DOI : 10.5958/2277-8934.2023.00002.4 *Vol 30 No 1, p 9-17*

MVA-BASED VACCINE EFFICACIES ON THE IMMUNE RESPONSE AND SEROPREVALENCE OF MERS-COV: A SYSTEMATIC REVIEW

Ahmed Alsaleem[1] and Mahmoud Kandeel[1,2]

[1]Department of Biomedical Sciences, College of Veterinary Medicine, King Faisal University, 31982 Al-Ahsa, Saudi Arabia

[2]Department of Pharmacology, Faculty of Veterinary Medicine, Kafrelsheikh University, 33516 Kafrelsheikh, Egypt

ABSTRACT

Modified vaccinia virus Ankara (MVA) is an attenuated type of poxvirus vaccine. Many vaccines against infectious diseases, which include influenza, HIV/AIDS, tuberculosis, cancer and lately, Ebola virus and MERS-CoV, have been developed using MVA as a viral vector. MERS-CoV encodes 4 structural proteins, nucleocapsid (N) or spike (S), also the membrane (M), in addition to (envelope) (E) (proteins), are all encoded by the MERS-CoV genome. Most viral vector-based MERS vaccines exhibit immunogenicity in vaccinated animals and use the full-length S or S1 protein of MERS-CoV as the coding antigen. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines were utilised. The research strategy used keywords, keyword combinations, MeSH terms, field tags, Boolean operators "AND" and "OR," and truncations. Search strings were built from these elements to ensure an accurate acquisition of the best output. The population, exposure, control, outcome, and studies (PECOS) criteria were used in this study. In a homologous vaccination regimen, MVA-MERS-S generated potent antibodies as well as specific B-cells, although T-cell responses showed a heterogeneous design among cohorts. A booster or third immunisation is significant because it increases the longevity and levels of antibodies and B-cells specific to MERS-CoV-S. Furthermore, the antibodies' levels and capacity to neutralise is improved after the late booster immunisation. Follow-up studies and large-scale clinical trials are required to confirm the circulation and immunity status against MERS-CoV in camels.

Key words: MERS-CoV, meta-analysis, MVA, spike protein, seroprevalence, vaccine

Modified vaccinia virus Ankara (MVA) is an attenuated type of poxvirus vaccine (Altenburg *et al*, 2014). Many vaccines against infectious diseases, including influenza, Middle East respiratory syndrome corona virus(MERS-CoV), HIV/AIDS, tuberculosis, cancer and lately, Ebola virus infection, are developed using MVA as a viral vector (Alharbi, 2019). The MVA strain of the vaccinia virus has 6 significant genomic deletions compared to the parental virus, which attenuated it by repetitive passage in chick embryo fibroblasts (CEFs). However, replication of MVA is low in most mammalian cells but robust in CEFs (Blanchard *et al*, 1998). MVA is a virus deficient in replication that has been tested in several experiments and has superior immunogenicity and profile of safety (Sutter and Staib, 2003). Primarily, this MVA vaccine has been used as a booster in heterologous prime-boost vaccination regimens, enhancing recombinant antigen-specific T-cells that have already been primed. Oftentimes, adenoviral vectors are employed in these schedules to accomplish the priming immunisation (Gilbert *et al*, 2006; Reyes-Sandoval *et al*, 2010).

Since its initial detection in 2012, the Middle East respiratory syndrome (MERS) virus has spread to many countries (Masood *et al*, 2020). There is evidence that human dipeptidyl peptidase 4 (hDPP4) is a functional receptor for MERS-CoV(Wang *et al*, 2013). There is still widespread concern about MERS-CoV infections in both humans and camels. Proof of human-to-human transmission of MERS-CoV has been found (De Wit *et al*, 2016). Humans who have any kind of contact with MERS-CoV-infected camels may be at a higher risk of contracting the virus. MERS-CoV remains a persistent threat to global health security, with new cases and outbreaks occurring regularly in the Middle East. MERS-CoV was shown to have a moderate to high prevalence, but a high seroprevalence. Despite the virus's extensive presence in camel herds, zoonotic

SEND REPRINT REQUEST TO MAHMOUD KANDEEL email: mkandeel@kfu.edu.sa

transmissions were less common (Kandeel, 2022). Effective interventions, such as vaccinations and medications are urgently needed to halt the spread of MERS-CoV among camels and prevent transmissions from camels to humans, as well as to treat and prevent infections among people.

MERS-CoV encodes four structural proteins nucleocapsid (N), spike (S), membrane (M)and envelope (E) proteins (Malik, 2020). Vector-based MERS vaccines exhibit immunogenicity in vaccinated animals and have their coding antigen as proteins S1 or the full-length spike protein (Zhou *et al*, 2018). Immunised mice produced MERS-CoV S-specific antibody responses in response to recombinant Ad5 vectors that encode the full-length or S1 extracellular domain of the MERS-CoV S protein, neutralising MERS-CoV infection *in vitro* (Kim *et al*, 2014). Infected mice have produced MERS-CoV-specific antibody responses, neutralising antibodies, and T-cell responses in response to Ad5 or Ad41 vectors expressing full-length S protein of MERS-CoV. MVA-MERS-S, an MVA-based viral vaccine that expresses viral S protein, showed effectiveness against MERS-CoV infection in Ad5/DPP4-transduced mice. MVA-MERS-S is a promising vaccination candidate due to its high rate of genetic stability and favourable development properties. Serum antibodies against MERS-CoV were abundant in vaccinated mice. Therefore, MVA-MERS-S could be used to advance research toward an urgently needed vaccine against MERS-CoV(Song *et al*, 2013).Vaccines with viral carriers like the measles virus, adenovirus and MVA can trigger strong humoral and cellular immune reactions (Rollier *et al*, 2011). In dromedary camels exposed to MERS-CoV, an MVA-based, full-length S MERS-CoV vaccine candidate (MVA-S) elicited mucosal immunity and decreased viral shedding (Haagmans *et al*, 2016; Volz *et al*, 2015). In addition, less infectious MERS-CoV particles and little to no viral RNA were found in the nasal cavities of immunised camels.

Developing MERS vaccine candidates has been challenging due to a lack of understanding of the mechanisms that determine protective immunity and the difficulties of conducting effective trials. Although, optimal animal models for discovering protective immunological correlates for MERS are still missing, current mice models show that antibodies and T-cells are crucial for building protective immunity. This study looks into how well MVA-based vaccines protect against MERS-CoV.

Materials and Methods

Study Design

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were used. The preparation for this work also included the use of PRISMA extensions published in the Cochrane Handbook for Systematic Reviews and Extensions – Chapter 4 (Page *et al*, 2021).

Search Strategy

This study identified Embase, Cochrane Central, PubMed, and Google Scholar as the electronic databases for research. The search strategy used keywords, keyword combinations, MeSH terms, field tags, Boolean operators "AND" and "OR," and truncations. Search strings were built from these elements to ensure an accurate acquisition of the best articles. Search strings used in the study included "MVA-Based"[All Fields] AND ("vaccin"[Supplementary Concept] OR "vaccin"[All Fields] OR "vaccination"[MeSH Terms] OR "vaccination"[All Fields] OR "vaccinable"[All Fields] OR "vaccinal"[All Fields] OR "vaccinate"[All Fields] OR "vaccinated"[All Fields] OR "vaccinates"[All Fields] OR "vaccinating"[All Fields] OR "vaccinations"[All Fields] OR "vaccination's"[All Fields] OR "vaccinator"[All Fields] OR "vaccinators"[All Fields] OR "vaccine s"[All Fields] OR "vaccined"[All Fields] OR "vaccines"[MeSH Terms] OR "vaccines"[All Fields] OR "vaccine"[All Fields] OR "vaccins"[All Fields]) AND "vaccin"[Supplementary Concept] OR "vaccin"[All Fields] OR "vaccination"[MeSH Terms] OR "vaccination"[All Fields] OR "vaccinable"[All Fields] OR "vaccinal"[All Fields] OR "vaccinate"[All Fields] OR "vaccinated"[All Fields] OR "vaccinates"[All Fields] OR "vaccinating"[All Fields] OR "vaccinations"[All Fields] OR "vaccination's"[All Fields] OR "vaccinator"[All Fields] OR "vaccinators"[All Fields] OR "vaccine's"[All Fields] OR "vaccined"[All Fields] OR "vaccines"[MeSH Terms] OR "vaccines"[All Fields] OR "vaccine"[All Fields] OR "vaccins"[All Fields]. Identified articles were sought to get the most relevant for this study.

Eligibility Criteria

The researchers selected eligibility guidelines for the selection of studies to be included in this study. The population, exposure, control, outcome, and studies (PECOS) criteria were used in this study. The population in the included studies had a high risk of getting MERS-CoV. Exposure considered for

inclusion was to be vaccination with an MVA-based vaccine. This study had no specific comparator for the control. The efficacy of the MVA-based vaccine was the outcome prioritised for this study. Study designs considered for this study were any studies that indicated the vaccination of MVA-based vaccines for MERS-CoV. Consideration was also done for articles with different study designs but had very relevant material. The studies had to have clinical or experimental trials for the MVA-based vaccines. Only English-published articles or those translated were considered for inclusion.

Data Extraction

Two researchers conducted the extraction of data. A pre-designed excel worksheet was used in the recording of extracted data. Information on the authors, year of publication, outcomes, and the results of the included studies was extracted. Engagement between the two researchers was constant to ensure the results' congruence. A third party quelled disputes that arose.

Quality Assessment

Utilising the Critical Appraisal Skills Program (CASP) standard checklist, the quality of the included studies was evaluated. Four sections were used to conduct the quality assessment. Three questions were used in Section I to verify the study designs of the included studies. Three questions were used to evaluate the listed studies' methodological soundness. Three questions were used to evaluate the results' validity, and two were used to evaluate their applicability. The answers "YES," "NO," and "CAN'T TELL" were used for evaluation. "Y," "N," and "CT" were used as the abbreviations for these answers. The studies were ranked for quality assessment, with 11 being the highest score possible, using the responses from the checklist. Studies of excellent quality received scores between 8 and 10. Scores of 6 and 7 were considered to be of moderate quality, while scores of 5 or less were considered to be of low quality. The questions used in the checklist are displayed in the table 1.

Results Analysis

This study made use of only one form of investigative analysis. The method used was a qualitative assessment and a systematic review. Literal analysis was also conducted from the included studies.

Table 1. CASP standard checklist used in this systematic review.

Validity of the Study Design
C1. Was the research question from the study focused?
C2. Was randomisation of the participants towards the interventions done?
C3. In conclusion, was there accountability of the participants?
Methodological Soundness of the Study
C4. Was blinding done for the following:
• The participants?
• The investigators?
• Results analysers?
C5. Were the study groups similar at the start of the trial?
C6. Was there a similarity in the level of care among the study groups and the participants?
Validity of the Results
C7. Was a comprehensive report on the effects of the interventions done?
C8. Was there a report on the precision of the effect of treatment or the estimate of the intervention?
C9. Were the efficacies of MVA-based vaccines identified?
Applicability of the Results
C10. Was there compatibility between the population and the results?
C11. Was there a benefit in the application of implantable cardioverter defibrillators compared to those without?

Results

Study Selection

One hundred and six articles were identified from the electronic databases used in the study strategy. Of these, 19 articles were excluded as duplicates. The remaining 87 studies were then screened using titles and abstracts to determine their suitability. Sixty-nine studies were excluded thus remaining only 18 studies. Further screening was conducted, which led to exclusion of 9 studies. Nine research/studies in total have been used in this systematic review. The PRISMA flow chart (Fig 1) shows the study selection process.

Study Characteristics

Information obtained from incorporated research/study was as shown in Table 2.

Quality Analysis

The study below shows the quality assessment conducted using the CASP checklist.

Table 1. Study characteristics including subjects, vaccine, test groups, outcomes and results summary.

Studies	Year	Subjects	Vaccine development	Test groups	Outcomes	Results summary
Alharbi *et al*	2022	Mice and Camels	(Encoding) (Full)-(length) (spike)of the proteins for(MERS)-(CoV) and(M.V.A)-(MERS)	Three mice groups (Group 1: (MVA)-wt)/(MVA)-(MERS); and the second (Group): (MVA)-(MERS)/(MVA)-(wt); including third Group: (MVA)-(MERS)/(MVA)-(MERS). 3 (camel)-(groups) (Group 1: (MVA)-(wt)/(MVA)-(MERS); second Group: (MVA)-(MERS)/(MVA)-(wt); including the third group: (MVA)-(MERS)/(MVA)-(MERS)	Humoral immune responses (Antibody (Ab))	A high dosage of (MVA-MERS) was included to induce stronger (Ab-responses) for the mice as well as camels and included(neutralising-antibodies). (MVA-MERS)–(vaccine), that was administered via (homologous) for (prime–boosting) regimen, inducing (high-levels) (neutralising) anti-(MERS-CoV) antibodies into mice as well as in the camels. (ELISA) in every(time-point): (Log10) end-point (titers)
Alharbi *et al*	2017	Mice	(Full-length) spike-for(MERS-CoV)	(Heterologous)(prime-boost) regimen – vaccines were given to the mice with (ChAdOx1) (MERS) together with boosting them with an (MVA-MERS) that was 28d.p.i. (Homologous-regimens) – vaccines were administered to mice which had (MVA-MERS) including boosting them with an (MVA-MERS) that was (21d.p.i).	Humoral immunogenicity	Both vaccine candidates with a sample of (14) including(28d.p.i) which induced higher levels for(S1), (specific) for (antibodies) with a mean-(endpoint)-(titre) of (Log10)=(4.8) and a (t.P.A) of (4.7) and without (tPA), besides controlled vaccines, (ChAdOx1)- encoding enhancing greener (fluorescent) (protein) of (ChAdOx1)-(e-GFP), with a mean-endpoint-titre of (Log10)=(1). (ELISA) with a mean-endpoint-titre (Log10)= (3.2)
Koch *et al*	2020	Humans	(Full-length) (MERS-CoV) with a (spike-glycoprotein)	Prime immunisation: Low dose group got; doses of (1×107) (plaque), (foming) units (P.F.U), High dose group; (1×10^8) (P.F.U). Booster shot: similar dose, which was 28-days later.	Frequency as well as the severity of adverse events, immunogenicity	Adverse events: (67, 10) out of (14) persons who participated in (the low-dose-group), (The high-dose-group) were (10) out of (12). Pain, fatigue, swelling, and indurations. (Sero-conversion) after booster: (9/12) of (low-dose-group) together with (111/11) of (high-dose-group). (Biding-antibody-titers) matched up (ME, RS-CoV) specific (neutralising) antibody). ELISA: (Spearman) matched up with(r=086) and (95%) CI, of (0·6960–0·9427), (p=0·0001)
Fathi *et al*	2022	Humans	(Fulpike)(glycol-protein) (MERS CoV-S)	(Homologous) primary immunisation: (Low) (dose) group); (1×10^7) (PFU), (High-dose-(group); (1×10^8) (PFU) Booster; after one year (±4months). Re-enrolment of ten persons to participate; three from(LD) and the other seven from (HD) groups: a dosage of (1×10^8)(PFU)	Safety and immunogenicity	Adverse events: (51) in (9/10) Participants. (40/51) were related to the vaccine. (Reactogenicity) and fatigue/malaise. (32/40) were mild cases. (37/40) were solicited. Occurrence of adverse events (AE): median 1 day. ELISA-optical-density (OD) (0.08) of (95%) CI (0.03–0.13). Elevated frequency and persistence for spike-specific (B-cells) were observed from a (late-booster-immunisation), which binds (immunoglobulin-G1) - (IgG1) as well as (neutralising-antibodies) and not (T-cell) response.

Table 1. Continued

Song *et al*	2013	Mice	Recombinant MVA, mature full-length S glycoprotein	Immunisation: (10^8) (PFU) (MVA)-(MERS) – (S) at zero and three weeks.	Immunogenicity	Every animal is given a (booster- immunisation) shortly after producing (circulating-antibodies) elevated levels, neutralising (MERS-CoV). (Serum-samples) did not detect (neutralising-antibodies) in a controlled animal that was inoculated via (non-recombinant) (MVA) and either (saline).
Volz *et al*	2015	Mice	(Full-length) (MERS-CoV) spikes (S) (protein) (MVA)-MERS) (S)	(Single-subcutaneous) that is (s.c.) immunisation via dosage with (10^7) and either (10^8) (PFU)	Immunogenicity	(MVA-MERS) – (S) brought out noticeable MERS-CoV) – (neutralising-antibodies). Immunisation used to boost (s.c.) gives results with elevated titers for (MERS-CoV) – (neutralising-antibodies). (Serum), (antibody) (titers) (log2).
Weskamm *et al*	2022	Humans	(MVA)-(MERS) – (S) that encodes (MERCoV) – (spike), (protein)	Administering of 3(homologous immunisations) during 0 to 28 days. (Booster-vaccination) were given at months (12) ± (4).		ELISA:r = 0.9383, p < 0.0001, 50% plaque-reduction neutralisation test (PRNT50),
Veit *et al*	2018	Mice	MERS CoV spike (S) protein	Female sets of (BALB/c) mices with (n=2) and (5) got immunised two times within intervals (21-days) for (10^8) (plaque forming units) (P.F.U) with recombinant (MVAMERS) – (N) and either (non-recombinant) (MVA) besides (P.B.S) used for (mock-vaccine).	Immunogenicity	Formation of (MVA-MERS) – (N) for the (recombinant-virus) using(CEF) infected with (MVA) together with (trans-infected) with (MVA) (vector-plasmid) (pIIIH5red) – (MERS) – (N).
Langenmayer *et al*	2018	Mice	(MERS-CoV) spikes (S) (protein)	Vaccination: (1×10^7) (PFU) together with(1×10^8)(PFU)	(MVA-MERS) – (S) distribution	(Real-time) (P.C.R) analysed via(> 240) (tissue-samples) detection(MVA-DNA)(pre-dominant) during injecting-site as well as draining (lymph nodes). The considerations of Level for (parenteral-site-inflammation) together with (hyperplasia) for (draining-lymph-nodes) incorporation with (immunological-response) into (vaccine-inoculation).

Discussion

MERS-CoV vaccines were created in addition to being tested *via* various animal models, including non-human primates, camels, and human clinical trials (Alharbi *et al*, 2017; Langenmayer *et al*, 2018). MVA-based vaccination that included full-length spike antigen testing was done in a mouse model and in dromedary camels (Alharbi *et al*, 2022). The vaccination elicited stronger T-cell mediated immune responses in mice, and increased levels for binding together with neutralising antibodies were obtained from camels. Homologous prime-boosters immunisation strategy in mice demonstrates the effectiveness of MVA viral vectors in priming and boosting. When compared to a heterologous prime-boost regimen, where for instance, primed with ChAdOx1based-vaccine together with boosting it with MVA encoding similar vaccine prompted elevated neutralising Abs of mouse models. It has been demonstrated that the use of MVA-based vaccine in prime and boost immunisation is suboptimal. Therefore, vaccines were established based on two alternative vectors, avoiding immune reactions unique to the priming vector. Additionally, it has been demonstrated that the vector-based vaccine is hindered by earlier immune reactions to a vaccine vector. Although, the MVA-based vaccine may not be influenced by pre-existing immunity

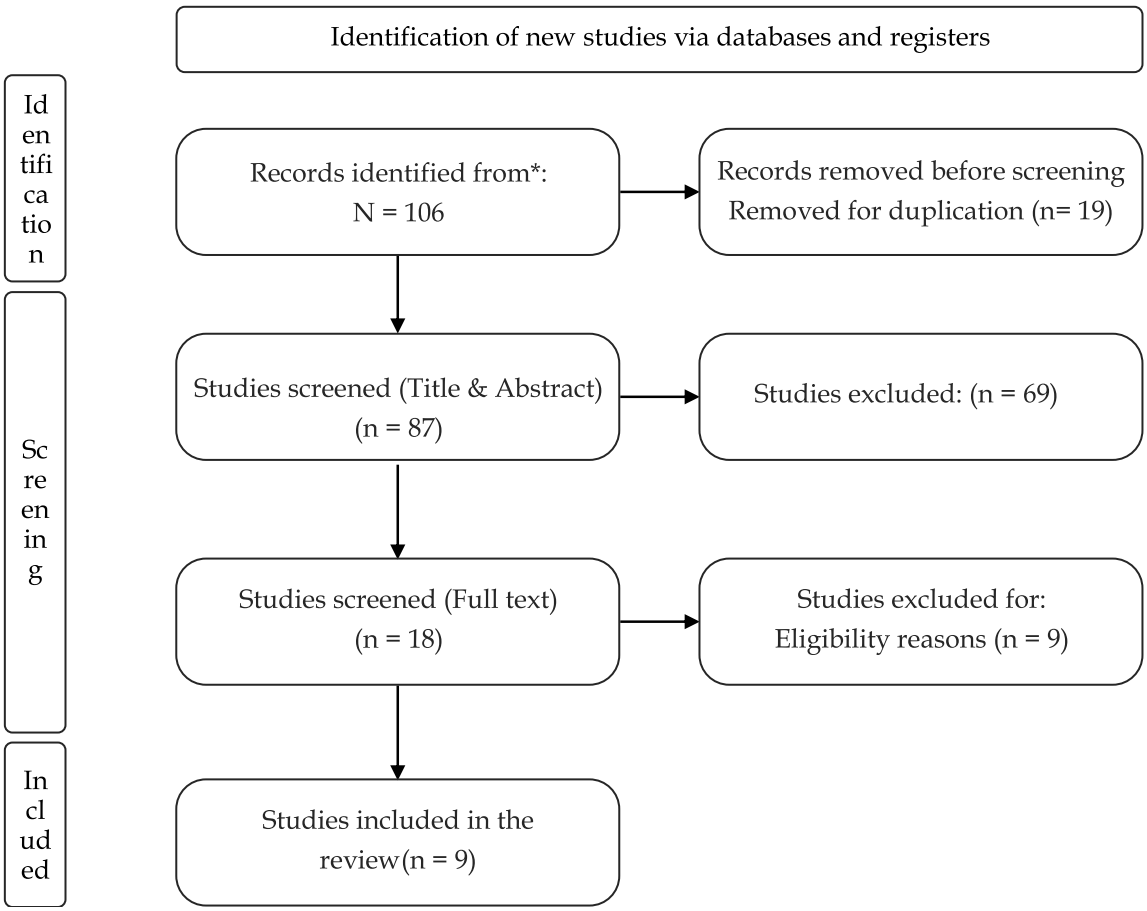

Fig 1. PRISMA flow diagram of studies in the systematic review.

Table 2. Quality Analysis of the included studies.

STUDY	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
Alharbi *et al*, 2022	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Alharbi *et al*, 2017	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Koch *et al*, 2020	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Fathi *et al*, 2020	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y
Song *et al*,	Y	CT	Y	Y	CT	Y	Y	Y	Y	Y	Y
Volz *et al*, 2015	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y
Weskamm *et al*, 2022	Y	CT	Y	Y	CT	Y	Y	Y	Y	Y	Y
Veit *et al*, 2018	Y	CT	Y	Y	CT	Y	Y	Y	Y	Y	Y
Langenmayer *et al*, 2018	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y

given that smallpox has been eradicated, there may still be problems with using MVA as a vector for numerous antigens or multiple vaccines, should any be approved in the future.

The anti-MERS-CoV spike Ab level was enhanced and unaffected by the anti-MVA-induced immune responses at prime. Anti-spike Ab levels that were at a high level in response to MVA-MERS were produced in mice with MVA-wt. This indicated that MVA has a potent effect on priming immunological reactions in animals with anti-MVA antibodies. The increase in anti-antigen Ab levels in homologous MVA vaccination regimens are often lower after the boost vaccination when compared to after prime immunisation. Most dromedary camels in Africa, the Middle East, and the Arabic Peninsula are MERS-CoV-seropositive. The prime vaccination with MVA did not appear to cause any elevation of spike Abs that were anti-MERS-CoV (Alharbi *et al*, 2022). After the boost vaccine, the immunisation displayed high neutralising and binding Abs levels.

The use of the tPA leader sequence being used by the F11 promoter and ChAdOx1 in MVA caused immunogenicity to increase slightly as compared to the use of mH5 promoter or no leader sequence, respectively (Alharbi, 2019). MVA vectored vaccine is the only MERS vaccine investigated in camels,

which also protected hDPP4 transgenic mice. Those were vaccinated with a requirement of two doses of a homologous prime-boost regimen administered both intramuscularly and intranasally to achieve protection partially and reduce viral shedding in camels.

According to Fathi *et al* (2022), in terms of the dynamics, kind, and severity of adverse events, the reactogenicity of the prime immunisation regimen and the booster vaccination appeared to be similar. Vaccination with two doses of MVA-MERS-S resulted in transient changes, specifically in haematologic parameters, which can be seen as a biological reaction to vaccination. A late MVA-MERS-S homologous booster was found to boost insert-specific immunity significantly. All participants in the study experienced an increase in anti-MERS-CoV-specific neutralising and binding antibody titers, regardless of whether they initially received low-dosage or high-dosage primary vaccinations, which was observed even if they had been unable to produce neutralising antibodies previously after the initial two-dose regimen.

In a study by Song *et al* (2013), all immunised animals developed significant circulating antibodies that neutralised MERS-CoV following booster immunisation. In contrast, control animals' blood samples given phosphate-buffered saline [PBS] or non-recombinant MVA injections did not show the presence of neutralising antibodies. According to Volz *et al* (2015) investigation, neutralising MERS-CoV antibodies were detectable following a single subcutaneous vaccination with 10^7 or 10^8 PFU of MVA-MERS-S. Increased titres of MERS-CoV-neutralising antibodies were the consequence of booster immunisations. Even a small dose of 10^6 PFU of MVA-MERS-S caused detectable neutralising antibodies. Similar antibodies were produced by MVA-MERS-S vaccination dosages of 10^7 and 10^8 PFU.

In Volz *et al* (2015) study, booster subcutaneous immunisations enhanced the quantity of IFN-γ-secreting MERS-S291-specific CD8+ T cells even more, especially with the lower dosage of 10^6 or 10^7 PFU of MVA-MERS-S. After single and prime-boost immunisations, intramuscular immunisations produced equal levels of CD8+ T-cell responses for all doses of the MVA-MERS-S vaccine. The intramuscular booster roughly tripled the amount of T-cell responses specific to MERS-S291. Histology revealed that the candidate vaccine in the investigation by Langenmayer *et al* (2018) did not cause organ lesions peripheral to the parenteral site or generalised lesions.

According to Weskamm *et al* (2022), the high-dose cohort experienced a seroconversion of 100% after receiving the first two doses of the MVA-MERS-S vaccine. These responses included T cell and antibody production. Immune reactions brought on by vaccination are frequently multilayered and can vary depending on the vaccine candidate. For instance, the strength and quality of immune response boosters can be affected by vector immunity, innate immunological reactions, and the stage of memory B cells (MBCs) maturation. It has been demonstrated that allowing more time between the prime and boost immunisations can significantly improve immunogenicity. It has been demonstrated that vaccines work better and produce stronger immune responses when the prime-boost period is increased from 28 to 84 days. Studies have shown that antigen-specific T-cell responses vary significantly between different cohorts. Antibody-secreting cells are effectively generated after the late boost.

According to Koch *et al* (2020), MVA-MERS-S vaccination generated both humoural and cellular immune responses to the MERS-CoV spike, mainly apparent after boost immunisation rather than prime immunisation. Most study participants reached baseline antibody levels by the end of the study, 6 months after vaccination, according to the humoral immune responses, which were assessed by ELISA and two different viral neutralisation assays. The humoral immune responses peaked at 42 and 56 days, were maintained through the 84^{th} day, and then declined to baseline levels.

In the Veit *et al* (2018) study, the MVA-MERS-N recombinant virus produced stable levels of antigen MERS-CoV N upon *in vitro* infection of cells from people, which indicated the unimpaired expression of the target gene at the level of late viral transcription using the synthetic, vaccinia virus-specific promoter PmH5. Additionally, antibodies from experimentally infected laboratory animals strongly recognised the MERS-CoV-N antigen generated in MVA-MERS-N infected cells, demonstrating that N-specific immune responses were potently activated upon MERS-CoV infection. According to descriptions of other viruses, the presence of N in these respiratory epithelial cells may lead to effective identification by innate and adaptive immune cells, causing strong protective immunity. As a result, the activation of N-specific immune responses in these animals highlights the MERS-CoV-N protein's potential use as a vaccine antigen. Veit *et al* (2018) claim that the MERS-CoV N protein can potentially effectively trigger CD8+

T cell responses specific to the virus. The MVA-MERS-N vector virus created by Veit *et al* (2018) for the study showed to be a stable recombinant virus that can be multiplied easily to produce vaccine formulations technically meeting all standards for further experimental or even medical development.

The study sought to investigate the efficacies of MVA-based vaccines on the seroprevalence of MERS-CoV. In a homologous vaccination regimen, MVA-MERS-S generated potent antibodies as well as specific B cells although, T-cell responses showed a heterogeneous design among cohorts. The late third immunisation is significant in that it increases the longevity and number of antibodies and B cells specific to MERS-CoV-S. After the late boost, the antibodies' levels and capacity to neutralise are reported to have stabilised. This supports a growing body of data that suggests late boosting may be a useful strategy for enhancing the immune response to CoVs generated by vaccination. This study showed that MVA-based vaccines on the seroprevalence of MERS-CoV were efficient.

Acknowledgement

The author extends his appreciation to the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (Project# GRANT2926).

Data availability statement

"All data are within manuscript. Further details can be requested from the corresponding author".

Funding statement

This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (Project# GRANT2926).

Conflict of interest disclosure

None

Ethics approval statement

Not apply

Patient consent statement

Not apply

Permission to reproduce material from other sources

Not apply

References

Alharbi NK. Poxviral promoters for improving the immunogenicity of MVA delivered vaccines. Human Vaccines and Immunotherapeutics. 2019; 15:203-209.

Alharbi NK, Aljamaan F, Aljami HA, Alenazi MW, Albalawi H, Almasoud A, Alharthi FJ, Azhar EI, Barhoumi T and Bosaeed M. Immunogenicity of high-dose MVA-based MERS vaccine candidate in mice and camels. Vaccines. 2022; 10:1330.

Alharbi NK, Padron-Regalado E, Thompson CP, Kupke A, Wells D, Sloan MA, Grehan K, Temperton N, Lambe T and Warimwe G. ChAdOx1 and MVA based vaccine candidates against MERS-CoV elicit neutralising antibodies and cellular immune responses in mice. Vaccine. 2017; 35:3780-3788.

Altenburg AF, Kreijtz JH, De Vries RD, Song F, Fux R, Rimmelzwaan GF, Sutter G and Volz A. Modified vaccinia virus ankara (MVA) as production platform for vaccines against influenza and other viral respiratory diseases. Viruses. 2014; 6:2735-2761.

Blanchard TJ, Alcami A, Andrea P and Smith GL. Modified vaccinia virus Ankara undergoes limited replication in human cells and lacks several immunomodulatory proteins: implications for use as a human vaccine. Journal of General Virology. 1998; 79:1159-1167.

De Wit E, Van Doremalen N, Falzarano D and Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. Nature Reviews Microbiology. 2016; 14:523-534.

Fathi A, Dahlke C, Krähling V, Kupke A, Okba NM, Raadsen MP, Heidepriem J, Müller MA, Paris G and Lassen S. Increased neutralisation and IgG epitope identification after MVA-MERS-S booster vaccination against Middle East respiratory syndrome. Nature Communications. 2022; 13:4182.

Gilbert SC, Moorthy VS, Andrews L, Pathan AA, McConkey SJ, Vuola JM, Keating SM, Berthoud T, Webster D and McShane H. Synergistic DNA–MVA prime-boost vaccination regimes for malaria and tuberculosis. Vaccine. 2006; 24:4554-4561.

Haagmans BL, van den Brand JM, Raj VS, Volz A, Wohlsein P, Smits SL, Schipper D, Bestebroer TM, Okba N, Fux R, Bensaid A, Solanes Foz D, Kuiken T, Baumgärtner W, Segalés J, Sutter G and Osterhaus AD. An orthopoxvirus-based vaccine reduces virus excretion after MERS-CoV infection in dromedary camels. Science. 2016; 351:77-81.

Kandeel M. Meta-analysis of seroprevalence and zoonotic infections of Middle East respiratory syndrome coronavirus (MERS-CoV): A one-health perspective. One Health. 2022; 15:100436.

Kim E, Okada K, Kenniston T, Raj VS, AlHajri MM, Farag EA, AlHajri F, Osterhaus AD, Haagmans BL and Gambotto A. Immunogenicity of an adenoviral-based Middle East Respiratory Syndrome coronavirus vaccine in BALB/c mice. Vaccine. 2014; 32:5975-5982.

Koch T, Dahlke C, Fathi A, Kupke A, Krähling V, Okba NM, Halwe S, Rohde C, Eickmann M and Volz A. Safety and immunogenicity of a modified vaccinia virus Ankara vector vaccine candidate for Middle East respiratory syndrome: an open-label, phase 1 trial. The Lancet Infectious Diseases. 2020; 20:827-838.

Langenmayer MC, Lülf-Averhoff A-T, Adam-Neumair S, Fux R, Sutter G and Volz A. Distribution and absence

of generalised lesions in mice following single dose intramuscular inoculation of the vaccine candidate MVA-MERS-S. Biologicals. 2018; 54:58-62.

Malik YA. Properties of coronavirus and SARS-CoV-2. The Malaysian Journal of Pathology. 2020; 42:3-11.

Masood N, Malik SS, Raja MN, Mubarik S and Yu C. Unraveling the epidemiology, geographical distribution, and genomic evolution of potentially lethal coronaviruses (SARS, MERS, and SARS CoV-2). Frontiers in Cellular and Infection Microbiology. 2020; 10:499.

Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R, Glanville J, Grimshaw JM, Hróbjartsson A, Lalu MM, Li T, Loder EW, Mayo-Wilson E, McDonald S, McGuinness LA, Stewart LA, Thomas J, Tricco AC, Welch VA, Whiting P and Moher D. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Revista Española de Cardiología (Engl ed). 2021; 74:790-799.

Reyes-Sandoval A, Berthoud T, Alder N, Siani L, Gilbert SC, Nicosia A, Colloca S, Cortese R and Hill AV. Prime-boost immunisation with adenoviral and modified vaccinia virus Ankara vectors enhances the durability and polyfunctionality of protective malaria CD8+ T-cell responses. Infection and Immunity. 2010; 78:145-153.

Rollier CS, Reyes-Sandoval A, Cottingham MG, Ewer K and Hill AV. Viral vectors as vaccine platforms: deployment in sight. Current Opinion in Immunology. 2011; 23:377-382.

Song F, Fux R, Provacia LB, Volz A, Eickmann M, Becker S, Osterhaus AD, Haagmans BL and Sutter G. Middle East respiratory syndrome coronavirus spike protein delivered by modified vaccinia virus Ankara efficiently induces virus-neutralising antibodies. Journal of Virology. 2013; 87:11950-11954.

Sutter G and Staib C. Vaccinia vectors as candidate vaccines: the development of modified vaccinia virus Ankara for antigen delivery. Current Drug Targets-Infectious Disorders. 2003; 3:263-271.

Veit S, Jany S, Fux R, Sutter G and Volz A. CD8+ T cells responding to the Middle East respiratory syndrome coronavirus nucleocapsid protein delivered by vaccinia virus MVA in mice. Viruses. 2018; 10:718.

Volz A, Kupke A, Song F, Jany S, Fux R, Shams-Eldin H, Schmidt J, Becker C, Eickmann M, Becker S and Sutter G. Protective Efficacy of Recombinant Modified Vaccinia Virus Ankara Delivering Middle East Respiratory Syndrome Coronavirus Spike Glycoprotein. Journal of Virology. 2015; 89:8651-8656.

Wang N, Shi X, Jiang L, Zhang S, Wang D, Tong P, Guo D, Fu L, Cui Y and Liu X. Structure of MERS-CoV spike receptor-binding domain complexed with human receptor DPP4. Cell Research. 2013; 23:986-993.

Weskamm LM, Fathi A, Raadsen MP, Mykytyn AZ, Koch T, Spohn M, Friedrich M, Bartels E, Gundlach S and Hesterkamp T. Persistence of MERS-CoV-spike-specific B cells and antibodies after late third immunisation with the MVA-MERS-S vaccine. Cell Reports Medicine. 2022; 3:100685.

Zhou Y, Jiang S and Du L. Prospects for a MERS-CoV spike vaccine. Expert Review of Vaccines. 2018; 17:677-686.