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The Middle East Respiratory Syndrome—How Worried Should We Be?

Stanley Perlman

Department of Microbiology, University of Iowa, Iowa City, Iowa, USA

ABSTRACT Ten years after the severe acute respiratory syndrome epidemic, a second coronavirus, the Middle East respiratory syndrome coronavirus (MERS-CoV), has been identified as the cause of a highly lethal pneumonia in patients in the Middle East and in travelers from this region. Over the past 9 months, since the virus was first isolated, much has been learned about the biology of the virus. It is now clear that MERS-CoV is transmissible from person to person, and its close relationship with several bat coronaviruses suggests that these animals may be the ultimate source of the infection. However, many key issues need to be addressed, including identification of the proximate, presumably zoonotic, source of the infection, the prevalence of the infection in human populations, details regarding clinical and pathological features of the human infection, the establishment of a small rodent model for the infection, and the virological and immune basis for the severe disease observed in most patients. Most importantly, we do not know whether a MERS-CoV epidemic is likely or not. Infection with the virus has so far resulted in only 91 cases and 46 deaths (as of 29 July 2013), but it is nonetheless setting off alarm bells among public health officials, including Margaret Chan, Director-General of the World Health Organization, who called MERS-CoV “a threat to the entire world.” This article reviews some of the progress that has been made and discusses some of the questions that need to be answered.

A NOVEL VIRUS IS IDENTIFIED IN SAUDI ARABIA

Middle East respiratory syndrome-coronavirus (MERS-CoV) was initially isolated in September 2012 from a patient in Saudi Arabia who had developed a lethal infection characterized by pneumonia and renal failure (1). A nearly identical virus was then isolated from a second Saudi Arabian patient with respiratory disease who had been flown to London for therapy. In retrospect, the first cases of MERS occurred in an extended family in Zarqa, Jordan, in April 2012. Virus was detected in two patients in that outbreak, but several other family members and health care workers developed respiratory disease. Virus was not isolated from these individuals. Renal failure was noted in some of the early reports, but it is not yet clear whether it was virus induced or occurred as a consequence of respiratory failure. Since those early days, several other clusters of infection have been identified, indicating that human-to-human transmission occurred, although spread may not be efficient (2). The incubation time for patients with confirmed disease was 5.2 days (confidence interval, 1.9 to 14.7 days), according to one study (3).

TRANSMISSION AND PREVALENCE

Transmission appears to occur more readily if the recipient is immunocompromised or has another comorbidity, such as diabetes. In the largest outbreak described thus far, 23 patients with laboratory-confirmed infection were followed in Al-Ahsa governorate in Saudi Arabia (3). Diabetes mellitus (74%), end-stage renal disease (52%), and lung disease (43%) were underlying illnesses in these patients. Transmission to family members and health care workers was documented in 1 to 2% of contacts, again demonstrating preferential infection of individuals with substantial comorbidities. Unlike another human respiratory coronavirus, the one that caused the severe acute respiratory syndrome (SARS) in 2002–2003, MERS-CoV has not preferentially infected health care workers. At present, it is not known if patients are able to transmit virus before the development of symptomatic respiratory disease. If this does occur, control of a large outbreak will be more difficult. The SARS epidemic was contained, in part, because

the majority of patients were infectious only after they developed pneumonia.

One year after MERS first came to light, all cases have been found to have a Middle East connection, with approximately 70% occurring in Saudi Arabia. Within Saudi Arabia, the first cases were recognized in the Al-Ahsa governorate, an area located in the eastern part of Saudi Arabia, which remains the epicenter of the outbreak. Cases have been identified in the United Kingdom, France, Germany, Italy, and Tunisia, in all instances in patients with a history of recent travel to the Middle East (Fig. 1). Global travel is extremely common, so it may be just a matter of time before MERS-CoV cases are identified on all continents. The Centers for Disease Control and Prevention has published definitions of confirmed and probable cases, which provides a uniform approach to evaluating patients with suspected disease (<http://www.cdc.gov/coronavirus/mers/case-def.html>).

One important caveat is that we do not know the extent of the infection within the wider community. Since most identified patients have underlying diseases, it is possible that MERS-CoV is a common infection, at least in Saudi Arabia, and that patients without significant comorbidities develop a mild respiratory disease or remain asymptomatic. Careful epidemiological studies, which are required to address this question, are hampered by the lack of validated diagnostic tools. Knowing the number of infected humans will provide critical information about the prevalence of MERS-CoV and about the likelihood of developing severe disease.

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Address correspondence to Stanley Perlman, Stanley-perlman@uiowa.edu.

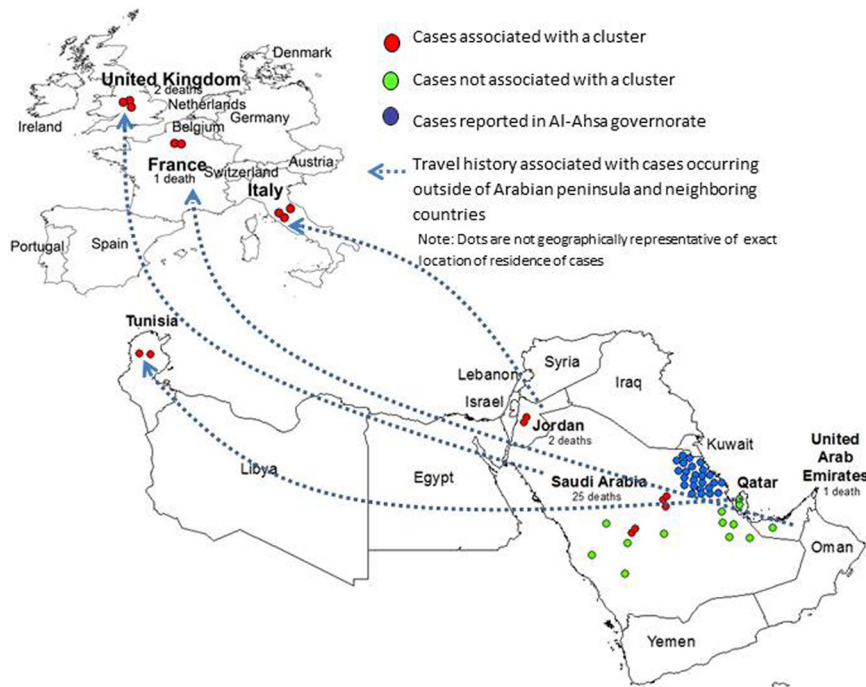


FIG 1 Map showing confirmed cases of MERS-CoV as of 7 June 2013 and place of likely exposure, based on travel history from the Arabian Peninsula or neighboring countries within 14 days of disease onset. Adapted from reference 23.

MERS-CoV IS A ZOONOTIC VIRUS

The genome of MERS-CoV was characterized within weeks of the isolation of the virus (4). Sequence analysis shows that it shares similarities with two previously identified betacoronaviruses, namely, BtCoV-HKU4, identified in *Tylonycteris* bats, and BtCoV-HKU5, present in *Pipistrellus* bats, but its closest neighbor is a betacoronavirus isolated from *Pipistrellus pipistrellus* bats in the Netherlands. During the 2002–2003 epidemic, SARS-CoV adapted to human populations to such an extent that it could no longer infect bat cells. (An alternative explanation is that we have not yet identified the bat species that actually served as the ultimate source for SARS-CoV and that cells from this species would be sensitive to SARS-CoV.) In contrast, MERS-CoV infects both human and bat cells, suggesting a possible direct bat-to-human route of transmission (5). However, since it is unlikely that most infected humans had direct contact with bats, it is more probable that another animal species common on the Arabian Peninsula, such as sheep, goats, cows, or even camels, serves as the direct source for infection. In support of the latter possibility, MERS-CoV neutralizing antibodies were detected in all dromedary camels sampled in Oman and even in a small percentage (14%) of camels in the Canary Islands (24). Identification of animals that serve as intermediate hosts would help define human populations at risk and might also allow culling or quarantine of infected animals, a method that was used successfully in Hong Kong to decrease the load of pathogenic avian influenza A virus in domestic poultry populations (6).

MERS-CoV IS A TYPICAL CORONAVIRUS

MERS-CoV is a large, positive-sense, single-stranded virus containing 30,119 nucleotides (4). The genome encodes both

nonstructural and structural proteins. Replicase-associated nonstructural proteins comprise two-thirds of the genome and are translated into a large polypeptide that is then cleaved into 16 proteins. These proteins are conserved in all coronaviruses, and partly as a consequence of the intense research efforts addressed at understanding SARS-CoV, the structure and function of many of these proteins are known. The structural proteins encoded at the 3' end of the genome are the same as those found in other coronaviruses and include a nucleocapsid protein, a spike glycoprotein essential for cell entry, and two membrane proteins involved in virus assembly and structure.

Interspersed between and within these four structural proteins are five accessory proteins that are unique to MERS-CoV. Accessory proteins are encoded by all coronaviruses, are not essential for virus replication, may be structural or nonstructural, and can be deleted without affecting pathogenesis (for examples, see references 7 and 8). A few are apparently involved in facilitating virus assembly or in immunoevasion, but the functions of the others are not known (9). MERS-CoV

accessory proteins share no homology with any known host or virus protein, other than those of the closely related BtCoV-HKU4 and -HKU5 strains of bat coronavirus (4).

THE HOST CELL RECEPTOR FOR MERS-CoV IS DIPEPTIDYL PEPTIDASE 4

Remarkably, the identity of the MERS-CoV receptor, dipeptidyl peptidase 4 (DPP4), was published only 6 months after the virus was first reported in the literature (10). Both bat and human DPP4 sensitize resistant cells for infection, supporting the notion that virus could cross species from bats to infect humans (5). DPP4, the receptor, is an ectopeptidase. Similar molecules are also used by other coronaviruses to enter cells, including human angiotensin-converting enzyme 2 (hACE2), which is used by SARS-CoV and HCoV-NL63, and aminopeptidase N (APN), which is used by several alphacoronaviruses. The catalytic sites of hACE2, APN, and likely DPP4 are not required for virus entry (10). Intriguingly, both hACE2 and DPP4 are shed from the cell surface (11, 12). Loss of hACE2 results in more severe pulmonary disease, while DPP4 is a neutrophil chemorepellent (13). Changes in DPP4 shedding that occur as a result of infection with MERS-CoV could influence the composition of the immune cell infiltrate, thereby ultimately affecting the outcome of the infection. DPP4 is also expressed on immune cells, including T cell lymphocytes (12), and is required for optimal function of these cells, but there is no evidence yet to suggest that MERS-CoV infection of lymphocytes occurs or plays a major role in pathogenesis. Curiously, CEACAM-1, the host cell receptor for mouse hepatitis virus (MHV), a murine coronavirus, is also expressed on T cells and is involved in signaling (14), but MHV does not infect T lymphocytes.

Events occurring immediately after MERS-CoV binds its DPP4

receptor appear to be similar to those reported for other coronaviruses. Cleavage of the surface glycoprotein must occur to expose the fusion peptide and to effect virus-cell membrane fusion and nucleocapsid release into the cytoplasm. Several host proteases, including cathepsin B and members of the TMPRSS family, have been implicated in this process (15).

MERS-CoV DOES NOT INDUCE IFN BUT IS MORE SUSCEPTIBLE THAN SARS-CoV TO IFN TREATMENT

A notable feature of the MERS-CoV infection is that the virus does not appear to induce an interferon (IFN) or proinflammatory innate immune response in primary human airway epithelial cells or in cultured cells (16, 17). A relative lack of IFN induction is a common feature of all coronavirus infections and may reflect both specific virus-mediated inhibition of cytoplasmic RIG-I-like sensing pathways and general evasion of cytoplasmic sensors via sequestration of viral macromolecular proteins inside membranous structures. In any case, this feature may allow MERS-CoV replication in the host prior to initiation of the immune response, contributing to enhanced virus levels and poor outcomes. On the other hand, MERS-CoV is more sensitive to IFN treatment than are other coronaviruses, such as SARS-CoV, suggesting a possible therapeutic intervention in infected patients (16, 17).

PRIMATES ARE THE ONLY NONHUMANS THAT CAN BE EXPERIMENTALLY INFECTED WITH MERS-CoV

The number of MERS-CoV-infected individuals is low, the availability of clinical samples is limited, and no autopsies have been reported. It is therefore crucial to begin development of an animal model for MERS, but thus far, experimental infection has been reported only in rhesus macaques (18). MERS-CoV-infected macaques develop a nonfatal mild pneumonia. The absence of severe respiratory disease and kidney disease in these nonhuman primates makes it imperative that additional animal models be developed. Thus far, there have been no reports of successful infection of mice or ferrets, but infection of these animals may be initiated or enhanced if the receptor for the virus, human DPP4, is expressed in lieu of the mouse protein. Notably, similar efforts to introduce the human receptor for SARS-CoV resulted in a transgenic mouse that developed an overwhelming neuronal infection (19, 20). These mice were useful for studies of vaccines and antiviral therapies but not for studies of pathogenesis. As mice engineered to express human DPP4 are developed, it will be important to minimize the likelihood of brain infection by careful attention to tissue-specific expression.

WHERE DO WE STAND NOW?

One year after the first case was identified, the total number of MERS cases remains low, with fewer than 100 cases reported. However, this is no reason for complacency. First, the virus has already spread from Saudi Arabia to Europe and is likely to spread throughout the world as infected patients travel for medical and other reasons.

Second, human-to-human transmission, initially considered to be a minor component of the disease process, has been documented in family and hospital settings. Early reports suggested that virus spread primarily to patients with underlying medical conditions, but recent documentation of spread to healthy persons, including health care workers, means that wider populations are at risk. This is a particular concern in Saudi Arabia, which is

both the primary site of MERS infection and the destination for the Hajj, the yearly pilgrimage in which several million Muslims travel to Mecca. Last year's Hajj did not result in an increase in MERS cases, and it is some comfort that Mecca is located across the country from the governorate of Al-Ahsa. However, large numbers of individuals will again make the Hajj this year in mid-October, a scenario that creates a substantial risk for widespread infection.

Third, like other coronaviruses, MERS-CoV may mutate to better adapt to human populations. Coronaviruses, unlike other RNA viruses, possess proofreading capabilities that enhance genome fidelity (21). However, the 2002-2003 epidemic made it clear that the SARS virus was able to rapidly adapt to infection of human populations (22). Mutations were detected throughout the SARS virus genome, but they appear to be most concentrated in the region of the surface glycoprotein involved in binding to the hACE2 molecule. There is every reason to suspect that MERS-CoV will undergo similar mutations that enhance both transmissibility between humans and replication within infected individuals.

WHAT ARE THE NEXT STEPS?

At this point, while the number of infected individuals is low, it is important to determine the extent and source of the infection. This information not only will provide a framework for establishing the relative importance of the disease and the potential for epidemic spread, but it may also facilitate direct interventions to eliminate reservoirs of infection. These analyses will require well-validated diagnostic reagents and access to human and animal blood samples in Al-Ahsa governorate in Saudi Arabia and elsewhere in the Middle East.

A second major goal will be to better understand the unique features of MERS. Does the high mortality rate reflect infection primarily of patients with substantial comorbidities? Does lack of recognition by host innate immune sensors result in high levels of virus in the lung and a dysregulated immune response, as occurred in patients with SARS? Does MERS-CoV inhibit interferon induction by novel mechanisms not utilized by other coronaviruses? Do the accessory proteins have novel mechanisms of action?

Third, development of a useful animal model that reproduces some of the features of the human infection will be critical for testing antiviral therapies and vaccines. A fourth goal will be to develop MERS-CoV-specific drugs and vaccines. The public health community learned a great deal from the SARS epidemic about approaches to treatment and prevention that might be effective against MERS-CoV.

Arguably, the most important outcome will be the development of drugs or vaccines that target a broad array of coronaviruses and not just a single virus. Events over the past 10 years show that coronaviruses are not only widespread in nature but also able to cross species to infect new hosts and can cause severe disease in humans. Having tools in hand before such events occur is critical for public health.

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