EDITORIAL

Another Decade, Another Coronavirus

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For the third time in as many decades, a zoonotic coronavirus has crossed species to infect human populations. This virus, provisionally called 2019-nCoV, was first identified in Wuhan, China, in persons exposed to a seafood or wet market. The rapid response of the Chinese public health, clinical, and scientific communities facilitated recognition of the clinical disease and initial understanding of the epidemiology of the infection. First reports indicated that human-to-human transmission was limited or nonexistent, but we now know that such transmission occurs, although to what extent remains unknown. Like outbreaks caused by two other pathogenic human respiratory coronaviruses (severe acute respiratory syndrome coronavirus [SARS-CoV] and Middle East respiratory syndrome coronavirus [MERS-CoV]), 2019-nCoV causes respiratory disease that is often severe.[1] As of January 24, 2020, there were more than 800 reported cases, with a mortality rate of 3% (https://promedmail.org/).

As now reported in the *Journal*, Zhu et al.[2] have identified and characterized 2019-nCoV. The viral genome has been sequenced, and these results in conjunction with other reports show that it is 75 to 80% identical to the SARS-CoV and even more closely related to several bat coronaviruses.[3] It can be propagated in the same cells that are useful for growing SARS-CoV and MERS-CoV, but notably, 2019-nCoV grows better in primary human airway epithelial cells than in standard tissue-culture cells, unlike SARS-CoV or MERS-CoV. Identification of the virus will allow the development of reagents to address key unknowns about this new coronavirus infection and guide the development of antiviral therapies. First, knowing the sequence of the genome facilitates the development of sensitive quantitative reverse-transcriptase–polymerase-chain-reaction assays to rapidly detect the virus. Second, the development of serologic assays will allow assessment of the prevalence of the infection in humans and in potential zoonotic sources of the virus in wet markets and other settings. These reagents will also be useful for assessing whether the human infection is more widespread than originally thought, since wet markets are present throughout China. Third, having the virus in hand will spur efforts to develop antiviral therapies and vaccines, as well as experimental animal models.

Much still needs to be learned about this infection. Most important, the extent of interhuman transmission and the spectrum of clinical disease need to be determined. Transmission of SARS-CoV and MERS-CoV occurred to a large extent by means of superspreading events.[4,5] Superspreading events have been implicated in 2019-nCoV transmission, but their relative importance is unknown. Both SARS-CoV and MERS-CoV infect intrapulmonary epithelial cells more than cells of the upper airways.[4,6] Consequently, transmission occurs primarily from patients with recognized illness and not from patients with mild, nonspecific signs. It appears that 2019-nCoV uses the same cellular receptor as SARS-CoV (human angiotensin-converting enzyme 2 [hACE2]),[3] so transmission is expected only after signs of lower respiratory tract disease develop. SARS-CoV mutated over the 2002–2004 epidemic to better bind to its cellular receptor and to optimize replication in human cells, enhancing virulence.[7] Adaptation readily occurs because coronaviruses have error-prone RNA-

dependent RNA polymerases, making mutations and recombination events frequent. By contrast, MERS-CoV has not mutated substantially to enhance human infectivity since it was detected in 2012.[8]

It is likely that 2019-nCoV will behave more like SARS-CoV and further adapt to the human host, with enhanced binding to hACE2. Consequently, it will be important to obtain as many temporally and geographically unrelated clinical isolates as possible to assess the degree to which the virus is mutating and to assess whether these mutations indicate adaptation to the human host. Furthermore, if 2019-nCoV is similar to SARS-CoV, the virus will spread systemically.[9] Obtaining patient samples at autopsy will help elucidate the pathogenesis of the infection and modify therapeutic interventions rationally. It will also help validate results obtained from experimental infections of laboratory animals.

A second key question is identification of the zoonotic origin of the virus. Given its close similarity to bat coronaviruses, it is likely that bats are the primary reservoir for the virus. SARS-CoV was transmitted to humans from exotic animals in wet markets, whereas MERS-CoV is transmitted from camels to humans.[10] In both cases, the ancestral hosts were probably bats. Whether 2019-nCoV is transmitted directly from bats or by means of intermediate hosts is important to understand and will help define zoonotic transmission patterns.

A striking feature of the SARS epidemic was that fear played a major role in the economic and social consequences. Although specific anticoronaviral therapies are still in development, we now know much more about how to control such infections in the community and hospitals, which should alleviate some of this fear. Transmission of 2019-nCoV probably occurs by means of large droplets and contact and less so by means of aerosols and fomites, on the basis of our experience with SARS-CoV and MERS-CoV.[4,5] Public health measures, including quarantining in the community as well as timely diagnosis and strict adherence to universal precautions in health care settings, were critical in controlling SARS and MERS. Institution of similar measures will be important and, it is hoped, successful in reducing the transmission of 2019-nCoV.

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

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Perspective

A Novel Coronavirus Emerging in China — Key Questions for Impact Assessment

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A novel coronavirus, designated as 2019-nCoV, emerged in Wuhan, China, at the end of 2019. As of January 24, 2020, at least 830 cases had been diagnosed in nine countries: China, Thailand, Japan, South Korea, Singapore, Vietnam, Taiwan, Nepal, and the United States. Twenty-six fatalities occurred, mainly in patients who had serious underlying illness.[1] Although many details of the emergence of this virus — such as its origin and its ability to spread among humans — remain unknown, an increasing number of cases appear to have resulted from human-to-human transmission. Given the severe acute respiratory syndrome coronavirus (SARS-CoV) outbreak in 2002 and the Middle East respiratory syndrome coronavirus (MERS-CoV) outbreak in 2012,[2] 2019-nCoV is the third coronavirus to emerge in the human population in the past two decades — an emergence that has put global public health institutions on high alert.

China responded quickly by informing the World Health Organization (WHO) of the outbreak and sharing sequence information with the international community after discovery of the causative agent. The WHO responded rapidly by coordinating diagnostics development; issuing guidance on patient monitoring, specimen collection, and treatment; and providing up-to-date information on the outbreak.[3] Several countries in the region as well as the United States are screening travelers from Wuhan for fever, aiming to detect 2019-nCoV cases before the virus spreads further. Updates from China, Thailand, Korea, and Japan indicate that the disease associated with 2019-nCoV appears to be relatively mild as compared with SARS and MERS.

After initial reports of a SARS-like virus emerging in Wuhan, it appears that 2019-nCoV may be less pathogenic than MERS-CoV and SARS-CoV (see table). However, the virus's emergence raises an important question: What is the role of overall pathogenicity in our ability to contain emerging viruses, prevent large-scale spread, and prevent them from causing a pandemic or becoming endemic in the human population? Important questions regarding any emerging virus are, What is the shape of the disease pyramid? What proportion of infected people develop disease? And what proportion of those seek health care? These three questions inform the classic surveillance pyramid (see diagram).[4] Emerging coronavirus-

Table 1. Pathogenicity and Transmissibility Characteristics of Recently Emerged Viruses in Relation to Outbreak Containment.

Virus	Case Fatality Rate (%)	Pandemic	Contained	Remarks
2019-nCoV	Unknown*	Unknown	No, efforts ongoing	
pH1N1	0.02–0.4	Yes	No, postpandemic circulation and establishment in human population	
H7N9	39	No	No, eradication efforts in poultry reservoir ongoing	
NL63	Unknown	Unknown	No, endemic in human population	
SARS-CoV	9.5	Yes	Yes, eradicated from intermediate animal reservoir	58% of cases result from nosocomial transmission
MERS-CoV	34.4	No	No, continuous circulation in animal reservoir and zoonotic spillover	70% of cases result from nosocomial transmission
Ebola virus (West Africa)	63	No	Yes	

* Number will most likely continue to change until all infected persons recover.

es raise an additional question: How widespread is the virus in its reservoir? Currently, epidemiologic data that would allow us to draw this pyramid are largely unavailable (see diagram).

Clearly, efficient human-to-human transmission is a requirement for large-scale spread of this emerging virus. However, the severity of disease is an important indirect factor in a virus's ability to spread, as well as in our ability to identify those infected and to contain it — a relationship that holds true whether an outbreak results from a single spillover event (SARS-CoV) or from repeated crossing of the species barrier (MERS-CoV).

If infection does not cause serious disease, infected people probably will not end up in health care centers. Instead, they will go to work and travel, thereby potentially spreading the virus to their contacts, possibly even internationally. Whether subclinical or mild disease from 2019-nCoV is also associated with a reduced risk of virus spread remains to be determined.

Much of our thinking regarding the relationship between transmissibility and pathogenicity of respiratory viruses has been influenced by our understanding of influenza A virus: the change in receptor specificity necessary for efficient human-to-human transmission of avian influenza viruses leads to a tropism shift from the lower to the upper respiratory tract, resulting in a lower disease burden. Two primary — and recent — examples are the pandemic H1N1 virus and the avian influenza H7N9 virus. Whereas the pandemic H1N1 virus — binding to receptors in the upper respiratory tract — caused relatively mild disease and became endemic in the population, the H7N9 virus — binding to receptors in the lower respiratory tract — has a case-fatality rate of approximately 40% and has so far resulted in only a few small clusters of human-to-human transmission.

It is tempting to assume that this association would apply to other viruses as well, but such a similarity is not a given: two coronaviruses that use the same receptor (ACE2) — NL63 and SARS-CoV — cause disease of different severity. Whereas NL63 usually causes mild upper respiratory tract disease and is endemic in the human population, SARS-CoV induced severe lower respiratory tract disease with a case-fatality rate of about 11% (see table). SARS-CoV was eventually contained by means of syndromic surveillance, isolation of patients, and quarantine of their contacts. Thus, disease severity is not necessarily linked to transmission efficiency.

Even if a virus causes subclinical or mild disease in general, some people may be more susceptible and end up seeking care. The majority of SARS-CoV and MERS-CoV cases were associated with nosocomial transmission in hospitals,[5] resulting at least in part from the use of aerosol-generating procedures in patients with respiratory disease. In particular, nosocomial super-spreader events appear to have driven large outbreaks within and between health care settings. For example, travel from Hong Kong to Toronto by one person with SARS-CoV resulted in 128 SARS cases in a local

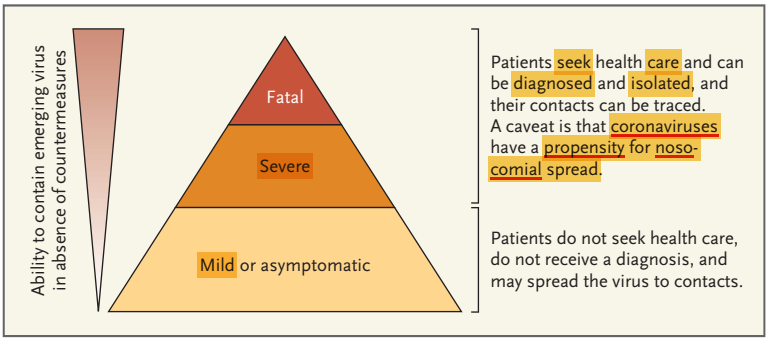

Figure 1. Surveillance Pyramid and Its Relation to Outbreak Containment.

The proportion of mild and asymptomatic cases versus severe and fatal cases is currently unknown for 2019-nCoV — a knowledge gap that hampers realistic assessment of the virus's epidemic potential and complicates the outbreak response.

hospital. Similarly, the introduction of a single patient with MERS-CoV from Saudi Arabia into the South Korean health care system resulted in 186 MERS cases.

The substantial involvement of nosocomial transmission in both SARS-CoV and MERS-CoV outbreaks suggests that such transmission is a serious risk with other newly emerging respiratory coronaviruses. In addition to the vulnerability of health care settings to outbreaks of emerging coronaviruses, hospital populations are at significantly increased risk for complications from infection. Age and coexisting conditions (such as diabetes or heart disease) are independent predictors of adverse outcome in SARS-CoV and MERS-CoV. Thus, emerging viruses that may go undetected because of a lack of severe disease in healthy people can pose significant risk to vulnerable populations with underlying medical conditions.

A lack of severe disease manifestations affects our ability to contain the spread of the virus. Identification of chains of transmission and subsequent contact tracing are much more complicated if many infected people remain asymptomatic or mildly symptomatic (assuming that these people are able to transmit the virus). More pathogenic viruses that transmit well between humans can generally be contained effectively through syndromic (fever) surveillance and contact tracing, as exemplified by SARS-CoV and, more recently, Ebola virus. Although containment of the ongoing Ebola virus outbreak in the Democratic Republic of Congo is complicated by violent conflict, all previous outbreaks were contained through identification of cases and tracing of contacts, despite the virus's efficient person-to-person transmission.

We currently do not know where 2019-nCoV falls on the scale of human-to-human transmissibility. But it is safe to assume that *if* this virus transmits efficiently, its seemingly lower pathogenicity as compared with SARS, possibly combined with super-spreader events in specific cases, could allow large-scale spread. In this manner, a virus that poses a low health threat on the individual level can pose a high risk on the population level, with the potential to cause disruptions of global public health systems and economic losses. This possibility warrants the current aggressive response aimed at tracing and diagnosing every infected patient and thereby breaking the transmission chain of 2019-nCoV.

Epidemiologic information on the pathogenicity and transmissibility of this virus obtained by means of molecular detection and serosurveillance is needed to fill in the details in the surveillance pyramid and guide the response to this outbreak. Moreover, the propensity of novel coronaviruses to spread in health care centers indicates a need for peripheral health care facilities to be on standby to identify potential cases as well. In addition, increased preparedness is needed at animal markets and other animal facilities, while the possible source of this emerging virus is being investigated. If we are proactive in these ways, perhaps we will never have to discover the true epidemic or pandemic potential of 2019-nCoV.

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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 another 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.)

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.

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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,[14] 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.[13]

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.

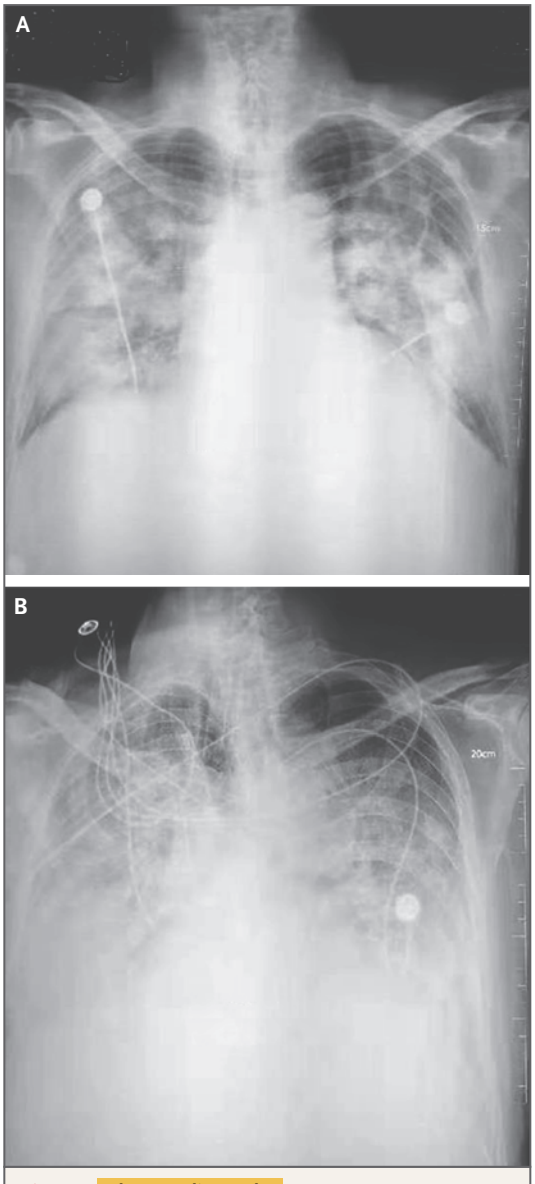

Figure 1. Chest Radiographs.

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.

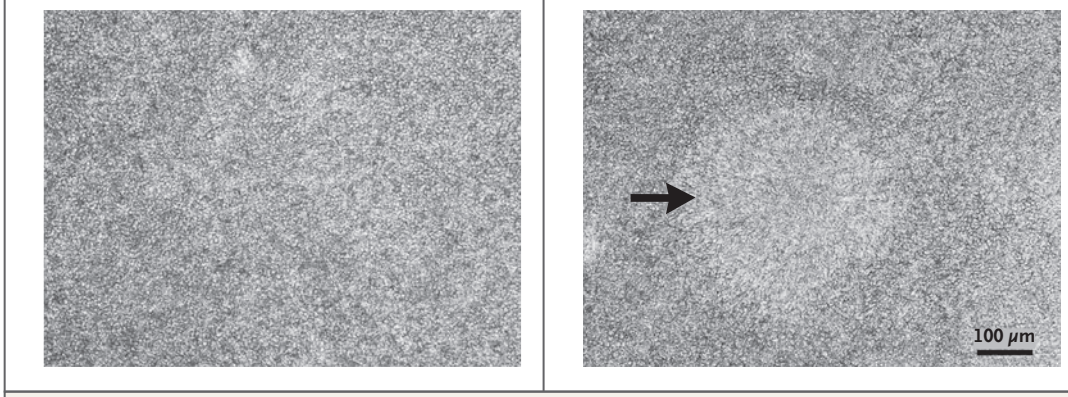

Figure 2. Cytopathic Effects in Human Airway Epithelial Cell Cultures after Inoculation with 2019-nCoV.

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 GASAID (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,

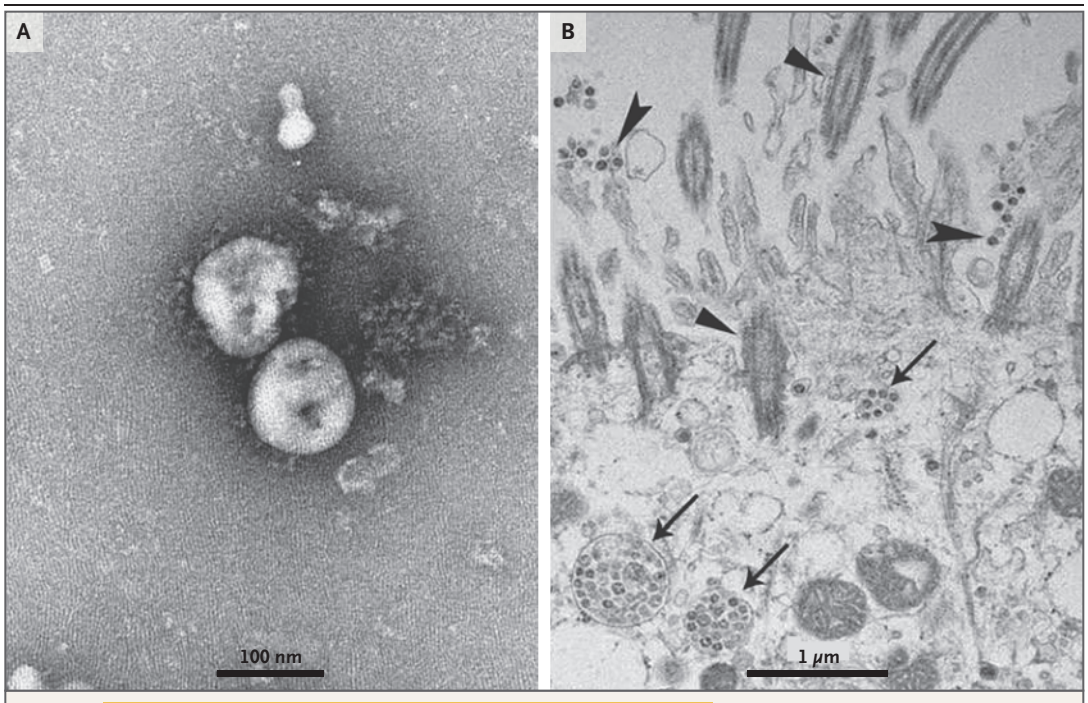

Figure 3. Visualization of 2019-nCoV with Transmission Electron Microscopy.
Negative-stained 2019-nCoV particles are shown in Panel A, and 2019-nCoV particles in the human airway epithelial cell ultrathin sections are shown in Panel B.

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 and formed an independent subclade 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 in lineage B of the subgenus sarbecovirus. 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 GASAID. 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 suc-

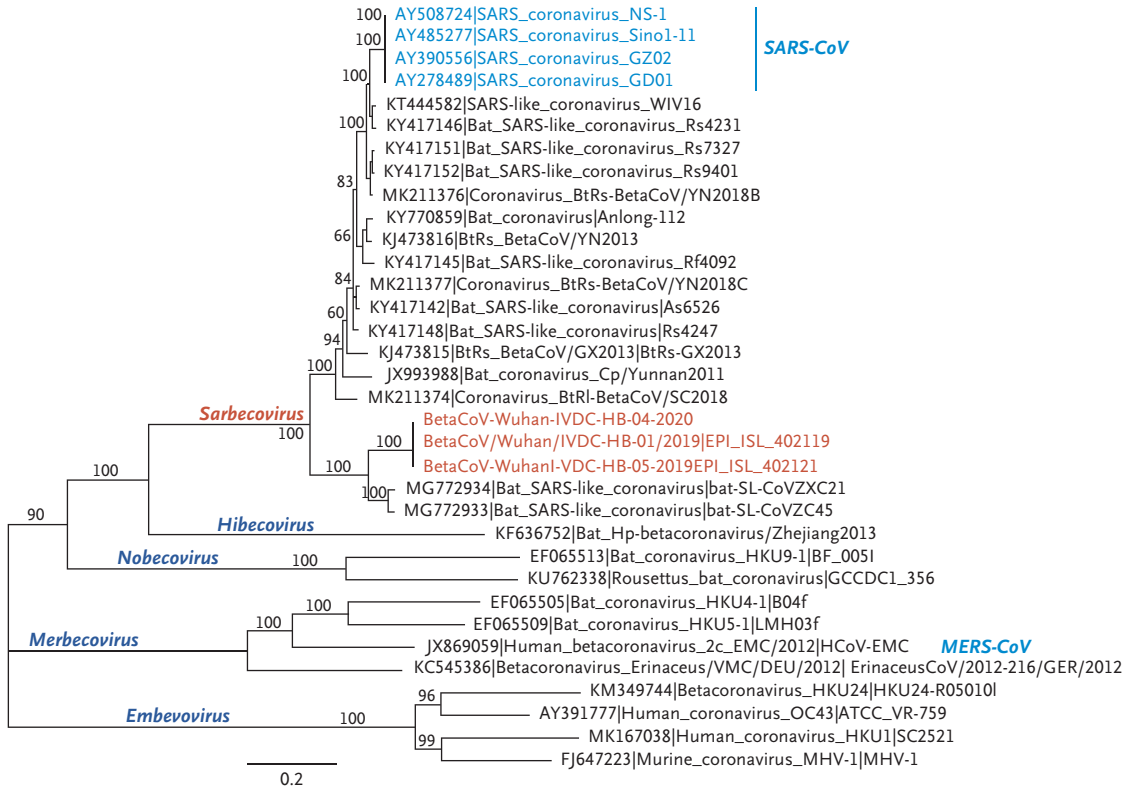

Figure 4. Phylogenetic Analysis of 2019-nCoV and Other Betacoronavirus Genomes in the Orthocoronavirinae Subfamily.

cessfully to identify infectious agents for many years. Unbiased, high-throughput sequencing is 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.[14] 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 sig-

nificance 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.

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

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