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Development of Middle East Respiratory Syndrome Coronavirus Vaccines - Advances and Challenges

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Abstract

Middle East Respiratory Syndrome Coronavirus (MERS-CoV) is an emerging pathogen with the potential to pose a threat to global public health. Sporadic cases and outbreaks continue to be reported in the Middle East, and case fatality rates remain high at approximately 36% globally. No specific preventive or therapeutic countermeasures currently exist. A safe and effective vaccine could play an important role in protecting against the threat from MERS-CoV. This review discusses human vaccine candidates currently under development, and explores viral characteristics, molecular epidemiology and immunology relevant to MERS-CoV vaccine development. At present, a DNA vaccine candidate has begun a human clinical trial, while two vector-based candidates will very soon begin human trials. Protein-based vaccines are still at pre-clinical stage. Challenges to successful development include incomplete understanding of viral transmission, pathogenesis and immune response (in particular at the mucosal level), no optimal animal challenge models, lack of standardized immunological assays, and insufficient sustainable funding.

Keywords: Middle East Respiratory Syndrome, MERS coronavirus, spike protein, receptor-binding domain, vaccine

Introduction

Confirmed and probable cases of Middle East Respiratory Syndrome (MERS) are defined by the World Health Organization (WHO) based on clinical signs and symptoms, radiological and

histological evidence of pulmonary parenchymal disease, epidemiologic linkage, and laboratory testing [1]. The etiological agent of MERS, MERS-Coronavirus (MERS-CoV), was first reported in a 60-year old male patient with acute pneumonia and subsequent renal failure in the Kingdom of Saudi Arabia (KSA) in 2012 [2]. Based on current understanding and sero-epidemiological evidence [3, 4], dromedary camels appear to be intermediate hosts, serving as an important pathway for animal-human transmission, although the precise mechanism remains unclear. Human-to-human transmission accounts for approximately 53-60% of total MERS cases [5], with droplets being considered to be the primary mode of transmission to family members and healthcare workers (HCWs) [6, 7]. Between September 2012 and January 2017, more than 1,900 laboratory-confirmed cases of MERS-CoV infection and approximately 690 deaths were reported to WHO from 27 countries, indicating a case fatality rate (CFR) of approximately 36% (**Figure 1**) [8, 9]. The majority of MERS-CoV infections (approximately 75%) have been reported from KSA. Three years after the initial description of MERS, MERS-CoV caused the largest outbreak outside KSA in the Republic of Korea in 2015. A recent epidemiological study comparing outbreaks in KSA and the Republic of Korea indicated higher morbidity and mortality in older males (≥70 years) in both countries [10]. The proportion of infected HCWs and nosocomial cases (i.e., hospital-linked contact history) was about 13% in KSA and the Republic of Korea [10, 11]. In summary, the most vulnerable populations appear to be HCWs and the elderly with underlying chronic diseases.

The Korean outbreak, in particular, raised concerns about spread to countries remote from endemic regions, reinforcing the potential importance of preventive and therapeutic measures against MERS-CoV infection. Concern about potential global dissemination was noted earlier,

with an imported case leading to patient-to-patient nosocomial transmission in France two years prior to the Korean outbreak. A 64-year-old male returning from the United Arab Emirates (UAE) was hospitalized with non-specific gastrointestinal but no respiratory symptoms [12]. This patient shared a hospital room with another patient, who became the only confirmed MERS case among 123 contacts of the index case, based on PCR testing of lower respiratory tract (LRT) specimens [13]. PCR testing of upper respiratory tract (URT) specimens was inconclusive, while serological assays were not performed, to define a case of MERS-CoV infection [12, 13].

The definition of a MERS case is critical, not only for disease control and prevention, but also to determine efficacy in clinical trials. The current WHO definition of a confirmed MERS case requires serological testing or real-time reverse transcriptase PCR (rRT-PCR) [1, 14]. PCR is both specific and sensitive, and has become the test of choice for coronavirus diagnosis, despite its limitations such as lack of correlation with viral load [15]. The assay mostly targets open reading frames (ORF) 1a and 1b, nucleocapsid (N) and spike (S) genes [16]. For screening, WHO recommends the ORF 1a assay as well as the upE assay targeting a region upstream of the envelope (E) gene. Both PCR targets are highly sensitive [14]. Although both URT and LRT samples are recommended by WHO [14], LRT samples are preferred for RT-PCR, especially when diagnostic testing is delayed by more than a week after symptom onset [17].

Seroconversion demonstrated by ELISA, immunofluorescent assays (IFA) and neutralization assay also meets the WHO case definition of MERS-CoV infection [1]. Serology testing for antigen (Ag) or antibody (Ab) detection has advantages of relatively short turnaround time and detection of infection in the absence of clinical signs and symptoms [18]. However, Abs may not

be detectable until 7-10 days after illness onset, which can result in delayed diagnosis [15, 19]. Recently, an Ag-capture ELISA targeting nucleocapsid protein (NP) of MERS-CoV has been developed, showing high sensitivity (< 1 ng/mL MERS-CoV-NP) and specificity (100%) [20]. In addition, Song *et al* developed a rapid immunochromatographic assay to detect MERS-CoV NP in dromedary camels with 94% sensitivity and 100% specificity, but further investigation is required before use in humans [21]. Other assays for Ab detection include neutralization assays, indirect fluorescent antibody assay, and ELISA. However, other techniques such as Western blot would be required to confirm antibody specificity if neutralization assay is not performed [15]. Currently, there is no standardized neutralization assay for MERS-CoV, and lack of well-characterized animal or human sera remains as a challenge.

Coronavirus infection, immune responses and monoclonal antibody

Viral characteristics and major proteins

MERS-CoV has four major structural proteins and spike (S) protein plays a significant role in cell entry through receptor recognition and membrane fusion [22]. S protein consists of two subunits – S1 containing the receptor binding domain (RBD) and S2 containing epitopes which are cross-reactive with homologous epitopes of other beta coronaviruses [23]. In the Korean outbreak, MERS-CoV mutations with reduced affinity of RBD for the host cell receptor, human dipeptidyl peptidase 4 (hDPP4 or CD26), were identified [24]. This finding might relate to a previous study suggesting that viral fitness and/or virulence may be impaired by immunological pressure such as neutralizing antibodies (NAbs) during human-to-human transmission [25]. According to the genomic analysis from the Korean outbreak [24], reduced affinity caused by a

mutant RBD (i.e., I529T mutant) lowered the efficiency of viral entry into CD26-expressing cells. This particular viral mutation appeared to be present in the index patient, whereas either wild type or another mutant was present through the second and third generations of human-to-human transmission. Reduced affinity and efficiency of viral entry caused by mutated RBD may be one of various factors contributing to decreased severity, and may explain differences in mortality rates in different epidemiological settings.

Lessons learned from SARS-CoV infection

Similar to MERS-CoV, the RBD of the SARS-CoV S protein is a primary target for immune responses including NAb response. Thus, information regarding SARS-CoV may provide useful lessons for MERS-CoV. The antibody response to SARS-CoV infection is relatively short. SARS-CoV-specific antibodies were undetectable in 21 of 23 convalescent patients 5-6 years post-infection [26]. On the other hand, memory T-cell responses tested by ELISPOT were detected in 14 of 23 surviving patients (~61%) and were undetectable in close contacts and healthy controls. A study of SARS-CoV neutralization plasticity demonstrated that neither a single RBD-targeting neutralizing mAb nor two neutralizing mAbs in combination blocked neutralization escape mutants [27], indicating that further study would be required to uncover NAb-mediated escape pathways.

Immune responses to MERS-CoV infection

MERS-CoV infection may trigger humoral immune responses including NAb induction, as well as cellular immune responses [19, 28]. In a study of 37 patients in KSA, serum IgG and NAbs

were detected in all survivors and inversely correlated with lower respiratory tract viral load [29]. A study of early serologic responses in 17 patients from the Korean outbreak showed robust IgG response in most patients within 3 weeks of illness onset [19]. In a different study in Korea, 14 patients recovering from MERS showed increased S1-specific serum IgG responses, while 5 non-survivors had relatively steady serum IgG titers [30]. The kinetics and duration of the serologic response to MERS-CoV infection has not been fully characterized. Early antibody responses have been associated with longer incubation periods and lower disease severity [19]. Moreover, cytokine profiles in 17 Korean patients included increased IL-6 and CXC-10 within 1-2 week of illness onset, with greater increase of both cytokines in patients with more severe disease [31]. Another study of MERS-CoV infected patients suggested that the absence of INF-α may be associated with impaired cellular (Th1) immune response in a deceased patient [28]. In addition, an animal study in interferon-α and MyD88 knockout mice demonstrated both efficient T-cell and B-cell responses were required to clear MERS-CoV [32]. Evaluation of MERS-CoV vaccine candidates may need to include measurement of both T-cell and B-cell immune responses.

Monoclonal antibodies against MERS-CoV

Research efforts have also focused on developing antiviral mAbs as prevention and therapy. MERS-CoV monoclonal antibodies currently under development have been summarized in the WHO roadmap [33]. Human mAbs prevent MERS-CoV binding to hDPP4, although different mAbs recognize different RBD epitopes [25, 34]. Three neutralizing human mAbs targeting the RBD of MERS-CoV S1 glycoprotein have been recently identified from a large phage-display

antibody library [34, 35]. Among these mAbs, m336 demonstrated potent neutralizing activity, showing IC_{50} = 5 ng/ml *in vitro* [34, 36]. Agrawal *et al*. [35] utilized a transgenic (Tg) mouse model expressing hDPP4 to assess prophylactic and therapeutic efficacy of m336. When Tg mice were treated with m336 12 hours post- MERS-CoV infection, all mice treated with higher dose (1 mg) survived but showed mild (<10%) and transient weight loss. Although m336 did not show full protection against morbidity, its prophylactic and therapeutic efficacy were greater than an irrelevant mAb (control) as assessed by weight loss and survival rate.

In a study by Luke *et al*. [37], two investigational MERS-CoV vaccines were used to produce transchromosomic (Tc) bovine human IgG (Tc hIgG), SAB-300 and SAB-301. Upon the administration of a single dose of SAB-301, 12 hours before and 24 and 48 hours after MERS-CoV EMC/2012 infection, viral lung titers decreased to near or below the limit of detection in hDPP4-transduced mice. These studies demonstrate the potential use of either monoclonal or polyclonal antibodies as passive immunotherapy to prevent and treat MERS-CoV infection.

In addition to mAbs targeting RBD epitopes, a novel mAb, 5F9, targeting the N-terminal domain (NTD) of the S1 subunit, has been generated in mice, showing neutralizing activity against wild type MERS-CoV EMC/2012 strain [38]. 5F9 had high binding affinity for NTD with EC_{50} of approximately 0.85 μg/ml and no cross-binding with RBD. These findings suggest that NTD also contains a neutralizing epitope for MERS-CoV.

Current status of MERS-CoV vaccine development

Animal models

Establishing accurate, reproducible and predictive animal models to evaluate the efficacy of MERS-CoV vaccine candidates remains challenging as MERS-CoV does not replicate in small animal models such as hamsters, ferrets and mice due to sequence differences in DPP4 in these animals [39, 40]. Although non-human primates (NHPs) such as rhesus macaques and common marmosets can be used as models [41], high cost and handling have been an obstacle [42, 43]. Clinical manifestations in rhesus macaques can be transient, with resolution of clinical signs and abnormal blood cell counts within 3-4 days post-infection [42]. Common marmosets appear to be more appropriate for vaccine efficacy studies as they develop a more severe MERS-CoV infection than rhesus macaques [44]. Dromedary camels are not optimal animal models for human infection because the virus primarily infects the nasal mucosa causing only mild symptoms and because their large size and geographically limited availability restrict their practical use [45]. A recent study by Adney *et al*. demonstrated viral shedding in nasal cavity and nAbs in intranasally infected alpacas and co-housed alpacas, suggesting that this species may substitute for camels in animal studies [46]. Transduced mice expressing hDPP4 using recombinant adenoviral vectors have been developed as a MERS-susceptible small animal model [47]. Transduced mice can be generated rapidly, but hDPP4 expression is transient and limited to the lungs. This technology has been used to demonstrate prevention and mitigation of viral replication by polyclonal IgG antibodies produced in Tc bovines, as discussed above [37]. A transgenic mouse model expressing hDPP4 has also been developed for MERS by Agrawal *et al*., the first study on the generation of a MERS-CoV transgenic mouse model [47]. Tg mice are the only available small animal model for severe or fatal MERS-CoV infection with respiratory manifestations and viremia [48]. Generation of Tg mice takes longer than transduced mice, and

hDPP4 is expressed non-physiologically (i.e., in tissues where it is not found naturally) although lesions are mainly found in the respiratory tract. Tg mice can be useful in screening antivirals and vaccines.

Current MERS-CoV vaccine approaches

Since 2012, MERS-CoV vaccine development has made some progress, but no approved vaccine against MERS-CoV is currently available. Vaccine candidates under development are listed in **Table 1** and include: DNA vaccines, protein subunit and peptide vaccines, vector-based vaccines, and live attenuated vaccines.

DNA vaccines

DNA vaccines are safe, produce stable antigen expression, and can induce humoral and cellular immune responses at relatively low manufacturing cost [49]. Compared to other vaccine platforms, however, DNA vaccines tend to elicit lower immune responses [50]. The most commonly reported adverse event in DNA vaccine studies is local pain at the injection site, and non-specific systemic symptoms such as malaise and pyrexia [51]. A DNA vaccine against SARS-CoV was shown to induce NAbs and effective T-cell responses in humans [52]. A study in rhesus macaques indicated that MERS-CoV DNA vaccine expressing the full-length S protein of MERS-CoV EMC/2012 administered via intramuscular injection with electroporation (EP) at 0, 3 and 6 weeks induced S-specific NAb response as well as T cell responses producing IFN-γ, TNF-α, and to a lesser extent, IL-2 in both low- and high- dose groups [53]. Protective efficacy was demonstrated in rhesus macaques with reduced viral load and no clinical and radiographic

signs of pneumonia compared to control animals [53]. These results led to the first Phase I clinical trial of a MERS-CoV vaccine utilizing the DNA-based vaccine developed by Inovio Pharmaceuticals and GeneOne Life Science Inc., which is ongoing at the Walter Reed Army Institute of Research in the United States [53, 54].

A prime-boost regimen using a DNA prime followed by protein boost has been used as an alternative approach. Wang *et al*. [55] showed that the combination of full-length S DNA vaccine (with EP) and S1 subunit protein in alum elicited NAbs against MERS-CoV in mice and NHPs. In mice, the full-length S DNA vaccine generated higher humoral response than the truncated, transmembrane-deleted S or S1 DNA regimens. NHPs immunized with S DNA/S1 protein or S1/S1 protein demonstrated significantly reduced pulmonary infiltrates and consolidation compared to unvaccinated NHPs. In both mice and NHPs, priming with either S1 protein or S DNA followed by S1 protein booster induced the highest NAb titers, compared with the S DNA only group. This was the first study demonstrating protective immunity induced by prime-boost combination regimen of MERS-CoV S DNA and protein in NHPs.

Protein subunit vaccines

Protein subunit vaccines are generally safe and well tolerated. Production consistency can be readily attainable due to defined immunogenic components [56-58]. Protein subunit antigens induce antibody responses with primarily CD4 T cell responses, and often require adjuvants [59, 60]. Studies of SARS-CoV have identified the S1 subunit, especially the RBD of SARS-CoV as a primary target for NAb in mice, NHPs, and humans [55, 61-65]. Similarly, for MERS-CoV, the full-length or fragments of S protein, particularly the RBD, has been used to demonstrate partial

efficacy in rhesus macaques [66, 67] as well as inducing NAbs in small animal models such as rabbits and mice [59, 60, 68-73]. A recent study showed that residues 377-588 of MERS-CoV S protein RBD were capable of eliciting potent humoral and cellular responses, which could be enhanced by adding MF59 adjuvant [60, 71]. As an extra-RBD target, the recombinant N-terminal domain (rNTD) of S protein was used as a vaccine candidate and showed humoral (IgG and NAb) and T cell responses in hDPP4-transduced mice when administered with alum or CpG adjuvant [70].

MERS-CoV S protein nanoparticles have also been developed as a possible vaccine candidate. Coleman *et al*. [74, 75] demonstrated that recombinant MERS-CoV S nanoparticles in combination with Matrix-M1 adjuvant induced NAbs, reduced lung viral titers and MERS-CoV M mRNA to baseline levels in transduced mice, suggesting that MERS-CoV replication was efficiently blocked. Matrix-M is a saponin-based adjuvant which has been shown to enhance humoral, cellular and protective responses with various pathogen vaccines including a H7N9 vaccine and Ebola virus vaccine [76, 77]. More studies are required to assess safety, immunogenicity and efficacy of nanoparticles in larger animal model(s) studies and/or human clinical trials for further development.

Vector-based vaccines

Vaccines using viral vectors such as Modified Vaccinia Ankara (MVA), adenovirus, and measles virus can elicit robust humoral and cellular immune responses [78-80]. However, the induction of antigen-specific immune responses may be hampered by pre-existing immunity to some vectors; the theoretical induction of hyper-inflammatory responses has also been raised [81, 82].

Vector-based MERS-CoV vaccines expressing different lengths of S protein are under development by various groups. An MVA-based, full-length S MERS-CoV vaccine candidate (MVA-S) was developed by the German Center for Infection Research and evaluated in transduced mice, demonstrating robust NAb response and reduction of viral replication in the lower respiratory tract [80]. MVA-S also induced mucosal immunity and reduced viral shedding in dromedary camels challenged with MERS-CoV [83]. Vaccinated camels demonstrated lower levels of infectious MERS-CoV particles and little to no viral RNA in the nasal cavity. A human clinical trial for this MVA-S vaccine candidate is planned.

A chimpanzee adenovirus (ChAdOx1)-based MERS-CoV vaccine developed by the Jenner Institute will also soon enter a clinical trial [84]. Chimpanzee adenovirus (ChAd), as a vaccine carrier, has some advantages such as limited to no cross-reaction with pre-existing immunity to human adenoviruses, ability to grow in human cell lines, and comparable immunological potency to human adenovirus serotypes in mice and NHPs [85]. ChAd has been shown to be safe and generally well tolerated. Rare reported SAEs such as thrombocytopenia or asymptomatic prolonged activated partial thromboplastin time were either unrelated to vaccine or were *in vitro* effects of anti-phospholipid antibody induction and did not indicate coagulopathy, respectively [86-88]. Several ChAd vaccines have been evaluated in mice and NHPs for various diseases including AIDS, malaria, Ebola, SARS, hepatitis C, rabies and cancer, demonstrating potent immunity and efficacy. A recent study investigated humoral and cellular immune responses of MERS-CoV candidate vaccines using ChAdOx1 and MVA [89]. Both ChAdOx1- and MVA-based MERS-CoV vaccines induced NAbs and cellular immune responses in BALB/c mice; a single dose of ChAdOx1 MERS-CoV vaccine with human tissue plasminogen activator (tPA)

elicited equivalent humoral immune response as two doses of MVA MERS-CoV vaccine with tPA. A measles virus (MV)-based MERS-CoV vaccine candidate expressing full-length S or a truncated soluble variant of S protein induced robust MV- and MERS-CoV-specific NAbs as well as T cell responses in INF-deficient mice [65, 78]. In the same study [78], protective efficacy was demonstrated against MERS-CoV challenge in immunized mice.

Live attenuated vaccine

Live attenuated vaccines have been shown to induce more potent immune responses and protection against viral challenge than non-replicating vaccines as they present antigens to the immune system in the manner of natural infection. Live attenuated vaccines can be constructed by deletion of genes responsible for virulence [90], but potential reversion to virulence may be a concern for regulatory agencies, and for use in immunocompromised individuals [91]. A live-attenuated MERS-CoV vaccine candidate has been developed based on a replication-competent but propagation-defective MERS-CoV strain [90]. Another engineered mutant virus lacking E protein (rMERS-CoV-ΔE) is not replication competent, but could be rescued in cells expressing E protein, suggesting that rMERS-CoV-ΔE mutant virus would be a potentially promising candidate.

Discussion

Since the first reported case of MERS-CoV in 2012, a limited number of MERS-CoV-specific patents have been filed [92]. These include nucleic acid sequence of MERS-CoV RBD in humans, mAb against MERS-CoV S protein, and RBD-Fc fusion protein. Investigation and

development of DNA-based and vector-based vaccine candidates have progressed substantially. Other approaches such as a modified mRNA vaccine similar to that being developed for Zika may be promising [93, 94]. Further studies may focus on improving MERS-CoV S protein vaccines by investigating new delivery technologies and optimizing strategies for administration and use of relevant adjuvants. Standardization of reagents and assays including neutralizing antibody assays (virus-based or pseudovirion-based) will be necessary. The development of potent adjuvants may mitigate concerns that vaccines against coronaviruses such as SARS-CoV and MERS-CoV may cause eosinophilic immunopathology and antibody-dependent enhancement of infectivity (ADEI) when NAb levels are low [95]. Eosinophilic immunopathology attributed to an inadequate vaccine-induced Th1 response in the absence of adjuvants was reported with a SARS-CoV vaccine in animals, and appropriate care should be taken in MERS-CoV vaccine development [95]. Currently a variety of adjuvants have been investigated for MERS-CoV vaccines: alum, MF59, Matrix-M1, CpG, and Poly (I:C) [67, 71, 75, 96]. The use of adjuvants such as alum, CpG, and delta-inulin-based polysaccharide adjuvant has been shown to increase NAb titers and ameliorate lung eosinophilic immunopathology [95, 97], suggesting that adjuvants may be utilized in MERS-CoV vaccine development to improve immunogenicity and safety.

Additionally, guidelines and standards being developed by WHO and others can serve as a guide for researchers, vaccine developers and public health authorities to develop MERS-CoV vaccines. Recently, WHO published a target product profile (TPP) for MERS-CoV vaccine, providing preferred and minimally acceptable characteristics for both dromedary camel vaccines and human vaccines; the latter for long-term protection and reactive use during outbreak [98].

The WHO R&D Blueprint for Action to Prevent Epidemics has prioritized MERS-CoV [98], illustrating the importance of preparedness for future outbreaks. The R&D Blueprint urges coordination of activities in sharing samples and data, organization of a repository for access to strains, standardized reagents, standardized NAb methodology, establishment of proper animal models, and conducting of clinical trials in outbreak regions.

Conclusion

The emergence of highly pathogenic coronaviruses including MERS-CoV has raised concerns, prompting the development of global strategies for disease prevention and control, particularly during emergency outbreaks. Although MERS-CoV has been prioritized by WHO for R&D, no preventive vaccines have been approved yet. Only brief human-to-human transmission has been reported, but the risk of future outbreaks resulting from viral adaptation and more efficient human-to-human transmission remains a potential threat to global health. In order to address challenges in availability of sustainable funding to accelerate R&D of vaccines for emerging pathogens including MERS-CoV, the World Economic Forum launched the Coalition for Epidemic Preparedness Innovations (CEPI) in 2016. CEPI is an international initiative involving governments, industry, philanthropic organizations and nongovernmental organizations for financial support and coordination of vaccine development. A better understanding of the pathogenesis, transmission and immune responses to virus in animals and humans is still sorely needed. Unclear identification of target population(s), variability of the S protein, the lack of standardized assays and/or appropriate animal models remain critical knowledge gaps.

Nevertheless, one DNA vaccine candidate has progressed to human clinical trials, and other vaccine candidates are soon to follow.

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Declaration of interest

The authors declare no conflict of interest.

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Figure 1. Global cases of MERS-CoV for 2012-2017 [9]

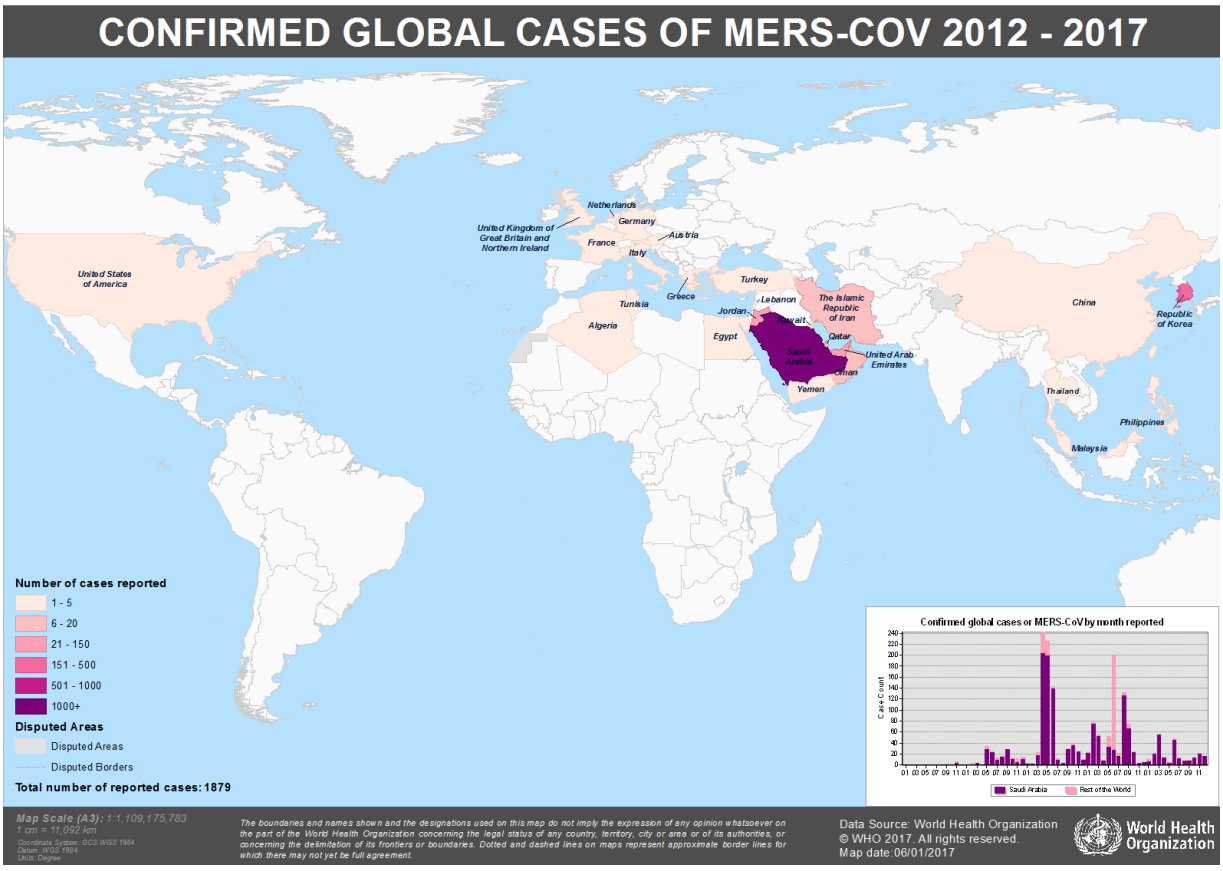

Table 1. MERS-CoV vaccines under development

Vaccine platform	Vaccine name	Viral antigen/Product design	Status	Animal models	Institution	Ref.
DNA	GLS-5300	Full-length S	Phase I	C57BL/6 mice, camels, rhesus	GeneOne Life Science, South Korea	[53]
Protein subunit	S-RBD-Fc	S1-RBD fused with human Fc	Preclinical	BALB/c mice, rabbits, hDPP4-mice	New York Blood Center, U.S.A., Fudan University, China	[47, 69]
	MERS-CoV rRBD	Truncated S1-RBD	Preclinical	BALB/c mice	China CDC	[59]
	rNTD	rNTD	Preclinical	BALB/c mice	China CDC	[70]
	MERS-S	Nanoparticles	Preclinical	BALB/c mice	Novavax, U.S.A.	[74, 75]
Heterologous prime-boost	S-DNA/S1 Protein	Full-length S (prime) + S1 subunit (boost)	Preclinical	BALB/c mice, Rhesus	U.S. National Institute of Health	[55]
Vector	ChAdOx1-MERS-S	Full-length S	Preclinical	Mice	Jenner Institute, UK	[84, 99]
	Ad5-S, Ad41-S	Full-length S	Preclinical	Mice	China CDC	[79]
	BNSP333-S1	RABV-S1	Preclinical	hDPP4-mice	Thomas Jefferson Univ., Univ. of Maryland, NIH, U.S.A.	[100]
	MERS-S/MERS-solS	Measles-full-length S/solS	Preclinical	IFNAR-/- mice	Paul Ehrlich Institute	[78]
	GreMERSfi	Ad5-full-length S/S1	Preclinical	BALB/c Mice	Greffex, U.S.A.	[101]
	MVA-	Full-length S	Preclinical	hDPP4-	German	[80]

	MERS-S			mice, planning for phase I	Center for Infection Research (DIFZ)	
Live attenuated	rMERS-CoV-ΔE	Recombinant MERS-CoV without E	In vitro	-	Universidad Autonoma de Madrid, Spain	[90]