Advancing Priority Research on the Middle East Respiratory Syndrome Coronavirus

David S. Hui[1] and Alimuddin Zumla[2]

[1]Division of Respiratory Medicine and Stanley Ho Center for Emerging Infectious Diseases, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong; and [2]Department of Infection, Division of Infection and Immunity, Centre for Clinical Microbiology, University College London, and NIHR Biomedical Research Centre, University College London Hospitals, London, United Kingdom

(See the brief report by Aburizaiza et al on pages 243–6, and the major article by Yao et al on pages 236–42.)

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Over a year since its first discovery, a new human disease, the Middle East Respiratory Syndrome (MERS), continues to be of major international concern due to its high fatality rate and lack of knowledge regarding its primary source and mode of transmission. It is caused by a novel coronavirus (CoV) MERS-CoV, initially named 2cEMC/2012 (HCoV-EMC) [1] and subsequently renamed as MERS-CoV [2] after international consensus [3]. It presents as a spectrum of respiratory diseases and is associated with a high case-fatality rate in persons with comorbid medical conditions [4, 5]. The first MERS case report was from Jeddah, Kingdom of Saudi Arabia (KSA), in September 2012 when MERS-CoV was isolated from a Saudi Arabian patient who died from a severe respiratory illness and multiorgan failure [2]. As of 15 November 2013, there have been 153 laboratory-confirmed cases of MERS, with 64 deaths (42% case-fatality rate), reported from 10 countries to the World Health Organization (WHO) [6, 7]. All cases were linked directly or indirectly to 1 of 6 countries in the Middle East: KSA, Qatar, Jordan, United Arab Emirates (UAE), Oman and Tunisia. Five countries outside the Middle East—the United Kingdom, France, Italy, Germany, and Tunisia—have reported patients who were either transferred for care or returned from a visit to the Middle East and subsequently became ill. Four of these countries—Italy, France, Tunisia, and the United Kingdom—have had secondary cases linked to the initial imported case [6, 7]. The majority of MERS-CoV cases to date (127 out of 153 cases) have been reported from KSA, occurring as family [8] or hospital [5] clusters, sporadic community cases, or detected with mild disease or asymptomatic infection on screening of healthcare workers who were in contact with MERS cases [9]. Human-to-human transmission of MERS-CoV has been well documented in KSA [5, 10], England [11], France [12], Tunisia, and Italy [6, 12]. The clusters detected so far are mostly small and there have been no reports of sustained transmission of MERS-CoV within the community.

Despite several multicountry collaborative research efforts with the government of KSA to define the demographic, clinical features, mode of transmission, and epidemiology of family and hospital clusters [4–10], several important priority research questions remain unanswered. It is unclear what the primary source and primary mode of transmission of MERS-CoV to humans is—critical information that is essential for developing interventions for reducing the risk of transmission, defining the epidemiology, and developing effective control measures. The cellular receptor for MERS-CoV has been identified as dipeptidyl peptidase 4 (DPP-4 or CD26) [11], and the structure of the receptor-binding domain of the virus spike protein complexed with DPP-4 was rapidly identified [13]. The receptor is conserved across mammals, suggesting several animal hosts, although no definitive animal reservoir for MERS-CoV has been identified. Studies of MERS-CoV genomes from MERS cases suggest the existence of a direct animal reservoir for MERS-CoV [10].

Bats are usual suspects for transmission of coronaviruses. A recent study [14] identified a small 190-nucleotide sequence of MERS-CoV, with maximum possibility of identity, in a fecal sample from an Egyptian tomb bat. Serological studies in animals have detected antibodies against the spike protein of betacoronaviruses [15], and this finding has led

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Correspondence: Alimuddin Zumla, MD, PhD, FRCP, Professor and Director, Infection and Immunity, University College London Medical School, Ctr. for Infectious Diseases and Intl. H., UCL Windeyer Institute, Room G41, 46 Cleveland St, London W1T 4JF, United Kingdom (a.zumla@ucl.ac.uk).

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researchers to conclude that MERS has, at some time, passed into camels [16]. Support for this theory comes from detection of anti-MERS-CoV antibodies in camels in Oman and Spain [17]. The authors of that study concluded that MERS-CoV or a virus very similar to MERS-CoV has widely circulated among camels in Oman. However, this does not provide definitive proof that camels are a source of MERS-CoV because viral nucleic acid tests of serum and fecal samples did not reveal the presence of MERS-CoV or viral particles. Furthermore, only one MERS case has been reported from Oman, which borders KSA. Coronaviruses constitute a large family of viruses that may cause a range of illnesses in humans, as well as a number of diseases in a variety of animals. Previous studies have shown, for example, that coronaviruses can cause severe symptoms in newborn camels [18], and may be likely that cross-reactive antibodies with a related coronavirus to MERS-CoV occurred. Further research is required to identify the specific source or reservoir of MERS-CoV, any other intermediate animal host(s) or other inanimate food source, and the mode of transmission to humans.

Despite these studies indicating the presence of anti-MERS-CoV antibodies to be widespread within animal populations in the Middle East, human studies have not shown widespread infection in local populations. The article by Asad Aburizazaiza and colleagues [19] in this issue of *The Journal of Infectious Diseases* contains data from a study where a staged approach utilizing an immunofluorescence assay (IFA), differential recombinant IFA, and a plaque reduction serum neutralization assay was used to detect MERS-CoV antibodies in serum samples from 130 blood donors and 226 abbatoir workers in Jeddah and Makkah during the 2012 Hajj pilgrimage. They conclude that there was no evidence of MERS-CoV circulation in the region, and suggest that a large percentage of the population is considered nonimmune. Although the data appear concordant with the apparent absence of MERS-CoV when screened by reverse-transcription polymerase chain reaction in 154 French pilgrims returning from the 2012 Hajj [20], these antibody studies do not provide proof of absence or presence of MERS-CoV because of limitations imposed by the restricted study design and small numbers studied. Because several groups have developed a range of serological tests for detecting MERS-CoV, there is a need to independently evaluate and validate the sensitivity and specificity of these assays against a blinded panel of serum samples from known positive and negative MERS cases, and against other tests that can identify the presence of MERS-CoV–specific nucleic acids. The availability of accurate, validated, sensitive, and specific serological tests is essential for conducting case-control studies, which are crucial to accurately defining the epidemiology and the potential impact of the MERS-CoV outbreak, and for surveillance purposes.

Many important questions about MERS-CoV remain unanswered [4]. The natural history, pathogenesis, host susceptibility factors, viral virulence, viral kinetics, periods of infectiousness, underlying mechanisms of protective immunity, optimal treatments, and factors governing treatment outcome remain unclear. Absence of these basic data is hindering the development of drug treatment, adjunct therapies, specific diagnostics, biomarkers, and vaccines. Whereas an animal source of the virus appears the most likely source, the route of transmission could be either direct or indirect contact, or the consumption of a contaminated food or food product. Available data indicate that MERS-CoV has not yet readily adapted to infecting humans, and human-to-human transmission is not sufficient or efficient for pandemic potential [21]. Despite extensive investigations and screening testing of contacts of MERS cases in KSA, only a few instances of transmission have been identified in healthcare workers [9]. Postmortem and histological studies have not been available, and introducing these, even noninvasive autopsies [22], would help advance the scientific knowledge base.

The availability of an animal model of MERS-CoV infection and disease is essential for understanding the pathogenesis, natural history, and immune responses and for developing effective therapies. In this issue of *The Journal*, Tanfeng Yao and colleagues [23] describe an animal model of MERS which they produced by using intratracheal infection of Rhesus macaque monkeys with MERS-CoV, resulting in the development of pneumonia, and showed MERS-CoV replication was largely restricted to the lower-respiratory tract. The infected monkeys showed clinical signs of disease, virus replication, histological changes, and neutralizing antibody production. Another recent study of a Rhesus macaque monkey model of MERS-CoV infection has shown similar findings [24]. Using a combination of intratracheal, ocular, oral, and intranasal inoculation with 7×10^6 50% tissue culture infectious dose of the MERS-CoV isolate HCoV-EMC/2012, the monkeys developed a transient lower-respiratory-tract infection. Clinical signs, virus shedding, virus replication in respiratory tissues, gene expression, and cytokine and chemokine profiles peaked early in infection and decreased over time. MERS-CoV caused a mild to marked multifocal interstitial pneumonia, with MERS-CoV replication occurring mainly in alveolar pneumocytes. This tropism of MERS-CoV for the lower-respiratory tract may explain the severity of the disease observed in humans and the limited human-to-human transmission.

The MERS-CoV rhesus macaque model will be instrumental in developing and testing vaccine and treatment options for an emerging viral pathogen with pandemic potential. Specific therapeutic interventions for MERS-CoV are not available and have not been clinically evaluated. Current patient management relies exclusively on supportive care, which, given the high case-fatality rate recorded so far [4], is not highly effective. Empiric treatment with antiviral drugs or drug regimens, or

immune therapies (which were used for severe acute respiratory syndrome [SARS]) [25] require clinical evaluation. A recent study [26] indicates that a 2-drug combination may be effective against MERS-CoV. Using small compound-based forward chemical genetics to screen known drugs against influenza, and also interferons, nelfinavir, lopinavir, and nitazoxanide because of their reported anticoronavirus effects, the authors identified mycophenolic acid, ribavirin, and interferons as exhibiting in vitro anti-MERS-CoV activity, and showed that the antiviral effect of interferon-β-1b was stronger than that of ribavirin. Using the Rhesus macaque monkey model for MERS-CoV infection, Falzarano et al [27] showed that treatment with IFN-α2b and ribavirin reduced virus replication, moderated the host response, and improved clinical outcome. Clinical evaluation of IFN-α2b and ribavirin should be considered for severe cases of MERS. Other treatment options for MERS-CoV that require further investigation include the cyclophilin inhibitors [28, 29] and convalescent plasma [30] from patients who have fully recovered from MERS-CoV. Convalescent plasma and related hyperimmune globulin may have had some apparent success during SARS [31] and during the influenza pandemic due to the 2009 influenza A (H1N1) virus [32].

With the current knowledge gaps, it is unknown whether MERS-CoV will remain a disease restricted to the Middle East with intermittent, sporadic outbreaks; progress to becoming a global pandemic; or burn out with time. Many priority research questions remain to be answered before the true pandemic potential and global impact of MERS-CoV can be accurately determined. Almost all patients who died or those who have been hospitalized with severe disease had other comorbid medical conditions [4]. The mortality rate and severity of disease are exaggerated to some degree by detection of such cases. The case-fatality rate has fallen in recent months due to the detection of milder and asymptomatic cases [7]. Determining the true spectrum of MERS-CoV infection and disease severity requires widespread viral testing, collection of clinical data, and serologic studies. Case-control studies are essential for defining the MERS-CoV outbreak, and validated accurate serological tests, which are sensitive and specific, are required to facilitate these. The most ominous characteristic of pandemic MERS-CoV strains would be progression to efficient human-to-human transmission. The number of sporadic MERS cases being reported has been small and indicates that the virus appears not readily capable of rapid human-to-human transmission. Despite extensive investigation and testing of hundreds of contacts by the KSA Ministry of health, only a few instances of transmission to healthcare workers or family contacts were identified [6, 7, 9]. Sequencing studies of all MERS-CoV genomes may reveal genetic features that will tell us if MERS-CoV has the ability to mutate and spread efficiently. The rapid sharing of genetic sequence information [10, 33] will provide valuable insights into the understanding of the molecular characteristics and transmission dynamics, which will assist in defining species specificity, ascertaining mutation rates and virulence, and also enabling discovery of drug targets, novel drugs, diagnostics, and vaccines.

Two million pilgrims from over 180 countries, and 1 million local KSA pilgrims, have recently visited Makkah and Madinah, KSA, to perform the 2013 annual Hajj pilgrimage, and have returned home after stays of between 2 and 8 weeks. Millions of others will visit KSA throughout the year for the mini-pilgrimage Umrah. While answers to priority research on MERS-CoV are being sought, the need for more coordinated surveillance and improved effective international cooperation between WHO, Middle Eastern governments, academic stakeholders, and pharmaceutical companies remains critical to tackling this ominous threat [34]. It is rather disconcerting that major knowledge gaps remain for the current MERS-CoV outbreak over a year after its first discovery. Once again, this illustrates that there remains a dire need for the establishment of robust public health and clinical infrastructures, effective global consortia, and a stable funding source for rapid and effective definition of new infectious diseases outbreaks and threats, and for prioritizing research, preparedness, and response efforts.

Note

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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