2020年问世；美国疾病预防控制中心早在1984出版发行了 *Biosafety in Microbiological and Biomedical Laboratories*，目前已经成为美国乃至全世界实验室生物安全领域的“金标准”，其第六版也即将面世。这些著作是实验室生物安全领域的经典必读工具书，均在保障实验室生物安全方面发挥了极为重要的作用。

2004年以来，我国的实验室生物安全工作进入到一个新的发展纪元，生物安全实验室的硬件和软件方面得到了飞速发展。面对当前新发、再发传染病持续出现，实验室生物安全相关风险依然存在的态势，编写一本适合我国国情的《实验室生物安全手册》，加强实验室

生物安全从业人员的培训，培养更多合格人才，就显得尤为重要。

作为中国疾病预防控制中心的“生物安全首席专家”，武桂珍研究员也是国际资深的实验室生物安全知名学者，三十多年默默深耕于传染病防控战线，她直接参与了严重急性呼吸综合征（SARS）、西非埃博拉、新型冠状病毒肺炎等数十起国内外重大公共卫生事件现场处置与协调工作。我国发生SARS病毒实验室感染事件后，她临危受命，在前无经验可鉴的情况下，组建了中国第一支实验室生物安全专业团队。十几年来，她一直不断探索创新、专业上深耕不辍，引领中国实验室生物安全的发展。武桂珍研究员也是最早走出国门与国际组织和同行开展生物安全交流的专家，目前担任亚太生物安全协会（A-PBA）主席。作为主编，武桂珍研究员还创办了我国生物安全领域的第一本英文专业期刊 *Biosafety and Health*（《生物安全与健康》），为我国生物安全领域的学术成果走出国门、与国际同行加盟交流、提升国际影响迈出了坚实的一步，为中国生物安全专家与国际同行开展广泛的学术交流搭建了一个专业平台。

为适应我国实验室生物安全工作发展的新形势和新需求，以武桂珍研究员为代表的一批长期从事实验室生物安全领域研究的专家共同编写了这本《实验室生物安全手册》。他们来自我国疾病预防控制系统、科研院所、高等院校、卫生事业管理机构和监督评审机构，充分体现了多学科、多部门的融合。他们工作在科研和管理一线，从不同的维度，用丰富的案例和娴熟的文字功底为读者解读和剖析实验室生物安全。

我相信《实验室生物安全手册》一定会成为实验室生物安全领域的又一经典著作，衷心希望《实验室生物安全手册》也将成为实验室生物安全领域的“干细胞”著作。本书可作为生物安全实验室一线工作人员的实用有效的参考工具书，必将规范和指导实验室生物安全，提升我国实验室生物安全管理水平，推动我国传染病预防控制工作健康发展。

高福

中国科学院院士
中国疾病预防控制中心主任
国家自然科学基金委员会副主任
2020年2月

前 言

实验室生物安全，顾名思义，就是与病原微生物实验室相关的生物安全。与病原微生物相关的研究、教学、临床、疾病控制、生产等生物医学实验室，均不可避免涉及已知或未知的有害生物因子操作活动。这些活动的目的本应是为人类健康创造有益的成果，但是由于风险评估不周、操作或防护不当、疏忽意外等多种原因，都可能引发实验室生物安全事故，造成严重危害。为使实验室生物安全的管理人员和从事实验室工作的人员进一步提高实验室生物安全意识和规范操作水平，有效避免实验室生物安全事件 / 事故的发生，我们组织国内实验室生物安全领域的专家学者编写了这本《实验室生物安全手册》。

《实验室生物安全手册》的编写突出了实用性和指导性，按照“看后即懂、懂后能做”的编写原则，站在实验室生物安全一线工作人员的角度，以在工作中可能遇到的实际问题为出发点，使工作在实验室生物安全一线的人员看到每一部分内容后，即能迅速、准确地掌握该部分涉及的工作相关知识和要点，知晓如何落实和执行各项要求。本书系统总结了近年来实验室生物安全管理的最新进展，覆盖了实验室管理的各个方面，真正有助于解决具体的实际问题，提高实验室生物安全工作的可操作性。

本书以手册的形式阐述实验室生物安全问题，便于一线工作人员随时随地参考使用。全书共分为 4 篇、18 章。第一篇概述部分主要介绍实验室生物安全总则和风险评估。第二篇实验室基本要求部分主要包括实验室建设要求、设施要求和关键设备要求。第三篇操作规范部分主要包括实验室常规操作技术规范，个人防护用品使用、消毒和灭菌，以及实验室在物理、化学和放射等方面的安全。第四篇运行管理部分包括了管理体系运行、菌（毒）种及样本的运输与保藏、实验室废物的处理、维护实验室设施、动物实验室的管理、实验室应急处置和实验室认证等内容。

本书的编写和出版得到公益性行业科研专项项目“新时期我国实验室生物安全主要问题及其对策研究”（201302006）和中国医学科学院医学与健康科技创新工程“重要新发突发传染病生物安全基础与防控技术研究”（2016-I2M-1-014）的资助。同时，也得到国家卫生健康委员会科技教育司以及国内实验室生物安全领域权威专家的大力支持和关心，在此一并表示衷心感谢。

希望本书能成为实验室生物安全一线工作人员的实用有效的工具书。由于时间仓促，水平有限，书中难免存在不足和问题，恳请各位读者、专家提出宝贵意见，以便再版时进一步修订完善。

武桂珍　王健伟

2020 年 2 月

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第一篇

实验室生物安全概述

第一章 实验室生物安全总则

实验室生物安全（laboratory biosafety）是指实验室的生物安全条件和状态不低于容许水平，符合相关法规、标准等对实验室生物安全责任的要求，可避免实验室人员、来访人员、社区及环境受到不可接受的损害。生物安全实验室（biosafety laboratory，BSL）是指通过实验室的设计建造、生物安全设备的配置、个人防护装备的使用，以及严格遵守预先制定的安全操作程序和管理规范等综合措施，确保操作生物危险因子的工作人员不受实验对象的伤害，确保周围环境不受生物因子污染，并保护实验对象（如病原、样本）不被污染的实验室。只有实验室的生物安全条件和环境处于良好状态，才能避免实验室人员的感染及环境的污染。

根据对操作病原微生物及相关因子所采取的防护措施，以及国际通行的实验室防护水平分级，我国将实验室生物安全防护水平分为一级、二级、三级和四级。一级防护水平为最低，四级防护水平为最高。一级和二级实验室为基础实验室。一级生物安全实验室主要操作已经确定不会引起成年人患病或实验人员感染的病原微生物。二级生物安全实验室操作病原体一般为第三类病原微生物，具有中度潜在危险。三级和四级实验室统称为高等级生物安全实验室。生物安全防护水平为三级的实验室适用于操作能够引起人类或者动物严重疾病，较容易直接或者间接在人与人、动物与人、动物与动物间传播的微生物；生物安全防护水平为四级的实验室适用于操作能够引起人类或者动物非常严重疾病的微生物，以及我国尚未发现或者已经宣布消灭的微生物。《病原微生物实验室生物安全管理条例》规定，三级、四级实验室从事高致病性病原微生物实验活动必须通过实验室认可，并获得卫生或兽医行政主管部门的病原体实验活动批准。

第一节 实验室生物安全的发展概况

一、国际发展情况

实验室生物安全的概念于20世纪50~60年代由美国科学家提出。当时，为防止生物战剂的泄漏，明确了对实验设施建设的建筑设计要求。20世纪70~80年代，实验室生物安全事故频发，促进了病原微生物操作规范、个人防护措施和实验室设施的有机结合。1979年，美国著名的实验室感染研究专家Pike指出："知识、技术和设备对防止大多数实验室感染是

策划编辑　苏晓曦　刘　彬
责任编辑　韩敬霖　刘　彬
封面设计　sense 盛世设计
　　尹　岩
版式设计　刘　茜

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《病原微生物实验室生物安全》

《病原微生物实验室生物安全》DVD教材采用图像、声音、文字、动画相结合的形式，向读者较为全面、系统讲解了实验室生物安全各方面知识和操作技能。本教材共计150分钟，由基础理论知识讲解、各级生物安全实验室使用操作演示和测试三大部分组成。实际操作演示以不同生物安全级别（BSL-1、BSL-2、BSL-3）实验室为模块，演示在不同级别生物安全实验室内的实验操作和相关设备使用。读者在完成全部学习内容后，可进入测试部分，验证学习效果。本教材可作为有关单位进行实验室生物安全培训的参考使用教材。

使用说明

本教材为DVD-ROM格式，运行环境需求：Windows 2000及以上的操作系统，最低分辨率800×600。

双击光盘目录下"TestSystem.exe"文件进行安装，具体使用方法查看"使用说明.txt"文件。

出 品 人：中国疾病预防控制中心
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技术顾问：车凤翔 梁米芳 海荣 段书敏 梁国栋 卢金星 王国治 叶强 何兆伟 吕京 祁建城
协助拍摄：中国疾病预防控制中心实验室管理处
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中国国际货运航空有限公司
监　　制：武桂珍 谢九如 赵赤鸿
总 监 制：侯培森

节目制作：北京科影音像出版社

出版监制：谢九如　出版策划：翟东强　统筹：彭冲　责任编辑：王旭

北京科影音像出版社
地址：北京市西城区新街口北大街74号
邮编：100035
电话：(010)66115507 66189908 66184452

北京科影音像出版社　出版

ISRC CN-A62-08-0032-0/V.R

ISBN 978-7-88056-476-1

《病原微生物实验室生物安全》

北京科影音像出版社　出版

1片装

病原微生物 实验室生物安全

北京科影音像出版社　出版
DISTRIBUTED BY BEIJING SCIENCE-EDUCATION AUDIOVISUAL PUBLISHING HOUSE

www.sci-edu.com

疫病防控和公共卫生领域科技创新战略研究报告

武桂珍

中国疾病预防控制中心

病毒病预防控制所

2023 年 5 月

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报告简介

受中华人民共和国科学技术部委托，武桂珍教授及其研究团队于2023 年 5 月完成了《疾病防控和公共卫生领域科技创新战略研究报告》。

武桂珍教授及其团队多年来深耕实验室微生物检测、消毒技术研发、生物安全装备研制等实验室生物安全关键技术，病毒溯源技术路径，疫苗研发生产中的生物安全管理，以及生物安全研究管理理论和实践等领域，成绩斐然。本报告在这些科研成果的基础上，通过大量调研，围绕加强国家疾病防控和公共卫生领域科技力量，梳理提炼了我国生物安全领域主要能力体系分析图，为科技主管部门建议了包括前沿基础研究、共性技术研究、重大工程建设、平台能力建设等方面的战略任务，并提出了一系列政策措施供参考。