BRIEF REPORT

Isolation of a Novel Coronavirus from a Man with Pneumonia in Saudi Arabia

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SUMMARY

A previously unknown coronavirus was isolated from the sputum of a 60-year-old man who presented with acute pneumonia and subsequent renal failure with a fatal outcome in Saudi Arabia. The virus (called HCoV-EMC) replicated readily in cell culture, producing cytopathic effects of rounding, detachment, and syncytium formation. The virus represents a novel betacoronavirus species. The closest known relatives are bat coronaviruses HKU4 and HKU5. Here, the clinical data, virus isolation, and molecular identification are presented. The clinical picture was remarkably similar to that of the severe acute respiratory syndrome (SARS) outbreak in 2003 and reminds us that animal coronaviruses can cause severe disease in humans.

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Coronaviruses are enveloped, single-stranded, positive-sense RNA viruses that are phenotypically and genotypically diverse.[1] Coronaviruses are widespread in bats around the world but can be found in many other species as well, including birds, cats, dogs, pigs, mice, horses, whales, and humans.[1] They may cause respiratory, enteric, hepatic, or neurologic diseases, with variable severity in various animal species. In humans, four respiratory coronaviruses — human coronaviruses (HCoV) 229E, OC43, NL63, and HKU1 — are known to be endemic. In addition, in 2003 a previously unknown coronavirus caused an outbreak of SARS in humans.[2-4] The diversity of coronaviruses is facilitated by the infidelity of the RNA-dependent RNA polymerase, the high frequency of RNA recombination, and the unusually large genomes for RNA viruses.[1,5] These factors not only have led to the diversity of known coronaviruses but also have facilitated the emergence of viruses with new traits that allow the organism to adapt to new hosts and ecologic niches, sometimes causing zoonotic events.

CASE REPORT

A 60-year-old Saudi man was admitted to a private hospital in Jeddah, Saudi Arabia, on June 13, 2012, with a 7-day history of fever, cough, expectoration, and shortness of breath. He had no history of cardiopulmonary or renal disease, was receiving no long-term medications, and did not smoke. The physical examination revealed a body-mass index (the weight in kilograms divided by the square of the height in meters) of 35.1, a blood pressure of 140/80 mm Hg, a pulse of 117 beats per minute, a temperature of 38.3°C, and a respiratory rate of 20 breaths per minute.

Chest radiography performed on admission showed low lung volumes, bilateral enhanced pulmonary hilar vascular shadows more prominent on the left, and accentuated bronchovascular lung markings. Multiple segmental, patchy, veiling

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for which final annotation remained in progress at the time of this report. The HCoV-EMC virus genome encodes the open reading frames common to betacoronaviruses, including open reading frame 1ab, which encodes many enzymatic products, the spike-surface glycoprotein (S), the small-envelope (E) protein, the matrix (M) protein, and the nucleocapsid (N) protein, as well as several nonstructural genes. The genome does not encode a hemagglutinin–esterase protein, in contrast to some other betacoronaviruses.

We compared the open reading frame 1ab gene product of HCoV-EMC with those of the other betacoronaviruses, HKU4 and HKU5, to test whether HCoV-EMC might belong to one of these known species or whether it represents a new species within the genus. The International Committee on Taxonomy of Viruses (ICTV) considers that viruses sharing more than 90% of sequence identity in the conserved replicase domains belong to the same species.[1] This 90% identity threshold serves as the sole species demarcation criterion. Since the identity of amino acid sequences in these conserved domains of open reading frame 1ab between HCoV-EMC and HKU4 and HKU5 was less than 80%, we concluded that HCoV-EMC represented a novel betacoronavirus species, although such classification requires formal ICTV approval.

DISCUSSION

The first decade of the 21st century has witnessed an increase in the number of coronaviruses that have been identified, along with a corresponding increase in the number of coronavirus genomes that have been sequenced. Such increases were due to the discovery of the SARS coronavirus, which resulted in a global outbreak of pneumonia in 2003 that affected persons in approximately 30 countries and resulted in about 800 deaths.[12] Before 2003, only two human coronaviruses were known, HCoV-229E and HCoV-OC43, both discovered in the 1960s.[13,14] After the emergence of the SARS-CoV in 2003, two additional human coronaviruses were discovered, HCoV-NL63 and HCoV-HKU1.[15-17] Here we report the isolation and characterization of the sixth coronavirus that apparently may infect humans.

On the basis of genetic data, the ICTV has identified four virus clusters within the Coronavirinae subfamily, of which three represent approved genera; alphacoronavirus, betacoronavirus, and gammacoronavirus. The five known human coronaviruses all belong to the genera alphacoronavirus (HCoV-229E and HCoV-NL63) and betacoronavirus (HCoV-OC43, HCoV-HKU1, and SARS-CoV).[2-4,13-16,18] HCoV-EMC is the first human coronavirus in lineage C of the betacoronavirus genus. Its closest relatives are coronaviruses HKU4 and HKU5, isolated from *Tylonycteris pachypus* and *Pipistrellus abramus* bats, respectively.[17]

As compared with other coronaviruses, HCoV-EMC was isolated and propagated relatively easily in Vero and LLC-MK2 cells. The only other human coronaviruses that replicate well in these monkey-cell lines are SARS-CoV and HCoV-NL63, which both use human angiotensin-converting enzyme 2 as their receptor. We hypothesize that one or more species of animals, possibly bats, were the reservoir host of this new coronavirus. Saudi Arabia harbors numerous bat species, including pipistrellus bats, which were found to carry BatCoV-HKU5 in Asia.

The patient's findings on chest radiography together with the clinical symptoms indicated acute respiratory distress syndrome (ARDS) with multiorgan dysfunction syndrome (MODS), similar to what has been described in severe cases of influenza and SARS.[19-21] These pneumonic changes did not respond to antibacterial treatment.[22] The patient was treated with oseltamivir for the possibility of infection with the H1N1 swine flu virus. Hematologic changes were evident in this patient in the form of lymphopenia, neutrophilia, and late thrombocytopenia. Abnormal hematologic variables were also quite common among patients with SARS. Lymphopenia was the most common finding in a cohort of 157 patients with SARS. In those patients, postmortem findings showed lymphopenia in various lymphoid organs with no features of bone marrow failure or reactive hemophagocytic syndrome.[23] The patient also had progressive impairment of renal function, similar to what had been described in some patients with SARS and possibly attributed to direct infection of renal tissue by the virus. The renal impairment in this case started on the 9th day of symptoms and progressed over the course of the patient's illness.

No symptoms were observed in the hospital among doctors and nurses caring for the patient, which suggests that the disease did not spread readily. However, staff members were not

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