Vaccines for the prevention against the threat of MERS-CoV

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Summary
First identified in 2012, Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV) is listed as a new Category C Priority Pathogen. While the high mortality of MERS-CoV infection is further intensified by potential human-to-human transmissibility, no MERS vaccines are available for human use. This review explains immune responses resulting from MERS-CoV infection, describes MERS vaccine criteria, and presents available small animal models to evaluate the efficacy of MERS vaccines. Current advances in vaccine development are summarized, focusing on specific applications and limitations of each vaccine category. Taken together, this review provides valuable guidelines toward the development of an effective and safe MERS vaccine. This article is written for a Special Focus Issue of Expert Review of Vaccines on “Vaccines for Biodefence”.

Keywords
Animal models; immune responses; MERS; MERS-CoV; neutralizing antibody; protection; spike protein; vaccines

Introduction
Since first emerging in Saudi Arabia in June 2012, cases of Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV) infection have been reported from 26 countries. Saudi Arabia has the largest number of MERS cases, followed by South Korea. As of February 02, 2016, 1,638 MERS cases, including 587 deaths (case fatality rate: ~36%), have been reported to the WHO [1–3]. Cases of MERS keep increasing in Saudi Arabia, and reach at 1,297 as of February 24, 2016 [4].

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Declaration of Interests
The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.
Similar to other coronaviruses (CoVs), including severe acute respiratory syndrome coronavirus (SARS-CoV) [5] and porcine epidemic diarrhea virus (PEDV-CoV) [6], MERS-CoV is a zoonotic virus and originates from bats, suggesting that bats are the most likely natural reservoir of MERS-CoV [7–10]. However, unlike SARS-CoV, which utilizes small animals, such as palm civets and raccoon dogs, as its intermediate hosts [11,12], MERS-CoV depends on dromedary camels [13–17]. Nevertheless, human-to-human transmission of MERS-CoV does occur, and unprepared healthcare facilities could become a major source for human infection and transmission (Fig. 1), as shown by the incidence of MERS-CoV cases in South Korea in 2015 [1,18–21]. Several family clusters infected with MERS-CoV have also been revealed [22–25]. Recently, MERS-CoV has been added to the NIAID list as a Category C Priority Pathogen [26]. This places it in the same category as SARS-CoV and, as such, it has the potential to be used as a biological weapon. Therefore, steps toward prevention strategies need to be taken, particularly the development of effective and safe vaccines.

CoV genera are classified as α, β, γ, and δ (Fig. 2) [2,27–31]. The δ CoV, a new genus in the Coronaviridae family, usually infects birds, including HKU16, HKU17 and HKU21, or mammals, such as porcine deltacoronavirus HKU15 and new strains recently identified in U.S., which causes porcine diarrhea [28,31–34]. Several α and β CoVs can infect humans, but only MERS-CoV and SARS-CoV have led to regional or global human outbreaks [1,2,20,35–40]. Although MERS-CoV and SARS-CoV are β CoVs, it is interesting to note that MERS-CoV is phylogenetically related to bat-CoVs HKU4 and HKU5 (Fig. 2) [2,41–43]. Unlike HKU5, HKU4 can bind to bat and human dipeptidyl peptidase 4 (DPP4), the receptor of MERS-CoV, to directly infect bat cells, or indirectly infect a number of human cells by exogenous stimulation [8,44,45]. Two mutations, S746R and N762A, in the surface spike (S) protein may be responsible for transmitting MERS-CoV from bats to humans (Fig. 1), thus explaining the origins of MERS-CoV in bats [7,9,44].

Similar to other CoVs, the MERS-CoV genome is a single positive-stranded RNA. It contains two large replicase open reading frames (ORFs), ORF1a and ORF1b, encoding two proteases, a papain-like protease and 3C-like protease, which are conserved in all other CoVs [2,43,46]. The downstream ORF2, ORF6, ORF7, and ORF8 of MERS-CoV genome are believed to encode S, envelope (E), membrane (M), and nucleocapsid (N) structural proteins, respectively, all having different functions (Fig. 3A–B) [2,43]. MERS-CoV E protein, for example, promotes virulence [46,47]. The surface S protein is composed of S1 and S2 subunits respectively responsible for cellular receptor DPP4 binding via the receptor-binding domain (RBD), and fusion of virus and cell membranes, thereby mediating the entry of MERS-CoV into target cells (Fig. 3C) [45,48–50]. The MERS-CoV RBD consists of a core structure, which is homologous to that of the SARS-CoV S protein RBD, and a receptor-binding motif, which is unique to MERS-CoV, thus determining viral pathogenesis and receptor recognition [51–54]. In addition to the aforementioned major structural proteins, the MERS-CoV genome also encodes several accessory proteins, including 3, 4a, 4b, and 5, which, however, might not be essential for virus replication [27,43,47]. Recent studies have revealed that whole-genome consensus sequences of MERS-CoV from dromedary camels and humans are identical, further confirming MERS-CoV transmission from dromedary camels to humans [15,55].
MERS-CoV infection and resulting immune responses

MERS-CoV infection may trigger antigen-specific humoral immune responses and neutralizing antibodies in camels and humans [56–59]. MERS-CoV- or S-specific antibodies, including those with neutralizing activity, were identified in dromedary camels from MERS-affected regions, including Saudi Arabia, Jordan, Qatar and the United Arab Emirates [13,55,56,60]. In addition, the seroprevalence of MERS-CoV-specific antibodies was shown to be significantly higher in individuals exposed to camels than that found among the general population [14]. Studies on 37 MERS-CoV-infected adult patients indicated that all survivals had serum IgG and neutralizing antibodies, and the levels of such antibodies were weakly but inversely correlated with viral loads in the lower respiratory tracts [61]. In South Korea, MERS-CoV-infected humans also demonstrated a clear kinetics of serologic responses, including robust antibody responses developed at the early stage of the disease onset, but delayed antibody responses with neutralizing activity associated with later, more severe stages of the disease [57]. The above studies suggest that humoral immune responses, including neutralizing antibodies, play an important role in preventing MERS-CoV infection.

In addition to B cell-mediated antibody responses, cellular immune responses mediated by specific T cells may play a supplementary role. The absence of IFNα in a patient who died from MERS-CoV infection could have impaired the production of antiviral adaptive IL-12- and IFN-γ-mediated Th1 immune responses, suggesting that IFNα might be important in the induction of robust cellular immune responses during the initial stage of disease progression [62]. Moreover, MERS-CoV infection may drive IL-17 production in humans, as well as the expression of cytokines and chemokines, including IL-12, IFN-γ, IP-10, and RANTES, in dendritic cells [63], effectively modulating innate immune responses.

Vaccine-induced immune responses and criteria for evaluating MERS vaccines

MERS vaccines can also induce humoral and cellular immune responses. Specifically, a good MERS vaccine should be able to induce strong humoral immune responses, particularly neutralizing antibodies, in vaccinated animals and humans, completely protecting immunized subjects from MERS-CoV challenge. Depending on the immunization routes, MERS vaccination may activate B cells to produce systemic IgG and/or secretory IgA (sIgA) antibodies, both of which can bind to the virus and, respectively, mediate systemic and mucosal immune responses [64–66]. Serum IgA could also be induced upon vaccination, particularly through the mucosal or intranasal (i.n.) route [65]. Antibodies with neutralizing activity can then neutralize MERS-CoV infection by blocking virus-target cell binding via cellular receptor DPP4, thus inhibiting virus entry (Fig. 4) [67,68]. It is likely that some B cells will become antigen-specific memory B cells capable of activation by further boost immunization or other stimulation factors to induce rapid recall antibody responses [69], but this outcome has not been extensively studied in MERS-CoV-directed vaccines.
Immunization of MERS vaccines could induce antigen-specific T cell immune responses as well. As such, CD4+ T cells can be activated to secrete Th1, including IL-2, IFN-γ, and TNFα, and/or Th2, such as IL-4, IL-5, and IL-10, cytokines, in turn promoting cytotoxic T cells, such as T lymphocytes or CD8+ T cells, to kill target cells infected with MERS-CoV (Fig. 4) [65,70–72]. However, in animal models, neutralizing antibody alone was able to protect against challenge from MERS-CoV [64,73]; therefore, T cell-mediated cellular immune responses, if needed, may play a supplementary role in preventing MERS-CoV infection [74,75].

An ideal MERS vaccine candidate should have high immunogenicity and strong potency, as judged by the ability to induce potent immune responses and neutralizing antibodies, as well as complete protection against MERS-CoV infection, with the lowest dosage and least injection time through an appropriate route. In addition, MERS vaccines need to maintain good safety without inducing virus-enhancing antibody or harmful immune responses, or causing immunopathological effects [27,75].

**Current animal models for evaluating the in vivo efficacy of MERS vaccines**

MERS vaccines need to be evaluated in appropriate animal models before proceeding to human clinical trials. Substantial progress has been made in the development of MERS animal models, including non-human primates (NHPs), such as rhesus macaques and common marmosets [76–79], as well as small animal models, such as hDPP4-transduced and transgenic (Tg) mice [73,80–83]. Table 1 summarizes the currently available animal models, their characteristics, and potential applications for evaluating the efficacy of MERS vaccines.

NHP models have been initially established as an effective vehicle for MERS-CoV infection and vaccine evaluation. Rhesus macaques infected with MERS-CoV developed lower respiratory tract symptoms with mild-to-moderate interstitial pneumonia, and virus replication mainly occurred in alveolar pneumocytes. Also, clinical signs of disease and neutralizing antibodies were produced in these animals upon virus infection [77,78]. In contrast, marmosets, as a new MERS-CoV infection model, developed a much more severe disease with progressive severe pneumonia, leading to significant viral replication in the lungs and partial lethality [79]. However, other reports demonstrated a mild-to-moderate nonlethal respiratory disease in common marmosets with limited additional clinical signs upon inoculation with MERS-CoV [87]. Except for NHPs, camels infected with MERS-CoV may present upper respiratory tract symptoms with virus replication and shedding in the upper respiratory tract of inoculated dromedary camels [16].

Unlike SARS-CoV, which easily infects commonly used laboratory animals, including Syrian hamsters, ferrets, and mice [88–91], MERS-CoV does not normally infect these animal species because of the differences in binding viral receptor [92–95]. Recently, a number of small animal models were developed for MERS-CoV [73,80–83]. For example, after prior transduction with adenovirus 5 expressing human DPP4 (Ad5-hDPP4), mice became sensitive to MERS-CoV infection and developed pneumonia accompanied by clinical disease and histopathological changes in the lungs [83]. In addition, a humanized
(HuDPP4) mouse model was established, in which mouse DPP4 was replaced by human DPP4 [86]. In particular, a human DPP4 transgenic (hDPP4-Tg) mouse model globally expresses the hDPP4 receptor, and it is fully permissive to MERS-CoV infection [73,81]. Infected animals developed progressive pneumonia and demonstrated significant weight loss and death upon virus infection. Virus replication was detected in lung and brain [80–82]. Thus, such small animal models provide an economical, readily available method of testing the efficacy of MERS-CoV candidate vaccines.

### Current advances in MERS vaccine development

No vaccines against MERS-CoV are currently available for human use. Nevertheless, progress has been made since the emergence of MERS-CoV in 2012, and a number of MERS vaccines have been developed and tested in preclinical stages [64,66,70,72,84,96,97], two of which are scheduled for human clinical trials [98,99]. These vaccines are based on recombinant virus; viral vectors, including modified vaccinia virus Ankara (MVA), adenovirus (Ad), and measles virus (MV); nanoparticles; DNA and DNA/protein; as well as subunit vaccines. Table 2 summarizes current MERS vaccines under development.

#### Recombinant MERS-CoV as vaccines

Unlike SARS vaccines that are usually developed based on attenuated or inactivated SARS-CoV, thus having a potential to recover virulence [11,105–108], MERS-CoV vaccines could be constructed based on recombinant viruses using reverse genetics. Accordingly, a recombinant MERS-CoV with expected marker mutations was generated using a panel of contiguous cDNAs spanning the entire viral genome and replicated to high titers with broad tissue tropism. Also, an engineered mutant MERS-CoV lacking the structural E protein was rescued and propagated in cells expressing the viral E protein in trans [46,47]. Using reverse genetics, it is possible to develop a replication-competent, propagation-defective MERS-CoV candidate vaccine, providing a platform for the design of live attenuated MERS-CoV vaccines. Since such recombinant MERS viruses still contain major virus components, their safety needs to be tested extensively, and their immunogenicity requires further evaluation in appropriate animal models.

#### Viral vector-based MERS vaccines

Similar to viral vector-based SARS vaccines [109–111], MERS vaccines can also be constructed using viral vectors that express major MERS-CoV proteins, normally the S protein. Several such MERS vaccine candidates have been developed and/or tested for efficacy in hDPP4-expressing mouse models or camels [64,97,100,101].

Ad5 or Ad41 vector expressing full-length S or S1 protein of MERS-CoV induced S-specific antibody responses and/or T-cell responses in a mouse model via the intramuscular (i.m.) or intragastric route, effectively neutralizing MERS-CoV infection in vitro [97,102]. Also, i.m. or subcutaneous (s.c.) vaccination of mice with a MVA-based full-length S vaccine elicited MERS-CoV-specific CD8+ T cell response and neutralizing antibodies, protecting hDPP4-transduced mice against MERS-CoV challenge [100,101]. Intranasally or intramuscularly administered MVA-S vaccine induced mucosal immunity, particularly the neutralizing
antibodies, in dromedary camels, resulting in significant reduction of excreted infectious virus and viral RNA transcripts after MERS-CoV challenge [64]. Similarly, a recombinant MV-based MERS vaccine expressing full-length, or truncated, S protein of MERS-CoV induced robust MERS-CoV neutralizing antibodies and T cell responses, protecting mice transduced with hDPP4 from MERS-CoV challenge [72].

Although able to elicit strong immune responses and/or protection, viral vector-based vaccines might have some unwanted limitations in terms of safety and potency. For example, pre-existing immunity to Ad in the general human population may cause some adverse effects by the induction of vaccine antigen-specific immune responses, thus reducing the overall efficacy of this vaccine type [112–114]. In addition, production of neutralizing antibodies against viral vectors themselves has been demonstrated in MV-S and MVA-S-based MERS vaccines [64,72]. Furthermore, full-length S protein of SARS-CoV encoded by the vectors can also induce non-neutralizing antibodies that may mediate enhancement of virus infection or cause harmful immune responses, such as inflammation and enhanced hepatitis [115–117], special attention should be drawn when developing MERS-CoV full-length S protein-based viral vectored vaccines.

**Nanoparticle-based MERS vaccines**

Nanoparticles can be used as a delivery vehicle to develop MERS vaccines. Nanoparticles containing MERS-CoV full-length S protein can be prepared and purified from pellets of infected baculovirus insect cells. In the absence of adjuvants, these nanoparticles induced a lower level of MERS-CoV neutralizing antibodies in mice, while in the presence of adjuvants, such as aluminum hydroxide (Alum) or Matrix M1, such neutralizing antibodies were significantly increased and maintained. Also, Matrix M1 significantly promoted the production of neutralizing antibodies as compared with Alum [96]. Thus, adjuvants are required for MERS nanoparticle vaccines, and different adjuvants function differently in promoting the immunogenicity of these vaccines. Thus far, efficacy and protection have not been evaluated for this vaccine type in MERS-CoV challenge models.

**DNA-based MERS vaccines**

Like the full-length S gene of SARS-CoV, DNA encoding full-length S protein of MERS-CoV can also be utilized to develop MERS vaccines [70,118]. Indeed, i.m./electroporation of mice and rhesus macaques with a synthetic DNA encoding full-length S protein of MERS-CoV elicited potent virus-neutralizing antibodies and cellular immune responses, as represented by the secretion of INF-γ, TNF-α, and/or IL-2 cytokines in CD4+ and/or CD8+ T cells, as well as the production of neutralizing antibodies in immunized camels. In addition, immunized NHPs were protected against MERS-CoV challenge without demonstrating clinical or radiographic signs of pneumonia [70]. Since such DNA vaccines encode MERS-CoV full-length S protein, the potential induction of virus-enhancing antibody and harmful immune responses is possible.

**DNA prime/protein boosted MERS vaccines**

In addition to a DNA-alone vaccination strategy, DNA priming followed by protein boosting could be used to develop MERS vaccines and, as a result, expand the immunogenicity and
efficacy generated by DNA. In this combinational vaccination strategy, DNA was constructed to encode full-length S protein of MERS-CoV, while protein was expressed as the viral S1 subunit [84]. Results demonstrated that i.m./electroporation priming of full-length S DNA and i.m. boosting of S1 protein of MERS-CoV with Ribi or Alum (aluminum phosphate, AlPO$_4$) adjuvant in mice and rhesus macaques, respectively, induced robust neutralizing antibodies against MERS-CoV infection, conferring protection of NHPs against MERS-CoV-induced radiographic pneumonia. However, because of the containment of full-length S DNA in the vaccination regimen, the potential of vaccine-caused immunopathology needs to be investigated.

Subunit MERS vaccines

Protein-based subunit vaccines against MERS-CoV have been developed [66,67,71,103]. While some subunit vaccines are designed on the basis of the full-length S1 protein [84], the majority of them are based on viral RBD [66,67,71,103,119]. These RBD-based vaccines are evaluated for immunogenicity and protective immunity in a number of MERS-CoV animal models, including hDPP4-transduced and hDPP4-Tg mice, as well as NHPs [71,73,74,103,119,120]. The antigenicity and functionality of these RBD proteins have also been extensively investigated.

In general, subunit vaccines might not induce immune responses as strong as those induced by other vaccine types mentioned above. However, the immunogenicity of subunit vaccines could be significantly promoted in the presence of an ideal adjuvant via an appropriate route [65,74]. In addition, it is also essential to maintain a suitable conformation of the protein antigen in the vaccine, such as the MERS-CoV RBD proteins [66,67]. For example, both s.c. and i.n. immunization of MERS-CoV RBD protein adjuvanted with Montanide ISA51 or Poly(I:C) induced long-term, high titers of S-specific systemic IgG, IgA, and mucosal sIgA antibodies, potently neutralizing MERS-CoV infection [65]. After comparing several different RBD fragments of MERS-CoV S protein, a fragment containing residues 377–588 of RBD elicited the highest neutralizing antibody in mice and rabbits and was therefore identified as a critical neutralizing domain [66,68]. Moreover, since MF59 adjuvant improved the ability of RBD protein to elicit the highest titer of neutralizing antibodies of all adjuvants tested, it is considered an ideal adjuvant to use with RBD subunit vaccines [74]. Even low doses of the RBD antigen plus MF59 adjuvant elicited sufficient neutralizing antibodies against MERS-CoV infection [104]. In the presence of MF59 adjuvant, this RBD protein protected Ad5-hDPP4-transduced and hDPP4-Tg mice from MERS-CoV challenge [73,74]. Clearly, the identified critical neutralizing domain of MERS-CoV RBD protein maintained good conformational structure, strong antigenicity to bind specifically to MERS-CoV RBD-specific sera and neutralizing monoclonal antibodies, as well as intact functionality to interact with soluble and cell-associated hDPP4 receptors [66,68,121].

In terms of safety consideration, subunit vaccines should be accounted as the safest vaccine type. They do not contain viral genetic materials, but only include essential antigens for eliciting protective immune responses, thus excluding the possibility of recovering virulence or inducing adverse reactions [122–126]. Different from the vaccines based on the full-length S or S1 protein, RBD-based MERS subunit vaccines contain the major neutralizing
epitopes and lack non-neutralizing immunodominant domains, thus having minimum risk to induce non-neutralizing antibodies with potential to cause harmful immune responses or enhancement of virus infection [27,66,75].

**Summary and conclusions**

MERS-CoV, a newly emerging infectious coronavirus and a new Category C Priority Pathogen, has caused high mortality in humans, thus posing continual threats to public health and global safety. Since the emergence of MERS-CoV in 2012, tremendous progress has been made in the development of MERS vaccines and the evaluation of their efficacy in suitable animal models. Presently, no MERS vaccines are available for human use. This review explains immune responses resulting from MERS-CoV infection, describes MERS vaccine criteria, and presents available small animal models to evaluate the efficacy of MERS vaccines. Current advances in vaccine development are summarized, focusing on specific applications and limitations of each vaccine category. These MERS vaccines were categorized as recombinant virus, viral vectors, nanoparticles, DNAs, DNAs/proteins, and subunit vaccines, denoting specific applications and limitations of each category. Taken together, this review provides valuable guidelines toward the development of an effective and safe MERS vaccine.

**Expert commentary**

Several MERS candidate vaccines in development have demonstrated the ability to induce immune responses and/or neutralizing antibodies that protect against MERS-CoV infection. Based on the limitations of some of these vaccine candidates, as discussed above, it might be fruitful to establish standards against which to measure the specific role of humoral and cellular immune responses relative to protection against MERS-CoV infection, and further evaluate the efficacy and correlation between immunogenicity and protection.

In addition to efficacy, safety is an important issue for any MERS vaccine. Experience garnered from SARS vaccine studies has demonstrated that vaccines based on the full-length S protein of SARS-CoV may induce non-neutralizing antibodies with enhancing effect on virus infection or harmful immune responses, or cause immunopathological effect, such as inflammation and increased severity [115,116]. Thus, when developing MERS vaccines based on the full-length S protein, precautions should be taken against the induction of harmful immune responses and/or virus-enhancing antibodies potentially resulting from its non-neutralizing epitopes in the immunodominant domains. Concomitantly, the immunopathological effects of these MERS vaccines should be investigated. Other safety tests, such as toxicity experiments, are also recommended before moving a MERS vaccine candidate to human clinical trials or patient use.

One may argue that no virus-enhancing antibody induced by full-length S protein of MERS-CoV has been reported so far. Indeed, there had been no report on antibody-mediated enhancement of SARS-CoV infection for 8 years since the virus was first identified in Guangdong Province, China in 2003. However, Jaume M et al [117] reported in 2012 that a SARS vaccine based on the full-length S protein could induce in mice virus-neutralizing
antibodies tested in Vero E6 cell culture, and virus-enhancing antibodies, via an FcγR-dependent manner, detected in cultures of THP-1, Raji, and Daudi cells that express FcγR. This finding suggests that virus-enhancing antibodies induced by the full-length S protein of MERS-CoV may be detectable if an appropriate assay system is used. Therefore, it would be especially important to investigate the potential of these MERS vaccines to induce virus-enhancing antibodies and harmful immune responses, and to cause immunopathological effects before moving a MERS vaccine candidate into human clinical trials. A lesson should be learned from the development of SARS vaccines – a shift from developing vaccines based on the full-length S protein at the beginning to developing RBD-based vaccines at the end.

Different from the full-length S protein, RBD of MERS-CoV S protein contains a critical neutralizing domain and lacks immunodominant domains with non-neutralizing epitopes, thus is safe and highly immunogenic to induce potent neutralizing antibodies and protective immunity against MERS-CoV infection. In comparison with other vaccine categories, such as recombinant viruses and viral vectored vaccines, subunit vaccines, including those based on the RBD, maintain higher safety profile due to the absence of viral genetic materials from the infectious viruses. The major conformational neutralizing epitopes in MERS-CoV RBD may attribute to RBD’s ability to induce neutralizing antibodies against both wildtype and mutant viral strains, since a viral strain with mutations in one epitope may still be sensitive to the neutralizing antibodies induced by other epitopes in RBD [75,84], demonstrating RBD’s capacity to elicit broad-spectrum neutralizing antibodies and cross-protective immunity. Therefore, similar to RBD-based SARS vaccines [11,127–133], subunit vaccines based on MERS-CoV RBD have the greatest potential for further development as an effective and safe vaccine candidate. It is noted that in addition to RBD, other regions, such as N-terminal domain, in S1 fragment of MERS-CoV may also possess some neutralizing epitopes [119,134]. Thus, combining RBD and S1 N-terminal domain in a subunit vaccine may result in synergistic effect in inducing broadly cross-neutralizing antibodies against divergent MERS-CoV strains.

At present, two full-length MERS-CoV S candidate vaccines, one based on MVA and the other on DNA, have been scheduled for clinical trials [98,99]. With the continual increase and extensive research of promising MERS vaccines in preclinical studies, more and more candidates with high efficacy and strong safety should be pushed forward to clinical trials for prevention of MERS-CoV infection.

**Five-year view**

In the next five years, more robust, affordable small animal models should be developed to help evaluate the efficacy of MERS vaccines. Comprehensive studies on the efficacy and safety of MERS vaccines are expected. Since MERS-CoV RBD-based subunit vaccines induce strong immune responses and neutralizing antibodies and maintain the highest safety profile, such vaccines are expected to increase in number, and with investment from government and Big Pharma, it is further expected that such vaccines will be brought to clinical trials in an expeditious manner and, upon approval, be used for preventing MERS-CoV infection in humans and for building biodefence stockpiles.
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**References**

Reference annotations

* Of interest

** Of considerable interest


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### Key issues

- Since its first identification in Saudi Arabia in 2012, Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV) has infected at least 1,638 persons worldwide, including 587 deaths, as of February 02, 2016, with Saudi Arabia and South Korea having the first and second largest MERS cases, respectively.

- MERS-CoV uses bats and dromedary camels as the most likely natural reservoirs and intermediate transmission hosts. Human-to-human transmission has been confirmed. Therefore, as a newly added Category C Priority Pathogen, MERS-CoV poses a threat to public health and global safety, highlighting the importance of developing effective and safe MERS vaccines.

- Among the four major structural proteins of MERS-CoV, spike (S) protein is the most important in viral pathogenesis. MERS-CoV depends on the S protein (S1 and S2 subunits) to bind the cellular receptor dipeptidyl peptidase 4 (DPP4) through the receptor-binding domain (RBD), followed by mediating MERS-CoV entry into target cells. As such, the viral S protein and RBD are major vaccine targets.

- Similar to MERS-CoV infection, MERS vaccines can trigger antigen-specific humoral, mucosal, and/or cellular immune responses. While cellular immune responses might be required to clear or kill virus, humoral immune responses, particularly neutralizing antibodies, play critical roles in protecting against MERS-CoV infection.

- Several animal models, including small animal models expressing hDPP4 receptor, have been developed to evaluate the efficacy of MERS vaccines.

- No MERS vaccines are available for human use. MERS vaccines under development are in preclinical stages, some of which are scheduled for human clinical trials. These vaccines are categorized as recombinant virus, viral vectors, nanoparticles, DNAs, DNAs/proteins, and subunit vaccines, the majority of which are based on the viral S protein.

- In addition to the major neutralizing epitopes in RBD, the full-length S protein also contains some immunodominant domains with non-neutralizing epitopes that can induce non-neutralizing antibodies, some of which may mediate enhancement of viral infection or harmful immune responses, or cause immunopathological effects, as those induced by the full-length S protein of SARS-CoV.

- The RBD in the S1 subunit of MERS-CoV S protein contains major neutralizing epitopes and lacks immunodominant domains with non-neutralizing epitopes, thus having much less risk to induce virus-enhancing antibody or harmful immune responses, and better potential...
than full-length S protein to be developed as an effective and safe MERS vaccine.
Figure 1. Potential MERS-CoV transmission routes and MERS-CoV-infection hosts
Bats are the most likely natural reservoir of MERS-CoV, and dromedary camels are potential intermediate hosts. Human-to-human transmission of MERS-CoV may easily occur through healthcare facilities or within family clusters.
Figure 2. Classification of coronavirus genera
The four coronavirus genera are α, β, γ, and δ coronaviruses. Each coronavirus genus contains different subclasses. Letters in blue indicate coronaviruses that have caused human infection.
Figure 3. MERS-CoV genome and schematic structure of viral proteins

(A) The MERS-CoV genome consists of 2 partially overlapping replicase open reading frames (OFR1a and 1b) and several downstream ORFs that encode viral functional structural proteins and other proteins with unknown function. (B) Schematic structure of major MERS-CoV structural proteins. (C) Schematic structure of MERS-CoV S protein. SP, signal peptide; RBD, receptor-binding domain; RBM, receptor-binding motif; FP, fusion peptide; HR1 and HR2, heptad repeat 1 and 2; TM, transmembrane domain; CP, cytoplasmic tail.
Immunization of MERS vaccines may activate naïve B cells to differentiate into plasma cells and produce serum IgG, IgA, and/or secretory immunoglobulin A (sIgA) antibodies to bind MERS-CoV. Antibodies with neutralizing activity will block binding between MERS-CoV and its receptor dipeptidyl peptidase-4 (DPP4) at the cell surface, thus inhibiting virus entry into target cells. Naïve CD4+ and CD8+ T cells can also be activated to produce cytokines and/or function as cytotoxic T lymphocytes (CTLs) to destroy MERS-CoV-infected target cells. Some memory B (Bm) and T (Tm) cells may be activated after further stimulation or boost vaccination, and play a role in humoral and cellular immune responses.
### Table 1

Current animal models being developed to evaluate the efficacy of MERS vaccines *

<table>
<thead>
<tr>
<th>Animal models</th>
<th>Symptoms</th>
<th>Characteristics</th>
<th>Vaccines tested</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus macaques</td>
<td>Animals developed lower respiratory tract infection; MERS-CoV caused mild to marked interstitial pneumonia, with virus replication in alveolar pneumocytes; presented clinical signs; produced neutralizing antibodies</td>
<td>Lacked severe disease; MERS-CoV tropism limited to lower respiratory tract</td>
<td>DNA, DNA/protein, subunit vaccines</td>
<td>[70,77,78,84]</td>
</tr>
<tr>
<td>Common marmosets</td>
<td>Animals developed severe pneumonia; extensive lesions and high viral loads in lungs detected</td>
<td>Severe, partially lethal, disease model</td>
<td>Not identified</td>
<td>[79]</td>
</tr>
<tr>
<td>Camels</td>
<td>Animals developed upper respiratory tract infection with virus replication and clinical signs</td>
<td>MERS-CoV tropism extended to upper respiratory tract</td>
<td>DNA, viral vector vaccines</td>
<td>[16,64,70]</td>
</tr>
<tr>
<td>Rabbits</td>
<td>Infectious virus detected in lungs and upper respiratory tract</td>
<td>No significant histopathological changes or clinical signs</td>
<td>Not identified</td>
<td>[85]</td>
</tr>
<tr>
<td>Ad5-hDPP4 mice</td>
<td>Animals developed pneumonia; showed clinical disease and histopathological changes in lungs</td>
<td>Transient expression; no mortality</td>
<td>Viral vector, subunit vaccines</td>
<td>[72,83]</td>
</tr>
<tr>
<td>Humanized (HuDPP4) mice</td>
<td>Expressed HuDPP4 in lungs; virus replication and pathology detected in lungs</td>
<td>No clinical signs of disease or mortality</td>
<td>Not identified</td>
<td>[86]</td>
</tr>
<tr>
<td>hDPP4-Tg mice</td>
<td>Animals developed progressive pneumonia; viral replication detected in lung and brain; produced neutralizing antibodies</td>
<td>Lethal disease model</td>
<td>Subunit vaccines; virus replicon particle (VRP) vaccines</td>
<td>[73,80–82]</td>
</tr>
</tbody>
</table>

* Ad5-hDPP4: adenovirus 5-human DPP4-transduced mice; hDPP4: human dipeptidyl peptidase-4; hDPP4-Tg mice: human DPP4-transgenic mice; MERS, Middle East respiratory syndrome; MERS-CoV, Middle East respiratory syndrome coronavirus.

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Table 2
Summary of current vaccines being developed to prevent MERS-CoV infection

<table>
<thead>
<tr>
<th>Vaccine categories</th>
<th>Immunogenicity and protection</th>
<th>Immunization routes</th>
<th>Adjuvant needed</th>
<th>Potential limitations</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant MERS-CoV vaccines</td>
<td>Not indicated</td>
<td>N/A</td>
<td>N/A</td>
<td>Possibility of recovering virulence</td>
<td>[46,47]</td>
</tr>
<tr>
<td>Viral vector-based vaccines</td>
<td>Induced antigen-specific humoral (IgG) and/or T cell immune responses, and neutralizing antibody in mice; protected hDPP4-transduced mice against MERS-CoV challenge; reduced MERS-CoV excretion after virus infection in dromedary camels</td>
<td>i.g., i.m., s.c., or i.n.</td>
<td>No</td>
<td>Pre-existing immunity; anti-vector responses; potential harmful immune responses by non-neutralizing epitopes of full-length S</td>
<td>[64,72,97,100–102]</td>
</tr>
<tr>
<td>Nanoparticles</td>
<td>Induced MERS-CoV neutralizing antibody in mice in the presence of adjuvants, particularly Matrix M1</td>
<td>i.m.</td>
<td>Yes: Alum, Matrix M1</td>
<td>Potential harmful immune responses by non-neutralizing epitopes of full-length S</td>
<td>[96]</td>
</tr>
<tr>
<td>DNA vaccines</td>
<td>Induced antigen-specific neutralizing antibody and cellular immunity in mice, NHPs and camels; protected NHPs from MERS-CoV challenge</td>
<td>i.m./AP system</td>
<td>No</td>
<td>Potential side effects; harmful immune responses by non-neutralizing epitopes of full-length S</td>
<td>[70]</td>
</tr>
<tr>
<td>DNA prime/protein-boost vaccines</td>
<td>Induced robust serum-neutralizing antibody in mice and NHPs from MERS-CoV challenge</td>
<td>i.m./AP system</td>
<td>Yes: Ribi, Alum, AlPO4</td>
<td>Potential harmful immune responses by non-neutralizing epitopes of full-length S</td>
<td>[84]</td>
</tr>
<tr>
<td>Subunit vaccines</td>
<td>Induced strong humoral and mucosal immune responses and potential neutralizing antibody in mice and/or rabbits; elicited T cell responses in mice; protected hDPP4-mice and NHPs from MERS-CoV challenge</td>
<td>i.m., s.c., or i.n.</td>
<td>Yes: Alum, MF59, Montanide, Poly(I:C)</td>
<td>Need to maintain suitable protein conformation; require appropriate adjuvant, route, or dose</td>
<td>[65,66,71,73,74,103,104]</td>
</tr>
</tbody>
</table>

*Alum, aluminum hydroxide; AlPO4, aluminum phosphate; AP system, AgilePulse® In Vivo Electroporation; hDPP4, human dipeptidyl peptidase-4; i.g., intragastric; i.m., intramuscular; i.n., intranasal; s.c., subcutaneous; MERS-CoV, Middle East respiratory syndrome coronavirus; S, spike.