

MINIREVIEW

The emergence of the Middle East Respiratory Syndrome coronavirus

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Excellent review on timely and newly emerging infectious diseases.

Keywords

MERS-CoV; coronavirus; epidemiology; molecular biology; intervention strategies.

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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 6 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.

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

The first human case of MERS-CoV was identified using a pancoronavirus 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

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

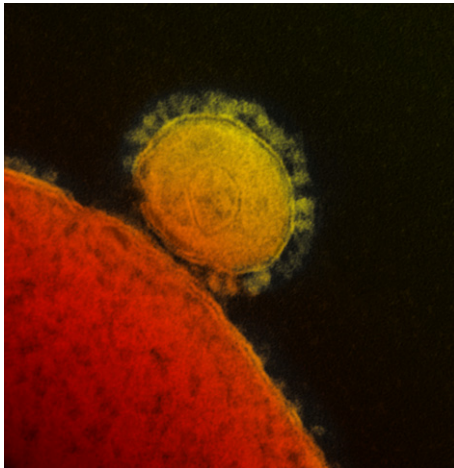


Fig. 1 False-color MERS-CoV particle visualized by electron microscopy. A MERS-CoV particle (yellow) attached to the surface of a cell (red). The characteristic MERS-CoV spike glycoproteins are clearly visible on the surface of the MERS-CoV particle.

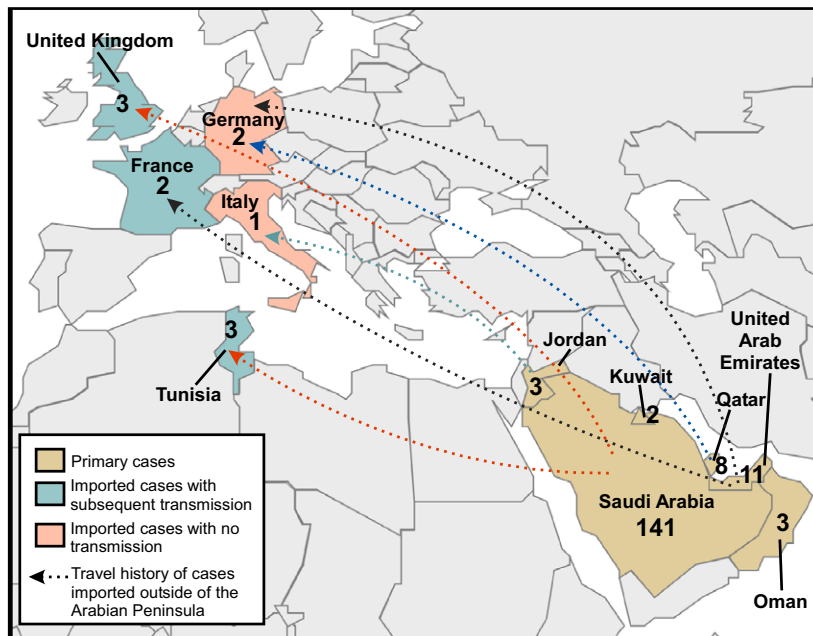
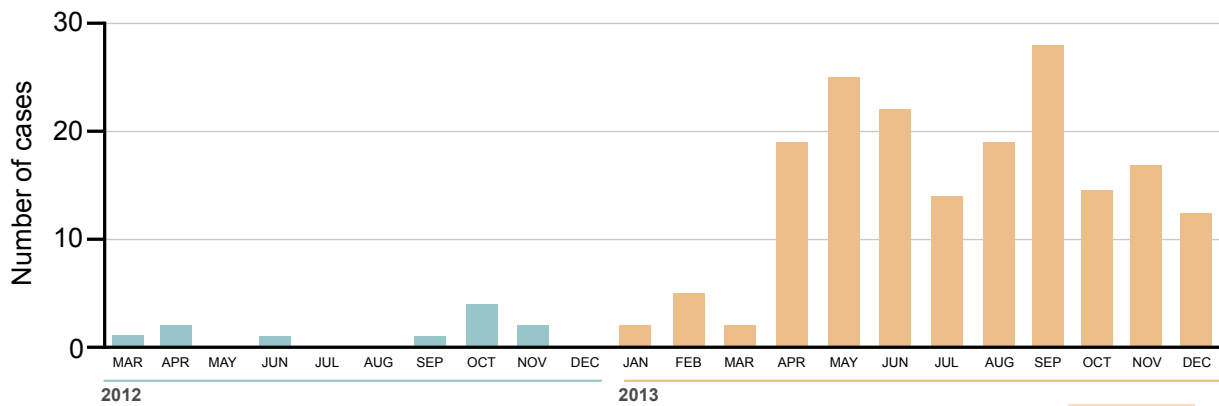
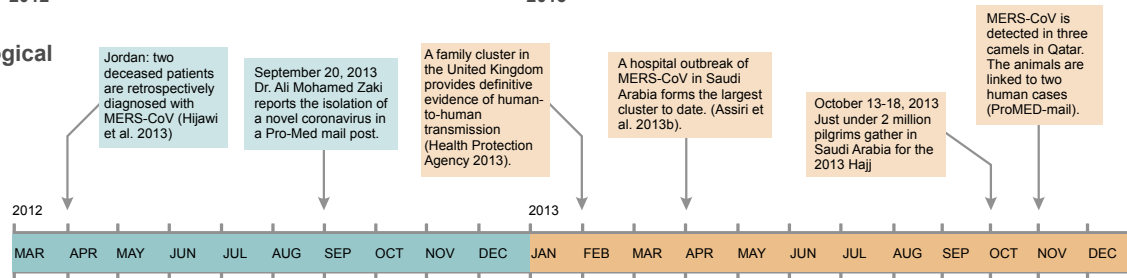


Fig. 2 Geographic distribution of the MERS-CoV outbreak. The geographic distribution of MERS-CoV cases up to February 1, 2014 is shown. Travel history of cases imported outside of the Arabian Peninsula is indicated with dotted arrows. Countries with primary MERS-CoV cases are shown in brown, countries with imported MERS-CoV cases and no confirmed human-to-human transmission are shown in pink, and countries with imported MERS-CoV cases and subsequent human-to-human transmission are shown in green.

MERS-CoV Cases by Date



Epidemiological Highlights



Scientific Highlights

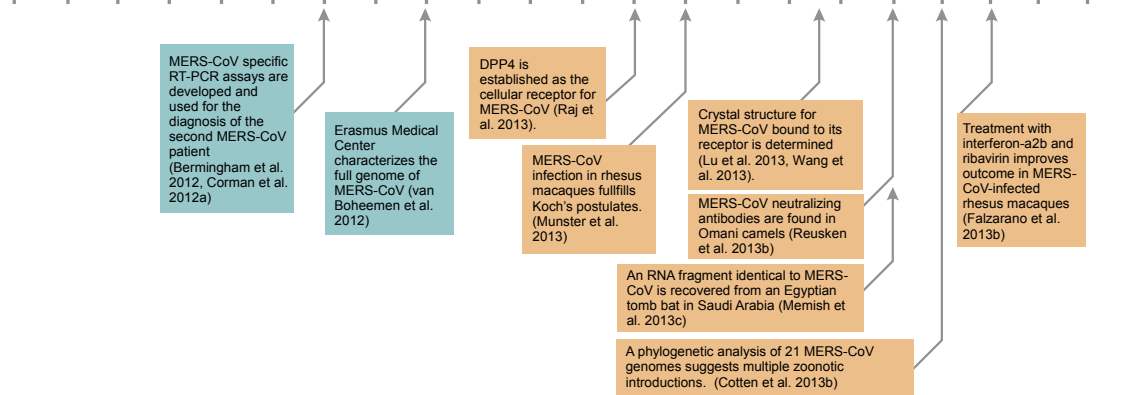


Fig. 3 Timeline of the MERS-CoV outbreak. Temporal distribution of MERS-CoV cases from March 2012 through December 2013. Significant outbreak events and scientific advances during this time period are highlighted below.

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.

MERS-CoV clinical features

The clinical manifestations of MERS-CoV range from subclinical infection to severe respiratory disease. Symptomatic patients often present with fever, myalgia, sore throat, shortness of breath, cough, and occasionally hemoptysis (Albarrak *et al.*, 2012; Assiri *et al.*, 2013a; Drosten *et al.*, 2013; Guberina *et al.*, 2013; Guery *et al.*, 2013; Health Protection Agency, 2013; Memish *et al.*, 2013b, c;

Puzelli *et al.*, 2013). Gastrointestinal symptoms such as diarrhea and vomiting are also common (Assiri *et al.*, 2013a; Guery *et al.*, 2013; Memish *et al.*, 2013b). Although coinfections have been limited, MERS-CoV coinfection with *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Candida* species, and influenza A virus (H1N1), rhinovirus, and herpes simplex virus type-1 has been reported (Zaki *et al.*, 2012; Assiri *et al.*, 2013a; Drosten *et al.*, 2013; Guery *et al.*, 2013; Health Protection Agency, 2013; Omrani *et al.*, 2013). All clinically described patients have shown abnormal chest radiographs with a spectrum of lower pulmonary infiltrates and consolidation consistent with viral pneumonia (Albarrak *et al.*, 2012; Zaki *et al.*, 2012; Assiri *et al.*, 2013a; Drosten *et al.*, 2013; Guberina *et al.*, 2013; Guery *et al.*, 2013; Memish *et al.*, 2013b). Over 60% of the first 144 MERS-CoV patients suffered severe disease, requiring intensive care such as mechanical ventilation and extracorporeal membrane oxygenation (The WHO MERS-CoV Research Group, 2013). Hematological abnormalities reported for clinical cases include thrombocytopenia (36%), lymphopenia (34%), lymphocytosis (11%), and neutrophilia (8%) (Assiri *et al.*, 2013a).

While kidney failure necessitating renal replacement therapy has been reported for a number of MERS-CoV cases (Albarrak *et al.*, 2012; Zaki *et al.*, 2012; Drosten *et al.*, 2013; Guberina *et al.*, 2013; Guery *et al.*, 2013; Memish *et al.*, 2013b; Omrani *et al.*, 2013), in the absence of postmortem examinations, there is no direct evidence of MERS-CoV replication in renal tissue. In many cases, renal involvement can be explained by pre-existing conditions, hemorrhagic complications, and organ stress due to extreme hypoxemia (Albarrak *et al.*, 2012; Drosten *et al.*, 2013; Guery *et al.*, 2013). Long-term sequelae of acute MERS-CoV have not yet been described.

Patient care

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.

The origin of MERS-CoV

Natural reservoir

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 perforates*; 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

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 (Albarrak *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

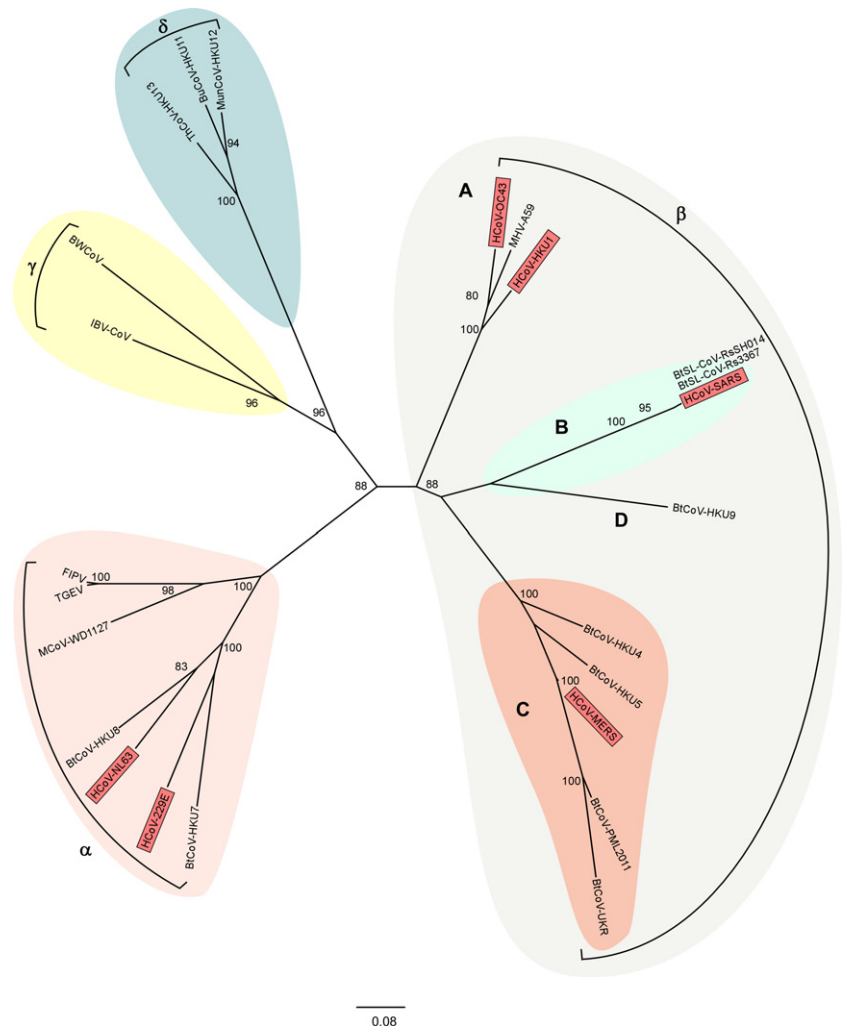


Fig. 4 Coronavirus phylogeny. Phylogenetic tree of coronaviruses with representatives of each of the four genera; *Alpha* (pink), *Beta* (gray), *Delta* (blue), and *Gammacoronavirus* (yellow). *Betacoronaviruses* are further subdivided into clades A through D, with clade B (green) containing SARS-CoV and clade C (orange) containing MERS-CoV. All known human coronaviruses are represented in red. Maximum-likelihood trees were generated with the MEGA5 software package using a 1231-nucleotide segment within the RdRp. Trees were visualized using Figtree. Bootstrap values above 75 are shown. CoV isolation origin abbreviations as follows: H: human; Bt: bat; BtSL: bat SARS-like; BW: beluga whale; IBV: chicken; FIPV: feline; TGEV: swine; M: mink; MHV: murine; Th: thrush; Bu: bulbul; Mun: munia.

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 of MERS-CoV

The respective roles of human-to-human and zoonotic transmission in the current MERS-CoV outbreak are not well understood (Fig. 5). Conclusive evidence of human-to-human transmission of MERS-CoV was first reported in a cluster of MERS-CoV cases in the United Kingdom, when an adult male who had travelled to Saudi Arabia transmitted the virus to two of his family members (Health Protection Agency, 2013). Overall, MERS-CoV human-to-human transmission chains have been self-limiting and irregular, and more than half of secondary MERS-CoV cases have originated in a healthcare setting (WHO, 2014). The largest cluster of MERS-CoV to date has involved 23 patients at three different healthcare facilities in the Eastern Province of Saudi Arabia, highlighting the potential of nosocomial transmission (Assiri *et al.*, 2013b). On the other hand, an early MERS-CoV patient transferred from Qatar to a specialist lung hospital in Germany was given intensive treatment for almost a month before the hospital learned of his MERS-CoV diagnosis. Extensive contact investigation and serological analysis of those potentially exposed to the



Fig. 5 Putative MERS-CoV transmission cycle. The putative transmission cycle for MERS-CoV. MERS-CoV likely originated from bats, acting as the natural reservoir. From the natural reservoir, MERS-CoV spilled either directly over to humans (green arrow) or via an intermediate host (dromedary camels, purple arrow). Currently, the exact route of zoonotic transmission of MERS-CoV into the human population remains unknown although the presence of MERS-CoV neutralizing antibodies and the detection of MERS-CoV in dromedary camels suggest that this species is likely to play a major role in the emergence of MERS-CoV. Phylogenetic analysis suggests that multiple introductions of MERS-CoV into the human population have occurred and both zoonotic transmission events and human-to-human transmission (blue arrows) drive the current MERS-CoV outbreak.

patient revealed no secondary infections (Buchholz *et al.*, 2013). Screening of MERS-CoV patient contacts has uncovered at least 18 instances of asymptomatic MERS-CoV infection in healthcare workers and other contacts, although the role these subclinical cases can play in the transmission of infection is unclear (Memish *et al.*, 2013a; The WHO MERS-CoV Research Group, 2013). Transmission of respiratory viruses is often directly associated with the amount of virus shed. The dynamics of MERS-CoV shedding throughout the course of disease have not been well characterized, but high viral loads detected in bronchoalveolar lavage samples from infected patients suggests that coughing and exudates from the lower respiratory tract could be important mechanisms of MERS-CoV human-to-human transmission (Drosten *et al.*, 2013; de Sousa *et al.*, 2013).

The zoonotic source of MERS-CoV continues to play a role in outbreak epidemiology through repeated introductions of virus into the human population (Cotten *et al.*, 2013a, b). The WHO has identified 62 sporadic cases of MERS-CoV, defined as having occurred with no known human exposure (The WHO MERS-CoV Research Group, 2013; WHO, 2014). In 14 early clusters, each primary case

was an adult male, suggesting that activities unique to adult males in the Arabian Peninsula may expose them to a virus source (Penttinen *et al.*, 2013). Interestingly, the rate of severe disease and death is higher for primary MERS-CoV patients than for secondary cases, despite a similar prevalence of comorbidities (The WHO MERS-CoV Research Group, 2013). This could be the result of higher doses of virus exposure among primary patients.

Transmission dynamics 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 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.

MERS-CoV biology

The 30 119-base pair genome of MERS-CoV consists of at least ten polycistronic open reading frames (ORFs), the organization of which follows that of coronaviruses in general (Masters, 2006; van Boheemen *et al.*, 2012). Over two-thirds of the 5' end of the coronavirus genome is composed of the replicase open reading frames ORF1a and ORF1b (Masters, 2006). In MERS-CoV, these ORFs are

translated into two polyproteins, one requiring a ribosomal frame shift, which are eventually cleaved into 16 putative nonstructural proteins (nsps; Sawicki *et al.*, 2007; van Boheemen *et al.*, 2012). The role of these nsps has not been empirically determined for MERS-CoV, but some function can be predicted based on conserved domains characterized in other coronaviruses. For instance, nsp12 putatively serves as the RdRp, and nsp14 is thought to function as the proofreading exoribonuclease (ExoN; van Boheemen *et al.*, 2012). Interestingly, coronaviruses are the only RNA viruses known to use a specific proofreading enzyme for the maintenance of high-fidelity viral RNA replication (Smith *et al.*, 2013). Downstream of the two large ORFs are at least nine ORFs encoding the structural proteins – spike, envelope, membrane, and nucleocapsid – and some accessory proteins (van Boheemen *et al.*, 2012). The translation of the downstream ORFs occurs via subgenomic mRNAs, a salient feature of the order to which coronaviruses belong: *Nidovirales* (Sawicki *et al.*, 2007).

Receptor binding of MERS-CoV

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

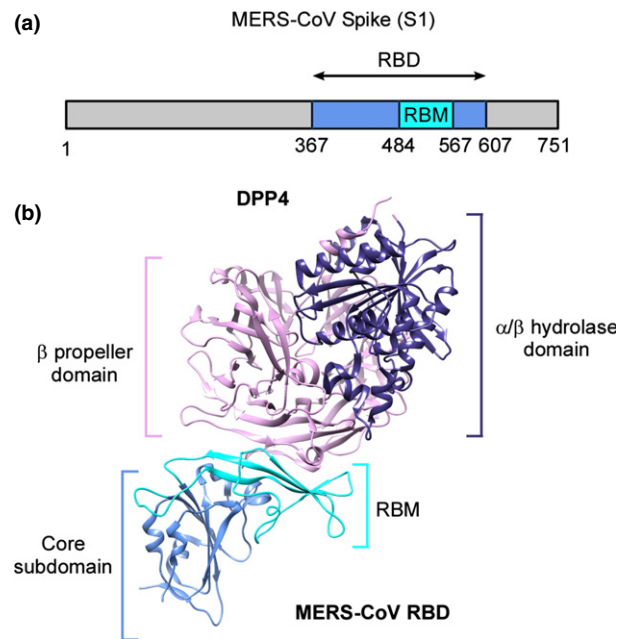


Fig. 6 MERS-CoV spike glycoprotein and DPP4 receptor interaction. (a) The linear organization of the S1 subunit of MERS-CoV spike glycoprotein with the variable RBD located at amino acid residues 367–607, with a RBM containing the critical amino acid residues for binding at residues 484–567. (b) The crystal structure of the MERS-CoV RBD coupled with the receptor dipeptidyl peptidase IV (DPP4). DPP4 is structurally divided into an alpha- and beta-hydrolase domain, and a beta-propeller domain. The beta-propeller domain of DPP4 (pink) interacts with the RBM region (light blue) of the MERS-CoV spike protein RBD. The schematic representation of the DPP4 – MERS-CoV spike protein RBD structure was generated using chimera and protein accession number 4KR0 (Lu *et al.*, 2013).

(Agnihothram *et al.*, 2013; Du *et al.*, 2013a, b, c; Gierer *et al.*, 2013; Song *et al.*, 2013).

Host interaction of MERS-CoV

Preliminary investigations of MERS-CoV host interactions and innate immune responses have been performed *in vitro*, *ex vivo*, and *in vivo* in a nonhuman primate model. MERS-CoV has been shown to replicate *in vitro* in human, bat, and porcine-derived cell lines, whereas cow, hamster, murine, rat, and canine cell lines were not susceptible (Müller *et al.*, 2012; Chan *et al.*, 2013b; Dijkman *et al.*, 2013; Kindler *et al.*, 2013; Raj *et al.*, 2013; Scobey *et al.*, 2013). While *in vitro* modeling holds some predictive value for MERS-CoV susceptibility *in vivo*, a more complete characterization of the mechanisms of cellular entry is necessary to understand host susceptibility (Leow, 2013). For instance, the species tropism of MERS-CoV appears to be restricted by variability in the cellular receptor. MERS-CoV is unable to replicate in mice, hamsters, or ferrets – common small animal models – and the MERS-CoV spike glycoprotein is unable to bind ferret-specific DPP4 (Coleman *et al.*, 2013; de Wit *et al.*, 2013a; Raj *et al.*, 2014).

Innate immunity

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; Zieleski *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).

MERS-CoV intervention strategies

Public health measures

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

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; Zielecki *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), and 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 μ 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 (Villaruel *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

Attempts to establish a small animal model for MERS-CoV 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**

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 bronchointerstitial 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

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 (T_H2) cell responses (Tseng *et al.*, 2012). While inactivated virus vaccines are particularly prone to inducing this type of T_H2-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 (Agnihothram *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 T_H1 cell responses, need to be pursued, and potential vaccines must be rigorously evaluated in animal models (Graham *et al.*, 2013).

Global response

In the year since its identification, MERS-CoV has not only spread across the Arabic Peninsula, but has been transported to the United Kingdom, France, Germany, Italy, Tunisia, and Spain (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). The initial detection of MERS-CoV was followed by the rapid development of MERS-CoV molecular and serological diagnostics (Corman *et al.*, 2012a, b). The emergence of MERS-CoV reminded the infectious disease community of the emergence of SARS-CoV and called for immediate public health preparedness and response. Research efforts have largely focused on the epidemiology of the outbreak and identification of putative natural and intermediate reservoirs and potential therapeutics. Some controversy has surrounded the identification and sharing of MERS-CoV isolates – the initial isolate was shared without the consent of the Saudi Arabian government, and material transfer agreements (MTA) of some isolates were said to be unnecessarily restrictive (Butler, 2013). An MTA has several purposes: it defines not only the material to be transferred, but who can use the materials and what the purpose of the transfer is. It also protects the intellectual property rights of each party by defining ownership of the original material and inventions resulting from the use of the material. Lastly, it designates liability of each party and ensures that the receiving party will use the material in a safe way (for instance, at the proper biosafety level). The controversies surrounding the initial detection of MERS-CoV highlight the importance of creating an international framework for rapid global sharing of virus strains and biologic materials during outbreaks. Similar controversies over ownership have arisen with H5N1 avian influenza (Fidler, 2008). Both the avian flu virus and the MERS-CoV cases strongly point to the need not only for prenegotiated transfer agreements but also for standardized 'best practices' guidelines for highly virulent emerging

disease materials where expedited sharing of material and data is of paramount importance.

Future perspectives

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



Fig. 7 Geographic distribution of dromedary camels. The global distribution of dromedary camels is indicated by yellow shading (Mukasa-Mugerwa, 1981).

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.

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