

Coronaviruses: Important Emerging Human Pathogens

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The identification of Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 reaffirmed the importance of understanding how coronaviruses emerge, infect, and cause disease. By comparing what is known about severe acute respiratory syndrome coronavirus (SARS-CoV) to what has recently been found for MERS-CoV, researchers are discovering similarities and differences that may be important for pathogenesis. Here we discuss what is known about each virus and what gaps remain in our understanding, especially concerning MERS-CoV.

MERS-CoV AND SARS-CoV EMERGENCES

The emergence of a highly pathogenic human coronavirus in the Middle East has sparked new interest in human coronaviruses around the world. Middle East respiratory syndrome coronavirus (MERS-CoV) was identified in 2012, almost 10 years after the highly fatal human severe acute respiratory syndrome coronavirus (SARS-CoV) emerged from China in 2003. Over the past 10 years, much has been learned about highly pathogenic coronaviruses from the investigation of SARS-CoV, which aids in our efforts to combat MERS-CoV; however, gaps in our understanding remain. Emerging pathogens present a unique challenge to science and medicine because generally little is known about them before they emerge from often-unrecognized zoonotic sources. Zoonotic transmission can occur by a spillover event from an animal to a human due to sustained or new close human contact or climate changes affecting the distribution of previously geographically restricted disease vectors such as insects. Viruses can also emerge from the evolution of a previously animal-restricted pathogen to one that can utilize a human receptor or the cellular machinery needed for infection. For most of these emerging pathogens, therapies and/or vaccination strategies have not been developed, and therefore, clinical treatment options for infected patients are limited to nonspecific supportive therapy.

There have been two known incidences of emergence of highly pathogenic coronaviruses. SARS-CoV emerged in 2003 in Guangdong Province, China, and spread to 37 different countries, causing 8,273 confirmed cases of infection, of which 775 (9%) were fatal (1). SARS-CoV spread to many countries in Southeast Asia, causing significant outbreaks in China, Hong Kong, Taiwan, Singapore, and Vietnam. Outside of this region, many other countries reported a small number of cases (for example, the United States of America had 27 confirmed imported cases and no deaths), but the only country outside of the region with a significant number of cases was Canada, with 251 confirmed cases and 44 deaths. Due to the implementation of infection control measures such as quarantine and blocks in air travel, SARS-CoV disappeared again in 2003, and no human infections have been seen since.

Then, approximately 10 years later, in 2012, MERS-CoV emerged in the Kingdom of Saudi Arabia (KSA) (2) with, as of 7 February 2014, 182 total confirmed cases, of which 79 (43%) were fatal. MERS-CoV is a betacoronavirus in the same family as SARS-CoV with a similar single-stranded positive-sense RNA genome

structure (Fig. 1A). Cases of MERS-CoV infection have been geographically restricted to the Arabian Peninsula, with the majority of cases occurring in KSA, Qatar, Jordan, Oman, United Arab Emirates, and Egypt. Outside of this region, there have been a smaller number of cases of infection in European countries (France, Italy, and the United Kingdom) in people who had traveled to the Arabian Peninsula or had been in contact with people who had. There is evidence of person-to-person transmission of MERS-CoV, with the only known source of infection being an already infected person. However, person-to-person transmission of MERS-CoV tends to occur only in situations where there is close, sustained contact with an infected person, for example, in the health care setting or where the secondary infection is in someone with another risk factor, such as immunodeficiency. The emergences of both SARS-CoV and MERS-CoV have highlighted the *Coronaviridae* as potentially important human pathogenic viruses.

SARS-CoV AND MERS-CoV RESERVOIRS

Almost immediately after SARS-CoV was identified, it was hypothesized that the virus emerged from an animal host, as there were strong epidemiological links to the live-animal markets of China. Screening of small mammals identified SARS-CoV RNA in masked palm civets (*Paguma larvata*) and a raccoon dog (*Nyctereutes procyonoides*) within a live-animal market in Guangdong, China (6), and further epidemiological evidence pointed to a strong link between SARS-CoV and exposure to masked palm civets (3). However, there was doubt as to whether these small mammals were the true hosts of SARS-CoV, not least because masked palm civets from the wild or from farms with no wet-market exposure were mostly negative for SARS-CoV (reviewed in reference 4). Bats were known to be an important reservoir for a number of human pathogens, and for many years it was possible to show that bats, specifically the horseshoe bats (*Rhinolophus* genus), had detectable antibodies to SARS-CoV proteins and carried coronaviruses that were phylogenetically related to SARS-CoV, known as SARS-CoV-like coronaviruses (SL-CoV) (5, 6).

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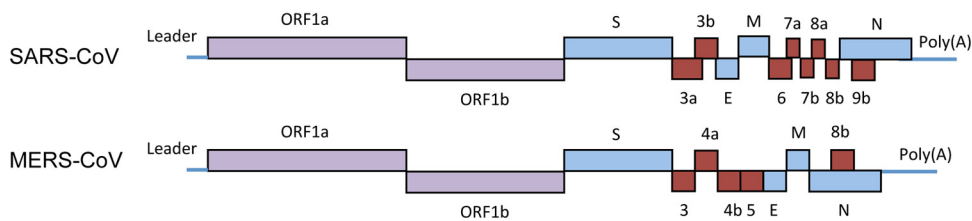
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A. Genome Structure



B. Transmission

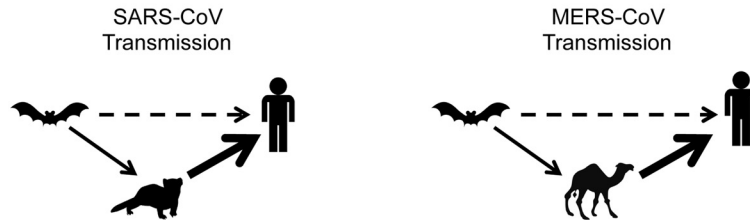


FIG 1 (A) Genome structures of SARS-CoV and MERS-CoV. The single-stranded positive-sense coronavirus genomes encode the structural proteins (blue) membrane (M), spike (S), envelope (E), and nucleocapsid (N), two replicase polyproteins (purple), ORF1a and ORF1b, and unique accessory proteins (red) that perform important functions in coronavirus replication and pathogenesis, such as blocking the innate immune signaling pathway. (B) Transmission routes. SARS-CoV transmission is thought to be from bats harboring SARS-like viruses to palm civet cats, which infected humans. SARS-CoV could also have been transmitted from bats to humans directly. MERS-CoV is thought to be transmitted from camels to humans, with the possibility that at some point bats infected camels. The dashed line identifies a low-level transmission event, the thin solid line identifies a potential transmission event, and the thick solid line identifies a probable transmission event.

However, all of the SL-CoVs had significant differences in genome sequences from human SARS-CoV, including in the receptor binding domains of the S proteins, and could not use the human SARS-CoV receptor, angiotensin-converting enzyme 2 (ACE2), to enter cells (reviewed in reference 4). Then, in 2013, two novel SL-CoVs, named RsSHC014 and Rs3367, that are more similar to SARS-CoV than any previous SL-CoV and are able to use ACE2 to enter cells were found in horseshoe bats (*Rhinolophus sinicus*) (7), suggesting that RsSHC014 and/or Rs3367 may be the direct ancestor of SARS-CoV in horseshoe bats and that these viruses are still in the bat population.

The reservoir for MERS-CoV is currently unknown. Studies in Oman and Egypt have shown that dromedary camels (*Camelus dromedarius*) have neutralizing antibodies to MERS-CoV (8), which immediately led to speculation that dromedary camels may be the intermediate host of MERS-CoV, and recent work has found MERS-CoV RNA in camels connected to an infected individual in Qatar (9). The isolation of live MERS-CoV from camels has not been achieved; however, it seems likely that camels are the reservoir of MERS-CoV and responsible for transmission to humans. Initial phylogenetic analysis of MERS-CoV showed that it is closely related to two known bat coronaviruses, BtCoV-HKU4 and BtCoV-HKU5 (10), and screening of bats for MERS-CoV-like coronaviruses (ML-CoVs) began. Studies have detected ML-CoVs in bat species in various countries (11); however, the full MERS-CoV sequence has not been isolated from any bat source. Bats may not be the only reservoir of MERS-CoV, as one study has found a close relative of MERS-CoV in the European hedgehog (*Erinaceus europaeus*) (12).

Therefore, SARS-CoV was transmitted to humans from Chinese horseshoe bats directly or via masked palm civets or raccoon dogs in the live-animal markets of China. MERS-CoV may also

have emerged from bats or other small animals and infected humans via dromedary camels (Fig. 1B).

VACCINATION AND TREATMENT FOR HIGHLY PATHOGENIC CORONAVIRUSES

There are no FDA-approved vaccines or treatments for highly pathogenic coronaviruses. Previous efforts to create a vaccine for SARS-CoV have utilized a number of approaches. Vaccines based on whole, inactivated SARS-CoV, spike subunits, recombinant viruses expressing SARS-CoV proteins, DNA plasmids expressing SARS-CoV proteins, or virus-like particles (VLPs) have all been tested *in vitro* and *in vivo* (reviewed in reference 13). Research on MERS-CoV vaccination strategies is in the early stages, and there are no approved vaccines for MERS-CoV. However, early studies using a modified vaccinia virus (14) and spike subunit vaccines (20) have been shown to induce MERS-CoV-neutralizing antibodies in mice.

Research on antiviral compounds that inhibit highly pathogenic coronaviruses is also in the early stages, and there are no specific anticoronavirus drugs that have been proven effective *in vivo*. In comparison to the sudden emergence of coronaviruses, however, the normal drug development process is long, and by the time a novel compound has gone through all the necessary clinical trials, the viral outbreak may be over. Therefore, there is a movement toward screening approved drugs against novel viruses *in vitro*. Approved drugs have known pharmacodynamics properties and pharmacokinetic tests and basic safety tests, so only antiviral efficacy needs to be established before they can be used in the clinic.

Development of an effective vaccination strategy against coronaviruses is an important area of research. More broadly, the development of vaccination and treatment strategies that

are quick and simple and require less regulatory approval is vital in the fight against emerging infectious diseases of all types, including highly pathogenic coronaviruses.

ANIMAL MODELS FOR EMERGING HIGHLY PATHOGENIC CORONAVIRUSES

A major block to the normal development of vaccines or drugs against emerging pathogenic viruses is the need for an animal model, preferably a small animal, to test the efficacy of a specific vaccine or drug *in vivo*.

The human strain of SARS-CoV, SARS-CoV (Urbani), is able to naturally infect mice but is unable to recapitulate the severe disease of adult human SARS-CoV infection (15). Passaging of SARS-CoV (Urbani) through BALB/c mouse lungs resulted in a lethal virus, named SARS-CoV (MA15) (15). Infection with SARS-CoV (MA15) results in lethal disease in young and adult mice, therefore providing a model for SARS-CoV infection in humans.

Of the small animals tested so far, neither mice (16) nor Syrian hamsters (17) could be infected with MERS-CoV. MERS-CoV has been shown to infect Rhesus macaques (*Macaca mulatta*), which have symptoms similar to those of human infections (18); however, use of nonhuman primates is not a practical way of quickly and cheaply developing vaccines and/or treatments for emerging infectious diseases. Recently, a mouse model using a human DPP4-expressing adenovirus vector to infect mouse lungs before infection with MERS-CoV was reported (19). This model shows replication and mild inflammation around airways. Further development of a stable mouse model is needed to further MERS-CoV research.

Broadly, however, because a small animal model exists for SARS-CoV and work is ongoing to develop one for MERS-CoV, there is potential for developing broad anticoronavirus vaccines and/or drugs using currently available tools.

FUTURE CORONAVIRUSES AND DIRECTIONS

The emergences of both SARS-CoV and MERS-CoV demonstrate the importance of the *Coronaviridae* as emerging human pathogens. When SARS-CoV disappeared, it was unknown whether there would be another event involving the sudden emergence of a coronavirus. The emergence of MERS-CoV nearly 10 years later has shown that highly pathogenic coronaviruses will continue to spill over from zoonotic sources into the human population.

Bats and other small mammals are rich sources of viruses, including coronaviruses, that can be transmitted to humans, so there is certainly potential out there for another coronavirus to jump from bat. Viral surveillance studies of animal species, including bats, rodents, and livestock, are essential to understand the potential human pathogens that exist in the environment before they can spill over. Therefore, there is a need to research these potential emerging viruses and also to develop broad-spectrum vaccination or therapeutic strategies to prepare for current and future emerging coronaviruses.

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