

TITLE:

The emergence of the Middle East Respiratory Syndrome coronavirus

ABSTRACT:

On September 20, 2012, a Saudi Arabian physician reported the isolation of a novel coronavirus from a patient with pneumonia on ProMED-mail. Within a few days the same virus was detected in a Qatari patient receiving intensive care in a London hospital, a situation reminiscent of the role air travel played in the spread of Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) in 2002. SARS-CoV originated in China's Guangdong Province and affected more than 8000 patients in 26 countries before it was contained six months later. Over a year after the emergence of this novel coronavirus—Middle East Respiratory Syndrome coronavirus (MERS-CoV)—it has caused 178 laboratory confirmed cases and 76 deaths. The emergence of a second highly pathogenic coronavirus within a decade highlights the importance of a coordinated global response incorporating reservoir surveillance, high-containment capacity with fundamental and applied research programs, and dependable communication pathways to ensure outbreak containment. Here we review the current state of knowledge on the epidemiology, ecology, molecular biology, clinical features and intervention strategies of the novel coronavirus, MERS-CoV.

Introduction:

Coronaviruses (family Coronaviridae, subfamily Coronavirinae) circulate in a diverse array of mammalian and avian reservoirs, including humans, bats, pigs, cats, dogs, rodents, and birds (Perlman & Netland, 2009). Coronaviruses (CoV) are classified into four genera (Alpha-, Beta-, Gamma-, and Deltacoronavirus) and are enveloped, positive-strand RNA viruses between 70 and 120 nm in size (Masters, 2006; de Groot, 2012). The spike glycoproteins that radiate from the virus envelope of the spherical particles are responsible for the characteristic crown-like appearance of coronaviruses (Fig. 1).

Four coronaviruses continuously circulate in the human population, all of which cause generally mild respiratory disease: HCoV-229E, HCoV-NL63 (Alphacoronavirus), HCoV-OC43, and HKU1 (Betacoronavirus; Hamre & Procknow, 1966; McIntosh et al., 1967; Fouchier et al., 2004; van der Hoek et al., 2004; Woo et al., 2005). In addition, there have been two zoonotic introductions of coronaviruses into the human population over the last decade, both associated with acute respiratory distress syndrome (ARDS) and high case fatality rates: severe acute respiratory syndrome CoV (SARS-CoV; Drosten et al., 2003; Kuiken et al., 2003) and Middle East respiratory syndrome CoV (MERS-CoV; Zaki et al., 2012). SARS-CoV caused the first pandemic of the 21st century, resulting in c. 8400 human cases and an 11% case fatality rate (SARS Epidemiology Working Group, 2003). In addition to the impact of SARS-CoV on infected individuals and the global public health community, the economic cost of the SARS-CoV outbreak event was estimated at \$16 billion (Brahmbhatt & Dutta, 2008). Although only 163 laboratory-confirmed cases of MERS-CoV are currently reported, the high case fatality rate and travel-related spread across multiple countries are reminiscent of the SARS-CoV pandemic.

Virus detection and case definition :: Epidemiology of MERS-CoV:

The first human case of MERS-CoV was identified using a pan-coronavirus reverse-transcriptase polymerase chain reaction (RT-PCR) assay (Zaki et al., 2012). MERS-CoV-specific quantitative RT-PCRs (qRT-PCR), targeting the region upstream of the E protein gene and the open reading frame 1b, were rapidly developed and have become standards in the laboratory testing and diagnosis of MERS-CoV (Corman et al., 2012a). Additional qRT-PCRs targeting the RNA-dependent RNA polymerase (RdRp) and nucleocapsid (N) genes have been developed as confirmatory assays (Corman et al., 2012b). The World Health Organization (WHO) case definition for MERS-CoV focuses on patients suffering febrile acute respiratory disease who have a direct epidemiological link to another confirmed case or are residents of or travelers to MERS-CoV-source countries (WHO, 2013c). Confirmatory laboratory testing requires a positive qRT-PCR of at least two specific genomic targets or a single positive target by qRT-PCR combined with sequencing of a second target (Centers for Disease Control and Prevention, 2013). In instances of inadequate testing or negative tests, a patient with a direct epidemiologic link to a confirmed MERS-CoV case is determined to be a probable case of MERS-CoV infection if they present with acute febrile respiratory illness.

Spatial distribution and demographics ::: Epidemiology of MERS-CoV:

While primary cases of MERS-CoV have been confined to six countries in the Middle East – Saudi Arabia, United Arab Emirates, Qatar, Jordan, Oman and Kuwait – travel-related cases have been identified in Tunisia the United Kingdom, France, Germany, and Italy (Fig. 2; Bermingham et al., 2012; Buchholz et al., 2013; Gulland 2013a, b; Health Protection Agency, 2013; Hijawi et al., 2013; Mailles et al., 2013; Memish et al., 2013b; Puzelli et al., 2013). Limited secondary transmission occurred after MERS-CoV introduction in Tunisia, France, and the United Kingdom, while imported cases of MERS-CoV infection in Germany and Italy did not lead to subsequent confirmed infections (Buchholz et al., 2013; Gulland, 2013a, b; Health Protection Agency, 2013; Puzelli et al., 2013). Over 80% of cases of MERS-CoV have occurred in Saudi Arabia, largely within the Riyadh and Eastern provinces (Centers for Disease Control and Prevention, 2013). As of January 20, 2014, there were 178 confirmed cases of MERS-CoV, 76 (43%) of which were fatal (Fig. 3). Although most cases have been clinically severe, contact surveillance has uncovered at least 27 subclinical or mild infections (Centers for Disease Control and Prevention, 2013). The case fatality ratio of MERS-CoV (43%) is much higher than that of SARS-CoV (CFR 11%) (SARS Epidemiology Working Group, 2003). The average age of MERS-CoV cases is 52 years, with a male-to-female ratio of 1.6–1 (The WHO MERS-CoV Research Group, 2013). Both the case fatality ratio and the male-to-female ratio have decreased as the incidence of MERS-CoV has increased, changes that may be attributed to improved case surveillance (Penttinen et al., 2013). Interestingly, over three quarters of MERS-CoV cases have occurred in patients with comorbidities (The WHO MERS-CoV Research Group, 2013). The most common comorbidities for MERS-CoV cases have been diabetes, hypertension, obesity, cancer, and chronic kidney, heart, and lung disease (Assiri et al., 2013a). While these comorbidities likely affect disease progression and outcome, the strong correlation of chronic disease and MERS-CoV may be biased by the high rate of these risk factors in the populations of the affected countries. In the Kingdom of Saudi Arabia, for instance, the prevalence of type-II diabetes across ages is 31.6%, the prevalence of obesity is 31.1% (Al-Daghri et al., 2011), and one quarter of adult males smoke (WHO, 2013a). Epidemiologic and pathogenesis studies will be necessary to discern how comorbidities impact susceptibility to, and progression of, MERS-CoV infection.

Patient care ::: MERS-CoV clinical features:

Clinical treatment for MERS-CoV patients has centered on intensive care. Drug treatment has consisted of broad-spectrum antibiotics and often oseltamivir, a drug targeting influenza A virus (Albarrak et al., 2012; Zaki et al., 2012; Assiri et al., 2013a; Drosten et al., 2013; Guery et al., 2013; Memish et al., 2013b; Omrani et al., 2013). Corticosteroids have been used for some patients, and antifungals were administered when necessary (Zaki et al., 2012; Assiri et al., 2013a; Drosten et al., 2013; Guery et al., 2013; Memish et al., 2013b; Omrani et al., 2013). Patients that progressed to severe acute respiratory distress were provided oxygen therapy, mechanical ventilation, or extracorporeal membrane oxygenation (Assiri et al., 2013b; Drosten et al., 2013; Guery et al., 2013; Health Protection Agency, 2013; Memish et al., 2013b; Omrani et al., 2013). Five of the 47 clinically described Saudi patients were treated with ribavirin, and one was given interferon- α (IFN- α); a few patients have also been infused with intravenous immunoglobulin (Assiri et al., 2013a). Few clinical case studies and no analyses of MERS-CoV patients receiving treatments such as ribavirin and immunoglobulin have been published.

Natural reservoir ::: The origin of MERS-CoV:

Rapid full-genome sequencing provided the first insight into the origin of MERS-CoV (van Boheemen et al., 2012; Cotten et al., 2013a). Phylogenetic analysis shows a close genetic relatedness between MERS-CoV and the group C Betacoronaviruses BtCoV-HKU4 and BtCoV-HKU5 detected in insectivorous bats (Woo et al., 2012), although molecular clock analyses suggest they are unlikely to be direct ancestors of MERS-CoV (Fig. 4; Lau et al., 2013). Since MERS-CoV's identification in 2012, closely related coronavirus sequences have been detected in bats in Africa, Asia, the Americas, and Eurasia, suggesting a widespread circulation of MERS-CoV-related viruses in the order Chiroptera (Annan et al., 2013; Anthony et al., 2013; De Benedictis et al., 2014; Ithete et al., 2013; Lelli et al., 2013; Wacharapluesadee et al., 2013). Investigations of samples from bats roosting in the vicinity of the first MERS-CoV case in Bisha, Saudi Arabia, revealed the presence of a 190-nucleotide RNA fragment with 100% match to the RdRp of MERS-CoV in the feces of an Egyptian tomb bat (*Taphozous perforatus*; Memish et al.,

2013c). Unfortunately, the short length of MERS-like CoV sequences identified in bats limits the strength of phylogenetic analyses and subsequent conclusions about the origin of MERS-CoV.

Intermediate host ::: The origin of MERS-CoV:

Direct contact between humans and bats is limited, and an intermediate species often plays a role in the transmission of emerging viruses from bats to humans (Field et al., 2001; Luo et al., 2003; Mahalingam et al., 2012; Nel, 2013). Anecdotal evidence of MERS-CoV patient contact with farm animals has been reported in a few cases (Albarak et al., 2012; Buchholz et al., 2013; Drosten et al., 2013; The WHO MERS-CoV Research Group, 2013), and so suspicions about the potential source of MERS-CoV have focused on livestock common to the Arabian Peninsula, such as goats, sheep, dromedary camels, and cows. The first evidence for the existence of an intermediate animal reservoir was the detection of MERS-CoV neutralizing antibodies in dromedary camels from Oman and the Canary Islands (Spain) (Reusken et al., 2013b). Subsequent studies have detected MERS-CoV neutralizing antibodies in dromedary camels from Egypt, Jordan, Saudi Arabia, and importantly, in camel serum collected in 2003 from the United Arab Emirates (Hemida et al., 2013; Perera et al., 2013; Reusken et al., 2013a; Meyer et al., 2014). While MERS-CoV neutralizing antibodies were not detected in any other species of livestock tested, including chickens, goats, sheep, and cattle, seropositivity among camels passed 90% in every location, even in 2003 (Hemida et al., 2013; Perera et al., 2013; Reusken et al., 2013a, b; Meyer et al., 2014). The high prevalence of neutralizing antibodies across age grades suggests pervasive and early infection of camels with MERS-CoV or a MERS-CoV-like virus. Recently, MERS-CoV virus was detected by RT-PCR in nose swabs from three camels in Qatar (Haagmans et al., 2013). The camels were epidemiologically linked to two human cases of MERS-CoV, and viral fragments sequenced from the camels showed high similarity to sequences from the human cases (Haagmans et al., 2013). While this datum provides more conclusive evidence that dromedary camels form part of the MERS-CoV outbreak picture, the direction of transmission is still unclear (Fig. 5). Transmission could have occurred from camels to humans, humans to camels, or concurrently from a third source to both humans and camels (Haagmans et al., 2013). Furthermore, the detection of MERS-CoV neutralizing antibodies in dromedary camels in regions with no reported human cases, such as Egypt and the Canary Islands, raises questions about the extent of MERS-CoV or MERS-CoV-like virus circulation in Africa, the Arabic peninsula, and minor Asia (Perera et al., 2013; Reusken et al., 2013b). Movement of camels between Africa and the Arabian Peninsula is common and could contribute to the spread of MERS-CoV between regions (Mukasa-Mugerwa, 1981; Perera et al., 2013).

Transmission dynamics of MERS-CoV ::: Transmission of MERS-CoV:

Because of the epidemiologic dynamics described above, the basic reproduction number (R_0) of MERS-CoV is uncertain. R_0

is a measure of the number of secondary cases generated by one case of disease in a naïve population – an R_0

of < 1 is self-limiting within a population. Using the epidemiological information from 62 probable cases of MERS-CoV infection, two different transmission scenarios were used to estimate the R_0

for MERS-CoV (Breban et al., 2013). One scenario was modeled on a large number of index patients per cluster, each generating a small transmission tree, reflecting the possibility that two epidemiologically linked cases could have been exposed to the same nonhuman source of MERS-CoV. This scenario yielded an R_0

of 0.60 and a yearly MERS-CoV introduction rate of 22.3 (Breban et al., 2013). The second scenario, which used a lower rate of introduction with higher human-to-human transmission, predicted an R_0

of 0.67 and a yearly introduction rate of 17.1 (Breban et al., 2013). A large-scale analysis of full-genome sequences of MERS-CoV has identified multiple zoonotic introductions of the virus and distinct genomes circulating in the same geographic spaces, providing evidence for multiple zoonotic introductions and transmission dynamics in agreement with a lower R_0

(Cotten et al., 2013b). Furthermore, a comparison of the R_0

s predicted for MERS-CoV and prepandemic SARS-CoV (R_0

of 0.8) suggests MERS-CoV has low pandemic potential (Breban et al., 2013). Another transmission model of 111 MERS-CoV cases predicted a similar R_0

(0.63), but warned that in the absence of control measures, the R_0

0 could range between 0.8 and 1.3 and allow for self-sustaining transmission (Cauchemez et al., 2013). Based on extrapolations of the incidence of disease in travelers returning from MERS-CoV-source countries, it was predicted that as many as 940 symptomatic cases of MERS-CoV may have occurred before August 8, 2013 (Cauchemez et al., 2013). Epidemiologic studies will be necessary to assess the prevalence and circulation of MERS-CoV infection in the human population.

Receptor binding of MERS-CoV ::: MERS-CoV biology:

By granting binding of the virus to the host cell, cellular receptors play an important role in determining the species and tissue tropism of coronaviruses (Thackray & Holmes, 2004; Masters, 2006; Tusell et al., 2007). The MERS-CoV spike protein is a 1353 amino acid type-I transmembrane glycoprotein presented as a trimer on the surface of the enveloped virus. After translation, the spike protein is cleaved into two domains: the S1 subunit responsible for receptor binding and the S2 unit that mediates membrane fusion (Ohnuma et al., 2013; Raj et al., 2013). The S1 spike glycoprotein binds to the surface enzyme dipeptidyl peptidase 4 (DPP4, also known as CD26). DPP4 is a type-II transmembrane glycoprotein that catalyzes the cleavage of N-terminal proline-containing dipeptides and aids glucose metabolism by proteolytic inactivation of incretins (Engel et al., 2003; Hiramatsu et al., 2003; Lambeir et al., 2003). DPP4 is the third exopeptidase found to act as a receptor for coronaviruses (Raj et al., 2013). Blocking the enzymatic activity of DPP4 does not affect MERS-CoV susceptibility, and so the significance of these enzymes as receptors for coronaviruses is thought to lie in their widespread expression on endothelial and epithelial tissues (Raj et al., 2013). DPP4 is relatively conserved between mammalian species, allowing MERS-CoV to bind to species as diverse as bats and humans (Müller et al., 2012; Raj et al., 2013). The receptor-binding domain (RBD) of MERS-CoV, the region of the spike protein that attaches to the DPP4 receptor, has been mapped to 240 amino acid residues in the S1 region of the spike protein (Chen et al., 2013; Lu et al., 2013; Mou et al., 2013; Wang et al., 2013). Co-crystallization of MERS-CoV spike protein and DPP4 revealed an interaction between the beta-propeller blades 4 and 5 of DPP4 and beta-strands 5, 6, 7, and 8 of the MERS-CoV RBD, also known as the receptor-binding motif (RBM; Fig. 6; Lu et al., 2013; Wang et al., 2013). The RBD of the spike protein induces neutralizing antibodies, making it an important target for the development of prophylactics and therapeutics (Agnihothram et al., 2013; Du et al., 2013a,b,c; Gierer et al., 2013; Song et al., 2013).

Innate immunity ::: Host interaction of MERS-CoV:

Infection experiments in human airway epithelial cells have shown that the global transcriptional host response is earlier and more robust after MERS-CoV infection than after SARS-CoV infection (Josset et al., 2013). Yet consistent with observations of SARS-CoV infection, MERS-CoV infection of human respiratory cells does not lead to a pronounced type-I IFN response (Kopecky-Bromberg et al., 2007; Kindler et al., 2013; Zielecki et al., 2013). Type-I IFNs play a key role in viral immunity, and pathogenic viruses often evade innate immunity through antagonist proteins that disrupt the IFN reaction (García-Sastre & Biron, 2006; Randall & Goodbourn, 2008; Taylor & Mossman, 2013). In MERS-CoV, inhibition of type-I IFNs has been demonstrated by the structural M protein and the accessory proteins encoded by ORF 4a, ORF 4b, and ORF 5 (Yang et al., 2013). The most potent inhibitor, ORF 4a, has been shown to inhibit type-I IFN activation by blocking the interaction between the RNA helicase sensor MDA5 and viral double-stranded RNA, a mechanism distinct from that employed by SARS-CoV (Kopecky-Bromberg et al., 2007; Niemeyer et al., 2013; Yang et al., 2013).

Efficient MERS-CoV replication has also been demonstrated in nonciliated bronchial epithelium, alveolar epithelial cells, endothelial cells, and macrophages in human ex vivo organ cultures (Chan et al., 2013c; Zhou et al., 2013). Consistent with the results obtained from human respiratory cell lines, MERS-CoV infection ex vivo does not lead to a strong type-I IFN response in these cultures (Chan et al., 2013c; Zhou et al., 2013). The combined in vitro and ex vivo data suggest that MERS-CoV actively interacts with and evades innate immune recognition pathways by the host (Kindler et al., 2013).

Ex vivo data support the results of viral dissemination and pathology, and cellular tropism established in the rhesus macaque model (de Wit et al., 2013b). Gene expression analysis in experimentally infected rhesus macaques showed differentially expressed genes in infected lung tissue associated with antiviral immunity, inflammation, and chemotaxis, including IL-6, chemokine C-X-C ligand 1, and matrix metalloproteinase. As expected, type-I IFNs were not

activated (de Wit et al., 2013b). The chemokine IL-8, a strong recruiter of neutrophils and other granulocytes, was induced in the macaques, perhaps explaining the increased numbers of neutrophils recorded in the blood of infected macaques and some human patients (Zaki et al., 2012; Assiri et al., 2013a; Guery et al., 2013; de Wit et al., 2013b).

Public health measures ::: MERS-CoV intervention strategies:

Mathematical transmission models highlight two important ways MERS-CoV can be controlled: reducing the rate of MERS-CoV introductions into the human population and breaking chains of human-to-human transmission. The reduction in the rate of MERS-CoV introductions in the human population requires a comprehensive understanding of the nonhuman source of MERS-CoV and the spatial and temporal dynamics of MERS-CoV circulation in this source. On the other hand, interrupting the human-to-human transmission cycle of MERS-CoV calls for an understanding of the parameters involved in transmission, such as virus shedding, stability, and routes of transmission. Although routes of transmission for MERS-CoV are not well understood, the spread of MERS-CoV between people in close contact settings suggests that direct contact and fomite transmission routes are likely to be involved. As stated above, the localization of MERS-CoV infection in the lower respiratory tract implicates coughing and other exudates as important sources of virus shedding. In addition, the environmental stability of MERS-CoV provides some information on the potential for fomite transmission. At low temperatures and humidity, both MERS-CoV and SARS-CoV virions retain viability on smooth surfaces much longer than many other respiratory viruses, including influenza virus H1N1, HCoV-229E, and HCoV-OC43 (Sizun et al., 2000; Chan et al., 2011; van Doremalen et al., 2013). Thus, temperature-controlled settings such as hospitals may be of particular risk for fomite transmission of MERS-CoV. Performing high-risk patient care procedures such as intubation and manual ventilation, along with inconsistent use of surgical masks, was associated with nosocomial transmission of SARS-CoV to healthcare workers (Ofner-Agostini et al., 2006; Nishiyama et al., 2008). Healthcare workers are a growing cohort of MERS-CoV cases, but while routes of exposure may be similar for both viruses, the MERS-CoV outbreak has not been characterized by the hospital-based super-spreader events which defined the epidemiology of SARS-CoV (Lipsitch et al., 2003). During infection with SARS-CoV, viral load in upper respiratory tract secretions remained low for the first 5 days of illness, not peaking in nasopharyngeal aspirates until about 10 days after onset of symptoms (Peiris et al., 2003; Cheng et al., 2004). Thus, SARS-CoV transmission could be prevented in the general population by basic public health and infection control measures, such as the isolation of patients in negative-pressure rooms, active surveillance and quarantine of contacts, and the provision of education and protective equipment for healthcare workers (Twu et al., 2003; Svoboda et al., 2004). Estimates of the serial interval of MERS-CoV infection in the largest cluster of MERS-CoV to date – 23 cases in a hospital setting – are slightly shorter than those for SARS-CoV (median 7.6 vs. 8.4 days), suggesting that transmission may occur earlier in the course of illness (Assiri et al., 2013b). Effective implementation of public health measures against the current MERS-CoV outbreak must integrate knowledge of shedding dynamics, exposure mechanisms, and virus viability.

Therapeutics ::: MERS-CoV intervention strategies:

Antiviral treatment specific to human coronaviruses has not been developed, and during the short-lived 2002–2003 SARS-CoV outbreak, supportive treatment regimens were not optimized (Stockman et al., 2006; Cheng et al., 2013). Commercially available drugs, such as type-I IFNs, lopinavir, and, at very high concentrations, ribavirin, were shown to inhibit cytopathic effect of SARS-CoV in *in vitro* studies, with a synergistic antiviral effect described for type-I IFNs and ribavirin (Chen et al., 2004; Tan et al., 2004; Birgit et al., 2005). These drugs were used to varying extents on SARS-CoV patients, often in combination with corticosteroids (Stockman et al., 2006; Cheng et al., 2013). Ribavirin is a structural analog of guanosine with broad-spectrum mutagenic effects on viruses. Despite its frequent use on SARS-CoV patients, ribavirin was not shown to be effective for patients, and adverse effects such as hemolytic anemia were common (Stockman et al., 2006; Cheng et al., 2013). Type-I IFN regimens for SARS-CoV patients were sometimes used as part of a multi-drug regimen, and one study of 22 SARS-CoV patients reported more rapid improvement of radiographic lung pathologies and better oxygenation in patients treated with IFN- α 1 (Loutfy et al., 2003; Stockman et al., 2006; Cheng et al., 2013). Speculations that immunopathology rather than uncontrolled viral replication contributed to lung deterioration during advanced SARS-CoV infection favored the widespread use of corticosteroids in its treatment (Nicholls et al., 2003; Peiris et al., 2003; Hui & Sung, 2004; Cheng et al., 2013).

Retrospective analysis of SARS-CoV patients showed no benefit of corticosteroid administration except when critical cases were analyzed alone and death-related variables were adjusted. Among these critical patients, corticosteroid therapy did significantly reduce case fatality (Chen, 2006). Similarly, corticosteroids have been used in some MERS-CoV patients with severe disease (Assiri et al., 2013a; Guberina et al., 2013; Memish et al., 2013b; Omrani et al., 2013). One previously healthy MERS-CoV patient who suffered ARDS, multidrug-resistant *Pseudomonas aeruginosa*, and suspected concomitant allergic bronchopulmonary aspergillosis improved rapidly when treated with the corticosteroid prednisolone (Guberina et al., 2013). The efficacy of corticosteroid treatment for other MERS-CoV patients with ARDS has not been reported. Therapies used during the SARS-CoV outbreak have shaped investigations of treatment regimens for MERS-CoV. MERS-CoV shows greater sensitivity than SARS-CoV to the antiviral effects of type-I and type-III IFNs in vitro and ex vivo (Kindler et al., 2013; Wilde et al., 2013; Ziebeck et al., 2013). This greater sensitivity could be explained by mechanistic differences of IFN antagonist accessory proteins between the viruses. For instance, MERS-CoV lacks a homologue of the SARS-CoV ORF6 protein, which blocks the STAT1-activating effects of IFNs and ultimately the transcriptional activation of downstream antiviral genes (Wilde et al., 2013). A comparison of five different IFNs has shown IFN- β to be the most potent inhibitor of MERS-CoV (Hart et al., 2013). Although high concentrations of ribavirin were effective in vitro in one study (Falzarano et al., 2013a), another study found no inhibitory effects of ribavirin at a dose translatable to current drug regimens in humans (Hart et al., 2013). When IFN- α 2b and ribavirin were used in combination against MERS-CoV, however, the 50% effective concentrations (EC50) of the drugs in combination were much lower than for the individual drugs. This additive effect decreased the drug requirements to concentrations potentially achievable in humans (Falzarano et al., 2013a). Interestingly, ribavirin is significantly more effective when the ExoN activity of coronaviruses is knocked out (Smith et al., 2013). Data indicate that in these knockout viruses, ribavirin has a greater ability to inhibit viral RNA synthesis and inosine monophosphate dehydrogenase (IMPDH), an enzyme necessary for the de novo synthesis of guanine nucleotides, which would explain the relatively high ribavirin dose needed to achieve inhibition of viral replication with MERS-CoV (Smith et al., 2013).

In a study to identify compounds that inhibit MERS-CoV, mycophenolic acid demonstrated a particularly high efficacy against MERS-CoV, with an EC50 of $< 10 \mu\text{M}$ (Chan et al., 2013a,b,c). Mycophenolic acid is an approved drug and, like ribavirin, acts by inhibiting IMPDH. While typically used as an immunosuppressant after tissue transplants, its antiviral activity has been attributed to an inhibition of viral RNA replication (Diamond et al., 2002).

Preliminary in vitro comparisons suggest that mycophenolic acid is a more potent inhibitor of MERS-CoV than ribavirin (Chan et al., 2013a; Falzarano et al., 2013a; Hart et al., 2013). Because of this, effective plasma drug levels may be better achievable for intravenous doses of mycophenolic acid than ribavirin (Chan et al., 2013a). Assumptions about the in vivo usefulness of drugs effective in vitro need to be made with care. Mycophenolic acid, for instance, is a potent inhibitor of T and B lymphocytes and dendritic cell maturation, effectively suppressing antigen presentation and immunoglobulin production (Villarroel et al., 2009). In a well-characterized animal model, the adverse immunomodulatory effects of the drug may outweigh any reduction in MERS-CoV virulence.

Animals model for MERS-CoV :: MERS-CoV intervention strategies:

Attempts to establish a small animal model for MERS-CoV in Syrian hamsters, mice, and ferrets have been unsuccessful (Coleman et al., 2013; Raj et al., 2013a, b; de Wit et al., 2013a). However, experimental infection studies in rhesus macaques showed that this animal species was susceptible to MERS-CoV (Munster et al., 2013). In rhesus macaques, MERS-CoV causes a lower respiratory tract infection reminiscent of mild to moderate human cases (de Wit et al., 2013b). Clinical signs of MERS-CoV-infected macaques included cough and increased respiration rate, and lung samples showed lesions characteristic of mild to marked pneumonia (Munster et al., 2013; de Wit et al., 2013b). Virus was detected throughout the respiratory tract and mediastinal lymph nodes, with viral loads higher earlier during infection. The primary sites of virus replication were type-I and type-II pneumocytes, main components of the alveolar architecture. Replication of MERS-CoV deep in the macaque lower respiratory tract may explain the low potential for transmission of MERS-CoV. Despite renal failure in some human patients, virus was absent from the kidney tissue of all macaques (de Wit et al., 2013b). A more recent characterization of MERS-CoV in rhesus macaques reported transient fever in infected monkeys and demonstrated a MERS-CoV-specific antibody response in the macaques starting at 7 days postinfection (Yao et

al., 2013). The rhesus macaque model of MERS-CoV infection is the only in vivo model established to date, and development of a small animal model is essential to conduct widespread research on pathogenesis and prophylactic and therapeutic countermeasures.

In vivo testing of antivirals ::: MERS-CoV intervention strategies:

Of the antiviral and immune modulatory compounds shown effective against MERS-CoV in vitro, only one treatment option has been examined in vivo. The efficacy of a combination IFN- α 2b and ribavirin treatment regimen was tested in MERS-CoV-infected rhesus macaques (Falzarano et al., 2013b). Doses were designed to achieve serum concentrations at or above the EC50 values determined in vitro. Treated animals did not show the clinical signs or hematological changes that developed in the untreated animals, such as breathing difficulties, decreased oxygen saturation levels, and increased neutrophil counts (Falzarano et al., 2013b). Gross pathology of lungs from treated animals was normal, while untreated animals displayed visible lesions. Histopathology revealed mild signs of bronchiointerstitial pneumonia in the treated animals, with more abundant alveolar edema and severe lesions seen in the untreated animals. The MERS-CoV viral load in lung samples was 0.81 log lower for treated animals compared to untreated animals. Furthermore, a lung-specific host response occurred in untreated macaques but not in treated animals, with increased levels of the cytokines and chemokines IL-6, IFN- γ , and MCP-1. Reduced expression of inflammatory genes was observed in the lungs of treated animals (Falzarano et al., 2013b). The significant improvement in clinical score for treated animals despite relatively similar viral loads between the groups suggests that immunopathology may be a factor in the severity of MERS-CoV infection. However, because the rhesus macaque model only recapitulates mild to moderate disease in humans, the effectiveness of these drugs against MERS-CoV-induced ARDS is unclear. Timing is also critical to the efficacy of treatment; most patients do not begin treatment until they are quite ill, whereas drug regimens in the macaques began only 8 h postinoculation. The translation of drug regimens from the laboratory to the clinic must address such discrepancies.

Prophylactics ::: MERS-CoV intervention strategies:

In the years since the SARS-CoV outbreak, attempts have been made to prepare for reemergence by establishing vaccines, and the advent of MERS-CoV has only heightened the need for effective coronavirus vaccines. Inactivated viruses, live-attenuated viruses, DNA vaccines, virus-like particles, and viral vector-based vaccines have all been shown to produce neutralizing antibodies to SARS-CoV (Chen et al., 2005; Zhao et al., 2005; Graham et al., 2012; Tseng et al., 2012). Unfortunately, SARS-CoV causes pulmonary immunopathology upon challenge after vaccination in animal models, presumably because they induce an immune response skewed toward T helper 2 (TH2) cell responses (Tseng et al., 2012). While inactivated virus vaccines are particularly prone to inducing this type of TH2-related hypersensitivity, immunopathology upon challenge has been observed with SARS-CoV-based viral vector vaccines (Deming et al., 2006), virus-like particle vaccines (Tseng et al., 2012), and DNA vaccines (Zhao et al., 2005). Investigations of the antigenic and serologic relationships of MERS-CoV to other coronaviruses have confirmed that neutralizing antibodies target the spike protein of MERS-CoV and are specific to MERS-CoV, while the N protein induces antibodies cross-reactive within its coronavirus subgroup (Agnihotram et al., 2013). The spike protein, and especially its RBD, is considered a key component in coronavirus vaccine design (Du et al., 2009, 2013b,c). A replication-defective vaccinia virus-based vaccine expressing the full-length spike protein of MERS-CoV has been shown to produce high levels of neutralizing antibodies in mice, but virus challenge cannot be applied to the mouse model (Song et al., 2013). Vaccination with recombinant protein containing a truncated RBD of the spike protein (amino acids 377–588) and the Fc of human IgG also induced high titers of neutralizing antibodies. The truncated RBD elicited higher levels of neutralizing antibodies than vaccination with a recombinant protein containing a larger fragment of the spike protein (amino acids 377–662). The authors suggest that non-neutralizing epitopes within the 588–662 region of the polypeptide may compete with neutralizing epitopes or destabilize the formation of the RBD (Du et al., 2013b). To maximize the protectiveness of neutralizing antibodies across MERS-CoV strains, the natural variation of MERS-CoV spike proteins needs to be characterized and considered in the design of vaccines (Graham et al., 2013). Live-attenuated viruses are another approach to MERS-CoV vaccine development. A MERS-CoV mutant lacking the structural E protein has been shown to be replication-competent but propagation-defective (Almazán et al., 2013). Development of safe and effective MERS-CoV vaccines must potentially overcome the challenges that arose for SARS-CoV. No in vivo testing of vaccines has been

performed to date; it remains therefore unclear whether challenge with MERS-CoV would illicit pulmonary immunopathology upon challenge. Creative approaches, such as the use of adjuvants that promote TH1 cell responses, need to be pursued, and potential vaccines must be rigorously evaluated in animal models (Graham et al., 2013).

Future perspectives ::: Global response:

Despite many advances in our understanding of the MERS-CoV outbreak, major questions remain. The epidemiology of MERS-CoV is still poorly understood. Although dromedary camels have been implicated as the most likely intermediate reservoir, more details on the genetic variation of MERS-CoV viruses circulating in camels and humans are necessary to identify camels as the definitive source of human MERS-CoV infections. The widespread distribution of dromedary camels across Africa, the Arabic peninsula, and South-West Asia highlights their potential to facilitate the outbreak's spread (Fig. 7), but information on the spatial and temporal patterns of MERS-CoV circulation in this species is needed. Because zoonotic introductions continue to play a role in the epidemiology of MERS-CoV in humans (Cotten et al., 2013b), an elucidation of the mechanisms of zoonotic transmission is essential and intervention strategies should focus on controlling these events.

Our clinical understanding of MERS-CoV infection is based on limited reports (Albarrak et al., 2012; Bermingham et al., 2012; Assiri et al., 2013a; Drosten et al., 2013; Guery et al., 2013; Memish et al., 2013a; Omrani et al., 2013). Of particular interest is the effect of comorbidities on MERS-CoV pathogenesis and patient outcomes. Preliminary evidence shows a direct relation between underlying comorbidities and disease severity (Assiri et al., 2013a). The observation that primary cases have been more severe than secondary cases, even when controlling for underlying conditions, might suggest that these primary cases were exposed to higher virus doses than secondary cases (The WHO MERS-CoV Research Group, 2013). This could also indicate that MERS-CoV is more readily transmissible from the intermediate reservoir to humans than from human-to-human and that milder MERS-CoV cases would be less likely to efficiently transmit MERS-CoV. Human-to-human transmission could increase if MERS-CoV becomes better adapted to humans. Prolonged MERS-CoV replication in immune-compromised patients could increase opportunity for the virus to acquire mutations enabling efficient transmission. The development and testing of MERS-CoV therapeutics is currently hindered by the absence of small animal models. In addition, phase I-III clinical trials need to be conducted before experimental vaccines and treatment options can be available to humans. Accordingly, research focusing on already-approved drugs for the treatment of MERS-CoV with result in faster implementation and wider availability of therapeutics in the clinic.

The increase in emerging infectious disease events over the last decades has made it apparent that a more complete understanding of the ecology, biology, and political economy of infectious disease emergence is necessary. Globalization, climate change, habitat alteration, and wildlife encroachment likely contribute to novel interactions between pathogens and hosts. Adequate preparation for future infectious disease outbreaks requires strong international relationships in research, monitoring and surveillance, and public health response. In 2005, after facing the emergence of SARS-CoV and avian influenza H5N1, the WHO (2013b) developed International Health Regulations for the coordination of global responses to emerging health threats. The WHO has structured its MERS-CoV response according to these regulations, forming an emergency committee on MERS-CoV, creating case definitions of infection, and providing frequent updates of the MERS-CoV outbreak through the IHR's global alert and response function. The Program for Monitoring of Emerging Diseases (ProMED-mail), which communicated the first report of MERS-CoV infection in a human, has also continued to provide the global health and research communities with updates of epidemiological reports and scientific findings. Capacity building, knowledge transfer, and training of the future generation of scientists are key factors in forming multidisciplinary preparedness for future infectious disease outbreaks. In this regard, special attention should be given to prevention and control of emerging infectious diseases in the developing world, the origin of the majority of infectious disease outbreaks.