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Newcastle disease virus-based MERS-CoV candidate vaccine elicits high-level and lasting neutralizing antibodies in Bactrian camels

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Abstract
Middle East respiratory syndrome coronavirus (MERS-CoV), a member of the Coronaviridae family, is the causative pathogen for MERS that is characterized by high fever, pneumonia, acute respiratory distress syndrome (ARDS), as well as extrapulmonary manifestations. Currently, there are no approved treatment regimens or vaccines for MERS. Here, we generated recombinant nonvirulent Newcastle disease virus (NDV) LaSota strain expressing MERS-CoV S protein (designated as rLa-MERS-S), and evaluated its immunogenicity in mice and Bactrian camels. The results revealed that rLa-MERS-S showed similar growth properties to those of LaSota in embryonated chicken eggs, while animal immunization studies showed that rLa-MERS-S induced MERS-CoV neutralizing antibodies in mice and camels. Our findings suggest that recombinant rLa-MERS-S may be a potential MERS-CoV veterinary vaccine candidate for camels and other animals affected by MERS.

Keywords: Newcastle disease virus, MERS-CoV, neutralizing antibodies, camels

1. Introduction
Middle East respiratory syndrome coronavirus (MERS-CoV), a member of the c lineage in the genus Beta coronavirus, causes high fever, pneumonia, acute respiratory distress syndrome (ARDS), as well as extrapulmonary manifestations including gastrointestinal symptoms, lymphopenia, acute kidney injury (Yeung et al. 2016), hepatic inflammation, and pericarditis (Wong et al. 2015). So far, MERS-CoV is responsible for 1800 laboratory-confirmed cases of human infection, including at least 640 deaths (WHO, http://www.who.int/emergencies/mers-cov/en).

The majority of human cases have been reported in the Middle East, likely due to the existence of dromedary camels, which have been confirmed to carry live MERS-CoV and may be the potential source of human infections (Azhar et al. 2014; Memish et al. 2014). Furthermore, due to globalization, geographical barriers are more easily bypassed than in the past. Since bats are the potential natural hosts of MERS-CoV (Annan et al. 2013; Memish et al. 2013) and are in limited contact with humans, the most effective strategy would be to suppress circulation of the virus in camels before MERS-CoV escalates into a global pandemic in humans. With the increasing number of...
MERS cases, there is an urgent need to develop vaccines or specific drugs targeted at epidemic MERS-CoV (Modjarrad et al. 2016; Zumla et al. 2016).

Newcastle disease virus (NDV) belongs to the genus *Avulavirus* in the *Paramyxoviridae* family. NDV is classified as lentogenic (nonvirulent), mesogenic (moderately virulent), or velogenic (highly virulent) according to their pathogenicity in poultry. Lentogenic strains, such as the LaSota, have also been applied as a vaccine vector targeting human and other animal diseases (Ge et al. 2007, 2010; Di Napoli et al. 2010a, b). NDV is innately advantageous as a potential vaccine vector for the following reasons: Firstly, NDV is antigenically distinct from the mammalian paramyxoviruses, it does not typically cause an productive infection in mammals. Secondly, the pre-existing immunity against mammalian paramyxoviruses does not interfere with the replication capacity of NDV. In addition, the safety profile of NDV has been confirmed in many non-human primates as well as humans (Bukreyev et al. 2006; Bukreyev and Collins 2008; Khattar et al. 2010; Kortekaas et al. 2010).

As a membrane-anchored structural protein of MERS-CoV, spike (S) protein mediates viral receptor binding and entry (Belouzard et al. 2012; Millet and Whittaker 2014). S protein is the primary target for anti-coronavirus vaccine design (Zhao et al. 2014), and studies have demonstrated that S protein is immunogenic and can induce neutralizing antibodies which plays crucial role in anti-CoV infection (Hofmann et al. 2004; Enjuanes et al. 2008; Du et al. 2009; Pascal et al. 2015). Currently, several MERS-CoV candidate vaccines, such as DNA vaccines (Muthumani et al. 2015), virus like particles (VLPs) (Wang et al. 2016) as well as recombinant viral vector vaccines. Of note, the recombinant viral vector MERS vaccines, such as modified vaccinia Ankara (Song et al. 2013) or adenovirus (Kim et al. 2014) demonstrated good immunogenicity and provided protection for mice, nonhuman primates (NHPs) and camels against MERS-CoV challenge. Herein, we generated a recombinant NDV LaSota virus expressing MERS-CoV S protein and evaluated its immunogenicity in mice and camels.

2. Materials and methods

2.1. Viruses and cells

BHK-21 and Vero-E6 cells were grown in Dulbecco’s minimal essential medium (DMEM) containing 10% fetal bovine serum (FBS). The NDV vector virus rLa was rescued from the genomic cDNA of the NDV LaSota vaccine strain as previously described (Ge et al. 2007). Recombinant NDV was grown and titrated in 9-day-old specific-pathogen-free (SPF) embryonated chicken eggs by allantoic cavity inoculation. A recombinant Vesicular stomatitis virus (VSV) vectored virus expressing MERS-CoV S protein and enhanced green fluorescence protein (eGFP), designated as VSVΔG-eGFP-MERS, was used to determine the induction of neutralizing antibodies by MERS-CoV. The recombinant VSV vectored virus (VSVΔG-eGFP-MERS) was generated by replacing the G gene of the recombinant VSV expressing eGFP with the MERS-CoV S gene as described previously (Li et al. 2006; Liu et al. 2015). VSVΔG-eGFP-MERS was grown and titrated in Vero E6 cells. Modified vaccinia Ankara expressing the T7 RNA polymerase (kindly provided by Dr. Bernard Moss, the National Institutes of Health, Bethesda, MD, USA) was grown and titrated in primary chicken embryo fibroblasts (Wyatt et al. 1995). All viruses were stored at ~70°C before use.

2.2. Plasmid construction and virus rescue

To construct the full-length recombinant NDV genomic cDNA, MERS-CoV (GenBank accession no. KF186567.1) S gene was amplified by PCR from synthesized cDNA (Invitrogen, Shanghai, China) by using the following primers: 5’-GACTGGTTAAAACCTAGAAAAATACGGGTAGAAGTCCGACCATGATAACCTCAGTTTCCTACTG-3’, and 5’-GACTGGTTAAAACCTCATTGTGAACATGAACCTTATGGTGTGTACATTAGTGAACATGAGATCCGGCTCGAG-3’, in which the NDV gene end and gene start sequences (underlined), the optimal Kozak sequence (italic) and the Pmel restriction sites (bold) were introduced. S gene was introduced into NDV genomic cDNA through a unique Pmel site in the P-M intergenic region. The resultant plasmid was designated as prLa-MERS-S and used for virus rescue following established protocol as described previously (Ge et al. 2007). The rescued virus was designated as rLa-MERS-S. The presence of S gene in the NDV genome was confirmed by sequencing of the entire viral genome. S protein expression in rLa-MERS-S infected cells was confirmed by indirect immunofluorescence and Western blot assay.

2.3. Assessment of virus pathogenicity

The pathogenicity of rLa-MERS-S in poultry was determined by mean death time (MDT), intracerebral pathogenicity index (ICPI), and intravenous pathogenicity index (IVPI) in embryonated SPF chicken eggs or in SPF chickens according to the OIE Manual (OIE 2004).

To assess the pathogenicity of the recombinant viruses in mice, 2 groups of 10 six-week-old female Balb/c mice (Vital River, Beijing, China) were intramuscularly (i.m.) injected with 1×10⁹ EID₅₀ (50% embryo infectious dose) rLa-MERS-S or rLa in 0.1 mL diluted allantoic fluid and intranasally (i.n.) inoculated with 3×10⁸ EID₅₀ rLa-MERS-S or rLa in 0.03 mL diluted allantoic fluid. The third group of 10 mice was i.m. injected with 0.1 mL and i.n. inoculated with 0.03 mL PBS.
as mock infection control. Mice were monitored daily for signs of illness, weight loss, or death.

2.4. Immunization of mice and camels

For mouse immunization, 10 six-week-old female Balb/c mice (Vital River, Beijing, China) were i.m. vaccinated with 1×10⁸ EID₅₀ rLa-MERS-S in 0.1 mL diluted allantoic fluid. Three weeks after the first dose, mice were boosted with the same vaccine at the same dose and via the same route. For serological assay, 2 weeks after the first dose (prime) and the second dose (boost), mouse blood samples were collected from the retro-orbital sinuses under isoflurane inhalation anesthesia.

Camel immunization was carried out in the Experimental Animal Center of the College of Veterinary Medicine of Inner Mongolia Agricultural University, China. Three groups (n=5 per group) of adult Bactrian camels (provided by College of Veterinary Medicine of Inner Mongolia Agricultural University) were immunized. Group 1 animals were i.m. immunized with 2×10⁹ EID₅₀ rLa-MERS-S in 2 mL diluted allantoic fluid. Group 2 animals were immunized i.m. with 2×10⁹ EID₅₀ rLa in 2 mL diluted allantoic fluid, and Group 3 animals were i.m. injected with 2 mL PBS. Three weeks after the first dose, the camels were boosted with the same vaccine at the same dose and via the same route. Serum was collected before vaccination and at week 0, 3, 5, 9 and 14 after priming.

2.5. Immunofluorescence

Indirect immunofluorescence assay (IFA) was used to detect S protein expression in rLa-MERS-S infected cells. BHK-21 cells were plated on cover slips in 35-mm-diameter dishes and infected with rLa or rLa-MERS-S at a multiplicity of infection (MOI) of 0.1. At 24 h post-infection, cells were fixed in 3% paraformaldehyde in phosphate-buffered saline (PBS) for 15 min at room temperature. Cells were blocked in PBS containing 1% (w/v) bovine serum albumin (BSA) at room temperature for 1 h. Cells were then incubated with mouse anti-S serum or chicken anti-NDV serum for 1 h at room temperature. Cells were then washed 3 times with PBS containing 0.05% Tween 20 (PBST) and stained with a FITC-conjugated goat anti-mouse antibody (Sigma, St. Louis, MO, USA) or an TRITC-conjugated rabbit anti-chicken antibody (Sigma, St. Louis, MO, USA) for 30 min. Cell nuclei were stained with DAPI after final wash. Stained cells were analyzed with a Leica TCS SP5 confocal laser microscope (Leica Microsystems, Wetzlar, Germany).

2.6. Western blot

BHK-21 cells were infected with rLa and rLa-MERS-S at a MOI of 2. Cells were collected and lysed at 36 h post-infection, cell lysates were subjected to 10% SDS-PAGE under denaturing conditions and transferred to polyvinylidene difluoride (PVDF) membrane for Western blot assay using chicken anti-NDV serum or mouse anti-S serum as primary antibody and horseradish peroxidase (HRP)-conjugated rabbit-anti-chicken IgG or goat-anti-mouse IgG as secondary antibodies, respectively (Sigma, St. Louis, MO, USA). The bands were visualized with ECL Plus Western Blotting Detection Reagents (GE Healthcare Life Sciences, Pittsburgh, PA, USA) on Kodak X-ray films.

2.7. Enzyme-linked immunosorbent assay (ELISA)

MERS-CoV S protein-specific IgG was measured by ELISA as described previously (Kong et al. 2012). Briefly, Vero-E6 cells were seeded onto two wells of a six-wells plate, and infected with VSVΔG-eGFP-MERS at a MOI=0.01. At 48 h post-infection, the cell pellet was collected and lysed by repeat pipetting, and the supernatant was coated in the ELISA plate at 4°C overnight. The plates were then washed and blocked with 2% BSA (w/v) at room temperature for 1 h. Serially diluted serum was added to the ELISA plate, and incubated at room temperature for 1 h. Plates were washed three times with PBST, then a 1:3,000 dilution of HRP-labeled goat anti-mouse IgG (Southern Biotech, Birmingham, AL) was added and incubated for another 1 h at room temperature. The plates were washed 5 times with PBST. For visualization, 50 µL of 3,3’,5,5’-tetramethylbenzidine (TMB) liquid substrate (Sigma, St. Louis, MO, USA) was added to each well for 5 min at room temperature; 50 µL of 2 mol L⁻¹ sulfuric acid was added to stop the reaction. OD values were determined with a Model 680 microplate reader (Bio-Rad, USA) at 450 nm. A standard curve was generated by coating with serially diluted purified unlabeled mouse IgG (Southern Biotech, Birmingham, AL) at known concentrations. A linear equation was built based on the standard IgG concentration and their OD values, thus the concentration of MERS-specific IgG could be determined according to the linear equation. For camel-specific IgG, the protocol of ELISA was the same as the ELISA for mouse serum; the difference was 1:3,000 diluted HRP conjugated rabbit anti-camel IgG antibody (Neoscientific, MA, USA) was used as secondary antibody. Due to the lack of purified camel IgG to generate standard curves, all camel sera ELISA results were expressed as relative which were used at 1:300 fixed dilutions.

2.8. Neutralization assay

To assess the MERS-CoV specific neutralizing antibodies, 25 µL of 2-fold serially diluted serum (heat inactivated
at 56°C for 30 min before use) was mixed with 25 µL of DMEM containing 5×10² TCID₅₀ VSVΔG-eGFP-MERS and incubated at 37°C for 1 h. Then the mixture was added to BHK-21 cells in quadruplicate wells of a 96-well plate. The eGFP-expressing cells were counted at 18 h post-infection under a fluorescence microscope. Neutralization titers were determined as the reciprocal of the highest dilution of serum that showed at least a 50% reduction in the number of fluorescent cells as compared with the negative control.

A recombinant NDV LaSota virus expressing the eGFP (designated as rLa-eGFP) was used to determine the neutralizing antibodies against vector. Briefly, 25 µL of 2-fold serially diluted serum was mixed with 25 µL of medium containing 2×10⁴ EID₅₀ of rLa-eGFP at 37°C for 1 h. After the incubation, 50 µL of the mixture was added to the BHK-21 cell monolayer in quadruplicate wells of a 96-wells plate. The GFP-expressing cells were counted at 16 h post-infection under a fluorescence microscope. Neutralization titers were expressed as the reciprocal of the highest dilution of serum that showed at least a 50% reduction in the number of fluorescent cells as compared with the negative control.

2.9. Statistical analysis

Two-way ANOVA with Bonferroni’s multiple comparison tests was used for statistical analysis. All P-values were two-tailed and considered statistically significant when the associated probability was less than 0.05.

3. Results

3.1. Generation of recombinant NDV expressing MERS-CoV S protein

Recombinant genomic cDNA of rLa-MERS-S was constructed by inserting the S gene of MERS-CoV between the P and M gene of NDV (Fig. 1-A). The resultant recombinant virus, rLa-MERS-S, was successfully rescued from the full-length genomic cDNA clone as described above. S protein expression was confirmed by Western blot analysis with mouse anti-S serum (Fig. 1-B). Indirect immunofluorescence further confirmed the S protein expression in infected BHK-21 cells. As shown in Fig. 1-C, non-infected BHK-21 cells (mock) were not stained by either mouse anti-S serum or chicken anti-NDV serum, infected cells were not stained by mouse anti-S serum, but were stained by chicken anti-NDV serum. By contrast, rLa-MERS-S infected cells were stained by both the mouse anti-S serum and the chicken anti-NDV serum.

The growth properties of rLa and rLa-MERS-S in eggs were examined next. As shown in Fig. 2, rLa-MERS-S reached peak titers of 9.7 logEID₅₀ mL⁻¹ at 72 h post-inoculation. The stability of the S gene within rLa-MERS-S was assessed by serial passaging of the virus in SPF chicken eggs over repeated passages. After 10 passages, the expression of S gene was assessed by RT-PCR and immunofluorescence, and results confirmed that S gene was stably maintained and expressed (data not shown).

3.2. Expression of S protein does not increase the virulence of NDV vector in poultry or mice

To investigate whether expression of S protein altered the pathogenicity of NDV vector, we assessed pathogenicity of rLa-MERS-S in poultry and mice. According to the OIE Manual, MDT, ICPI, and IVPI tests were used to assess the pathogenicity of NDV strains in poultry. NDV strains can be classified into three groups based on their MDTs: velogenic (<60 h), mesogenic (60–90 h), and lentogenic (>90 h). Our results showed the MDT of rLa and rLa-MERS-S were both greater than 120 h, indicating that these 2 viruses were lentogenic (Fig. 3-A). All rLa and rLa-MERS-S inoculated chickens remained healthy during the observation period. The ICPI values for rLa and rLa-MERS-S were 0.4 and zero, respectively; while the IVPI values for rLa and rLa-MERS-S were both zero. These data indicated that rLa-MERS-S and rLa were of low pathogenicity to SPF chickens and embryonated chicken eggs, suggesting that the insertion of the S gene in NDV did not increase the virulence of the NDV vector.

Next, to investigate the safety of the recombinant viruses in mammals, we inoculated mice i.m. with 1×10⁸ EID₅₀ of rLa or rLa-MERS-S in 0.1 mL diluted allantoic fluid, and at the same time inoculated the mice i.n. with 3×10⁷ EID₅₀ of rLa or rLa-MERS-S in 0.03 mL diluted allantoic fluid. Mice were observed daily for two weeks for signs of weight change or other indicators of illness. The rLa and rLa-MERS-S infected mice showed similar changes in body weight to mock-infected mice and no signs of disease were observed in any animals (Fig. 3-B).

3.3. rLa-MERS-S induces significant MERS-CoV specific IgG and neutralizing antibodies in mice

Mice MERS-specific IgG1, IgG2a and total IgG levels were determined by ELISA (Fig. 4-A, I). Notably, the total IgG antibody levels were significantly boosted after the second dose (P<0.01). Furthermore, rLa-MERS-S could induce both the IgG1 (Th2) (Fig. 4-A, II) and IgG2a (Th1) antibody responses (Fig. 4-A, III) after the second dose with a slightly Th2-biased in immunized mouse. The ELISA result was in accordance with the result of NDV and MERS VNA assays. Significant NDV VNA was detected in all mice (Fig. 4-B), but MERS-CoV VNA was detected only in the blood of mice inoculated with rLa-MERS-S, not rLa (Fig. 4-A) after the second dose (P<0.01). These results demonstrated that
rLa-MERS-S was highly immunogenic in mice.

3.4. rLa-MERS-S elicits high-level, long-lasting MERS-CoV-specific neutralizing antibodies in camels

The levels and duration of the recombinant virus-induced neutralizing antibody responses are shown in Fig. 5. Camel IgG against S protein was measured by ELISA after the primary and secondary immunization (Fig. 5-A). Similar to ELISA antibodies, the neutralizing antibodies were boosted, and gradually decreased after the second dose. For rLa-MERS-S immunized camels, MERS-CoV neutralizing antibodies were detected after the second dose (5 weeks) and lasted for at least 9 weeks (Fig. 5-A). There was no significant difference between 9 and 14 weeks in the level of neutralizing antibodies in the rLa-MERS-S groups (Fig. 5-B, II). NDV neutralizing antibodies were detected after the primary immunization and were boosted after the second dose (Fig. 5-B, I). No statistical difference in NDV neutral-
Fig. 3 Pathogenicity evaluation of the recombinant viruses in chickens and mice. A, pathogenicity assay in SPF eggs and chickens. MDT, the mean death time; ICPI, intracerebral pathogenicity index; IVPI, intravenous pathogenicity index. B, weight change in mice inoculated with recombinant and vector viruses. Groups of 10 mice were inoculated intramuscularly (i.m.) with $1\times10^8$ EID$_{50}$ (in 100 μL) and intranasally (i.n.) with $3\times10^7$ EID$_{50}$ (in 30 μL) of rLa (Newcastle disease virus (NDV) LaSota virus) or rLa-MERS-S (a recombinant NDV LaSota virus expressing the Middle East respiratory syndrome coronavirus (MERS-CoV) spike protein), and observed and weighed daily for 14 days. All mice survived in the duration of the experiment. Body weight changes for each group are shown as ratios of the body weight at day 0, which was set as 100.

| Viruses         | Pathogenicity in eggs and chickens |
|-----------------|-----------------------------------|
|                 | MDT | ICPI | IVPI |
| rLa             | >120| 0.4  | 0    |
| rLa-MERS-S      | >120| 0    | 0    |

Fig. 4 Immunization of mice. Groups of 10 mice each received 2 doses of $1\times10^8$ EID$_{50}$ (50% embryo infectious dose) of rLa-MERS-S, rLa intramuscularly with a 3-week interval. Blood samples were collected 2 weeks after the first dose (prime) and the second dose (boost) for antibody assays. The ELISA antibodies of mouse total IgG (A, I), IgG1 (A, II), IgG2a (A, III) against the MERS-CoV S protein for each group. The sera neutralization titers to Newcastle disease virus (NDV) (B, I) and to Middle East respiratory syndrome coronavirus (MERS-CoV) (B, II) in the different groups. VNT, virus neutralization test. Data for the neutralization titers to MERS-CoV and to NDV were presented individually with the mean±SD of 10 mice for each group. Two-way ANOVA with Bonferroni’s multiple comparison tests: *, $P<0.05$; **, $P<0.01$. 
4. Discussion

MERS-CoV is a deadly emerging infectious pathogen that poses a serious public health threat. Development of MERS-CoV vaccines is important both to protect susceptible animals and to reduce animal-to-human transmission (Haagmans et al. 2016; Sabir et al. 2016). In this study, a recombinant NDV, rLa-MERS-S, expressing MERS-CoV S protein was constructed. rLa-MERS-S maintained high growth titers in embryonated eggs and low pathogenicity in poultry and mice. The recombinant viruses induced significant MERS-CoV-specific neutralizing antibodies in mice and long-lasting MERS-CoV specific neutralizing antibodies in camels. Our findings demonstrated the potential of rLa-MERS-S as a candidate veterinary vaccine against MERS-CoV infection.

Camels and other MERS-CoV-reservoir animals that are in close contact with humans may facilitate human infection and lead to sustained outbreak during pandemic outbreaks (Perera et al. 2013; Chu et al. 2014). The MERS-CoV reservoir-harboring capacity by camels draws our attention, as to date there are an estimated 300,000 Bactrian camels in China. Consequently, given the highly threatening zoonotic potential of MERS-CoV, we previously carried out a serological and virological surveillance study in the camel herds of West Inner Mongolia Autonomous Region of China (Liu et al. 2015). Although our study did not identify any MERS-CoV RNA and antibodies in the camel herds, constant vigilance is still required. Furthermore, those results underscore the necessity in developing a reserve vaccine against MERS-CoV.

The role of neutralizing antibody against S protein of MERS-CoV in controlling viral infections has been well documented. Haagmans et al. (2016) showed that orthopoxvirus-vectored MERS-CoV vaccines (MVA-S) were effective in eliciting antibodies against MERS-CoV and could significantly reduce the amount of excreted infectious virus and viral RNA transcripts upon MERS-CoV challenge. In addition, Kim et al. (2014) reported that recombinant adenoviral vectors encoding MERS-CoV S protein (Ad5.MERS-S) or Ad5.MERS-S1) could induce neutralizing antibodies in mice. The success of these viral-vectored MERS-CoV vaccines demonstrated that protection against MERS-CoV infection in camels could be achieved by live-attenuated vectored-MERS-CoV vaccines. While both MVA- or adenovirus-vectored MERS vaccines are safe and effective, their production process is complicated and costly, thus may not be suitable for use in developing countries. In contrast, NDV-vectored vaccines have several advantages: Their safety and efficacy profiles have been confirmed in many studies, are easy to culture and can be grown to high titers in chicken eggs, do not require complicated cell culture equipment, and are thermostable when lyophilized. These features make NDV-based MERS-CoV vaccines feasible and accessible especially in rural and other economically disadvantaged areas. Previous studies indicated that recombinant NDV based vaccines could induce good mucosal immune responses in upper respiratory tract in birds and mammals (Martinez-Sobrido et al. 2006; Ge et al.
2007) through intranasal immunization. In present study, due to the very limited camel numbers and the difficulties to practicing intranasal instillation in camels, we did not use intranasal immunization. While considering MERS-CoV’s pathogenicity respiratory system, it is necessary to further explore and compare the immunogenicity of rLa-MERS-CoV given by intranasal and intramuscular route. These studies were scheduled in our institute’s large animal facility in future.

In the present study, we determined that immunization with rLa-MERS-S induced a slightly higher level of IgG1 than IgG2a antibodies against S protein of MERS-CoV. The result is in agreement with the study by Zhang et al. (2016) in which the authors demonstrated that IgG1 played a more important role in MERS-CoV protection in DPP4 transgenic mice. Furthermore, this observation suggests that both Th1 (IgG2a) and Th2 (IgG1) antibody responses may be critical in inhibiting MERS-CoV infection. However, we cannot exclude the possibility that Th2 (IgG1) antibodies may play a dominant role in neutralizing MERS-CoV infection.

Our results demonstrated that rLa-MERS-S induced comparable MERS neutralizing antibody levels to MVA-S, which may indicate that rLa-MERS-S is protective. Due to the unavailability of MERS-CoV, it was impossible for us to perform neutralization assays using live MERS-CoV. Instead, we used a VSV-based chimeric virus, VSVΔG-GFP-MERS, to mimic MERS-CoV in a serum neutralization test. The recombinant virus used the S protein, instead of the G protein, as its sole membrane anchored protein to attach and enter cells. Meanwhile, since the virus was engineered to express an eGFP gene as an additional transcription unit, the positively infected cells emitted green fluorescence. Our results showed that VSVΔG-GFP-MERS was replication-competent and could stably express S protein and eGFP protein. These features render it a reliable surrogate for MERS-CoV in evaluating the neutralizing antibody capacity in the unavailability of live MERS-CoV. Due to the unavailability of a large animal high-containment facility, it was not possible to conduct a camel challenge study in the current setting, but may be considered in the future. Nevertheless, our results thus demonstrated the potential of rLa-MERS-S to serve as a candidate MERS-CoV veterinary vaccine.

5. Conclusion

The present study demonstrated that Newcastle disease virus-vectored MERS-CoV vaccine (rLa-MERS-S) elicits high-level and lasting neutralizing antibodies in mice and camels. The rLa-MERS-S could be a potential MERS-CoV veterinary vaccine candidate for camels and other animals affected by MERS-CoV.

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