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Efficacy assessment of an MVA vectored Rift Valley Fever vaccine in lambs

Núria Busquets [a], Gema Lorenzo [b], Elena López-Gil [b], Raquel Rivas [a], David Solanes [a], Iván Galindo-Cardiel [c,d], F. Xavier Abad [a], Fernando Rodríguez [a], Albert Bensaid [a], George Warimwe [e], Sarah C. Gilbert [e], Mariano Domingo [a,c], Alejandro Brun [b,*]

[a] Centre de Recerca en Sanitat Animal, CReSA, UAB-IRTA, 08193 Barcelona, Spain
[b] Centro de Investigación en Sanidad Animal, CISA-INIA, Valdeolmos, 28130 Madrid, Spain
[c] Departament de Sanitat i d'Anatomia Animals, Universidad Autónoma de Barcelona (UAB), 08193 Barcelona, Spain
[d] WorldPathol S.L., 50018 Zaragoza, Spain
[e] The Jenner Institute, University of Oxford, OX3 7DQ Oxford, United Kingdom

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ABSTRACT

The present study has evaluated the protection conferred by a single subcutaneous dose of a modified vaccinia virus Ankara (MVA) vectored vaccine encoding the Rift Valley Fever virus (RVFV) glycoproteins Gn and Gc in lambs. Three groups of six to seven lambs were immunized as follows: one group received the vaccine (termed rMVA-GnGc), a second group received an MVA vector (vector control) and a third group received saline solution (non-vaccinated control). Fourteen days later, all animals were subcutaneously challenged with 10^5 $TCID_{50}$ of the virulent RVFV isolate 56/74 and vaccine efficacy assessed using standard endpoints. Two lambs (one from the vaccine group and one from the vector control group) succumbed to RVFV challenge, showing characteristic liver lesions. Lambs from both the vector control and non-vaccinated groups were febrile from days 2 to 5 post challenge (pc) while those in the rMVA-GnGc group showed a single peak of pyrexia at day 3 pc. RVFV RNA was detected in both nasal and oral swabs from days 3 to 7 pc in some lambs from the vector control and non-vaccinated groups, but no viral shedding could be detected in the surviving lambs vaccinated with rMVA-GnGc. Together, the data suggest that a single dose of the rMVA-GnGc vaccine may be sufficient to reduce RVFV shedding and duration of viremia but does not provide sterile immunity nor protection from disease. Further optimization of this vaccine approach in lambs is warranted.

1. Introduction

Rift Valley Fever virus (RVFV) is a mosquito-borne bunyavirus that causes a zoonotic disease. The virus is composed of a tripartite RNA genome, comprising large (L), medium (M) and small (S) genome segments. The viral nucleoprotein N and the virulence-associated non-structural protein NSs are encoded in the S segment in ambisense orientation while the L segment encodes the viral polymerase, and the M segment encodes at least two non-structural proteins (termed NSm and 78 kDa) and the structural glycoproteins Gn and Gc (Bouloy and Weber, 2010; Gerrard and Nichol, 2007). RVFV is widely distributed in the African continent, the Arabian Peninsula (Balkhy and Memish, 2003) and Indian Ocean islands (Gerdes, 2004; Pepin et al., 2010; Sissoko et al., 2009), where it has caused major disease outbreaks in both human and livestock. Newborn lambs and gestating ewes are extremely vulnerable to RVFV infection, which results in very high rates of mortality and abortion, respectively. RVF is now considered an emerging threat for European countries due to trade and globalization and the ability of the virus to replicate in and be transmitted by numerous mosquito species (Pepin et al., 2010). As well as improving virus detection it is desirable to develop better vaccines against RVF in susceptible animal species. Currently licensed vaccines do not meet the safety standards required for use in Europe.

Two classical RVF vaccines are available which have been used to control recent outbreaks in South Africa; a formalin inactivated vaccine (Harrington et al., 1980) and a live attenuated virus strain (Smithburn, 1949). Both these vaccines retain some flaws such as low immunogenicity and potentially adverse side effects, respectively. A live-attenuated vaccine termed 'Clone 13' has been licensed for use in several countries in Africa, being safer for

* Corresponding author. Tel.: +34 916202300; fax: +34 916202247.
E-mail address: brun@inia.es (A. Brun).

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pregnant ewes (Dungu et al., 2010) although it has been shown that Clone 13 replicates and is excreted in saliva in competent mosquito species (Amraoui et al., 2012; Muller et al., 1995). In addition, there is a need for human vaccines that could be deployed among populations at risk including farmers, veterinary and/or medical personnel at risk after a RVF outbreak.

Current knowledge in the mechanisms involving the induction of immunity against RVFV in mammals may help in the design of novel vaccines with the potential for use in humans. Some of the most promising approaches tested to date for the generation of RVF vaccines are based on the use of attenuated recombinant viruses rescued by means of reverse genetics (Bird and Nichol, 2012; Morrill et al., 2013a,b; Oreshkova et al., 2013). Other approaches have used live viral vectors such as adenoviruses (Holman et al., 2009; Warimwe et al., 2013), alphaviruses (Gorchakov et al., 2007; Heise et al., 2009), paramyxoviruses (Kortekaas et al., 2010) and members of the *Poxviridae* family (Papin et al., 2011; Soi et al., 2010; Wallace et al., 2006) to deliver immune relevant RVFV antigens. Recently, we observed that a single immunization with a recombinant modified vaccinia virus Ankara encoding the RVFV glycoproteins Gn and Gc (rMVA-GnGc) can protect mice against lethal RVFV challenge (Lopez-Gil et al., 2013). In the present work we evaluate the immunogenicity and protective efficacy of the rMVA-GnGc vaccine in sheep, a major target species for the virus.

2. Materials and methods

2.1. Virus and vaccines

The South African virulent RVFV 56/74 isolate used for sheep challenge studies was kindly provided by the Agricultural Research Council-Onderstepoort Veterinary Institute (South Africa). This virus was originally isolated from cattle (Barnard and Botha, 1977) and subsequently propagated 3 times in chicken embryo-related cells and seven times in MDBK cells. Two additional passages in BHK-21 cells were performed to generate a viral stock. $TCID_{50}$ titers of the virus stock were determined on Vero cells by the method of Reed and Muench (Reed and Muench, 1938). The MVA vaccine was generated by homologous recombination in MVA infected chicken embryo fibroblast (CEF) cells of a recombinant plasmid encoding GFP as marker and RVFV glycoprotein GnGc sequences in a bi-cistronic expression cassette as described (Lopez-Gil et al., 2013). The virus was grown in permissive DF-1 cells (ATCC# CRL-12203), the supernatants and lysed cells harvested and virus pelleted by ultracentrifugation through 36% sucrose cushion. Titration of vaccine virus was performed in DF-1 cell monolayers. Both vaccine virus and challenge virus stocks were stored at −80 °C until use.

2.2. Experimental design, clinical records and sampling procedure

Animal procedures were approved by the Ethical and Animal Welfare Committee of *Universitat Autònoma de Barcelona* (UAB) in accordance with the EU directive 2010/63/EU for animal experiments. The experiments were conducted at Biosafety Level 3 (BSL-3) facilities of the *Centre de Recerca en Sanitat Animal (CReSA*-Barcelona). Nineteen lambs (Ripollesa breed) of both sexes, aged 5–7 weeks at the time of vaccination were used. All animals were in-farm treated with insecticide to eliminate ectoparasites and then moved to the BSL-3 facility 5 days prior to the vaccination. Lambs were fed as per procedures used in conventional farms, with water supply provided *ad libitum*.

All lambs were housed in the same animal box. The lambs were distributed into three groups. The first two groups, composed of 6 lambs each, were immunized with 10^8 plaque-forming units (pfu) of the rMVA-GnGc vaccine (vaccine group) or with an rMVA only encoding GFP (vector control group), respectively. The third group (infection control; $n = 7$) received saline solution. In all groups, lambs were inoculated subcutaneously using 25G needle injection, behind the elbow. One lamb from the vaccine group (#124) accidentally received an incomplete dose of the rMVA-GnGc vaccine.

Two weeks after immunization the lambs were challenged with 10^5 $TCID_{50}$ of RVFV 56/74 subcutaneously. Clinical signs, including rectal temperature and behaviour, were recorded daily until the end of the experiment. Animals were considered febrile if rectal temperature was above 40.2 °C. This threshold was derived based on the mean plus three standard deviations of the rectal temperatures recorded seven days before the challenge. All lambs were necropsied and selected organs fixed in 10% formalin for histopathological studies.

Blood, as well as nasal and oral swabs were collected every two days post-challenge for viral RNA extraction and/or virus isolation. After collection, the blood samples in EDTA were directly frozen to −80 °C and nasal and oral swabs were mixed in DMEM prior −80 °C storage. Serum samples were obtained from each animal on the day of challenge and at days 5, 9 and 16 post-challenge for analysis of anti-RVFV antibodies.

2.3. Immunological assays

Specific anti-nucleoprotein N antibodies were detected using a competitive ELISA (ID-VET) as per manufacturer's instructions. Neutralizing antibody titers were determined as described (Lopez-Gil et al., 2013). Briefly, heat inactivated serum samples were serially diluted (twofold), starting at a dilution of 1:20 in DMEM medium containing 2% FBS, mixed with an equal volume (50 µl) of medium containing 10^3 pfu of a stock of the MP-12 RVFV strain. After one hour incubation at 37 °C, this mixture was added to Vero cell monolayers seeded in 96-well plates. After 3 days at 37 °C, cells were fixed and stained with 2% crystal violet and 10% formaldehyde. The neutralization titer of each sample was defined as the reciprocal of the highest serum dilution resulting in 50% neutralization.

2.4. Virus detection and isolation

RNA from nasal and oral swabs was extracted using Trizol® (Invitrogen) and from blood using Tri-reagent® (Sigma), according to manufacturer's instructions. RVFV RNA from samples was detected using a TaqMan one-step RT-qPCR specific for RVFV L segment as described previously (Busquets et al., 2010). Positive samples on RT-qPCR were processed for virus isolation as follows. Blood samples were first lysed by dilution in sterile distilled water and 10× phosphate buffered saline (PBS) added to restore physiological conditions (final blood dilution 1:20). Hundred µl of log2 dilutions (starting at 1:40) were added to 96-well cultured Vero cells in quintuplicate. After incubation for 90 min supernatants were aspirated and substituted with fresh media (DMEM supplemented with 2% FBS). After 6 days the cells were fixed and stained with 10% formaldehyde and 2% crystal violet. $TCID_{50}$ end point titers were calculated by the Reed and Muench method (Reed and Muench, 1938). Twofold dilutions of infected swabs, either nasal or oral, were prepared in cell culture medium with antibiotics and incubated onto Vero cells in quadruplicate and processed as described above.

2.5. Statistical analyses

Statistical comparisons were made using the Kruskal–Wallis test and adjustment for multiple testing done by the Dunn's post

hoc test. All calculations were performed using the GraphPad 5.0 software (Prism).

3. Results

3.1. Clinical and pathological findings in lambs

After immunization no fever, abnormal behavior or apparent clinical display was observed in any of the lambs. However, morbidity was observed over the duration of the experiment in all groups upon challenge with the RVFV 56/74 virus. To quantify the extent of morbidity among the groups, a clinical score was applied based on severity of illness (Fig. 1). The earliest clinical display observed after challenge was mucosal hyperemia, noticed in all lambs as early as day 2 post-challenge (pc), being more evident in the MVA control and saline groups as compared to the vaccine (rMVA-GnGc) group. By day 4 pc this manifestation reached similar intensity in lambs from the vaccine group. (Fig. 1A). Later, more severe signs of disease were observed in all groups. The MVA control group displayed such signs from days 4 to 11 pc, but severity of illness among the rMVA-GnGc vaccinated lambs had decreased by day 5 pc. Lambs receiving saline also displayed clinical signs for a longer duration than the rMVA-GnGc group, but their overall clinical severity was lower than that observed in the MVA control group (Figs. 1B–D).

Bilateral conjunctivitis was a clinical feature found in some lambs across all groups. Three lambs showed even more severe signs such as lack of appetite, lethargy and respiratory distress. Two of these died (lambs #125 and 130), one from the vaccine group and one from the MVA control group, at days 4 and 5 post challenge, respectively. At necropsy, both dead animals revealed as fatal lesion a moderate to severe hepatomegaly with multiple friable whitish foci that were diagnosed as severe multifocal hepatic necrosis, a characteristic injury of RVF disease. Accumulation of transudate at the pleural and peritoneal cavities was also found, as well as moderate segmental hemorrhagic enteritis. Also, splenomegaly and swelling of peripheral and visceral lymph nodes with petechiae and edema was found in both dead lambs. The third lamb (#132), also from the MVA control group, survived although showing extremely pale mucosa. At day 11 post challenge all remaining sheep in the rMVA-GnGc vaccinated group displayed no clinical signs, whereas in both control groups some lambs still displayed signs of disease (Fig. 1B–D).

Rectal temperatures were taken daily both after immunization and challenge. As expected, pyrexia was absent in all lambs from all groups before the challenge. After virus inoculation, all lambs showed an increase in temperature albeit differences were observed between the groups (Fig. 2). Among rMVA-GnGc vaccinated lambs fever peaked at day 3 post challenge (Fig. 2D) with the exception of the lamb that received an incomplete dose of the vaccine (lamb #124, see Methods), which peaked at day 2 instead, suggesting a vaccine dose effect. In this group only one lamb (#121), showed a secondary onset of fever on day 5 post challenge (Fig. 2A). This lamb was the only sheep from this group showing a strong bilateral conjunctivitis at day 7 post challenge. The duration of fever lasted longer in both control groups (from day 2 to day 5), with an earlier peak of fever at day 2 post challenge (Figs. 2B and C). In these control groups the onset of fever was asynchronous, with some lambs peaking at 3, 4 and 5 days post challenge. Lamb #132 from the MVA control group had a secondary peak of fever on day 9 (Fig. 2B) and showed pale mucosa until the end of the study.

3.2. Virus detection and isolation

To determine more precisely the level of efficacy achieved by the vaccine, blood and swabs samples taken from lambs at different days post challenge were analyzed by RT-qPCR and virus titration in cell culture (Table 1). Viral RNA in blood was not detected at day 1 post challenge. However it was detected at day 3 post

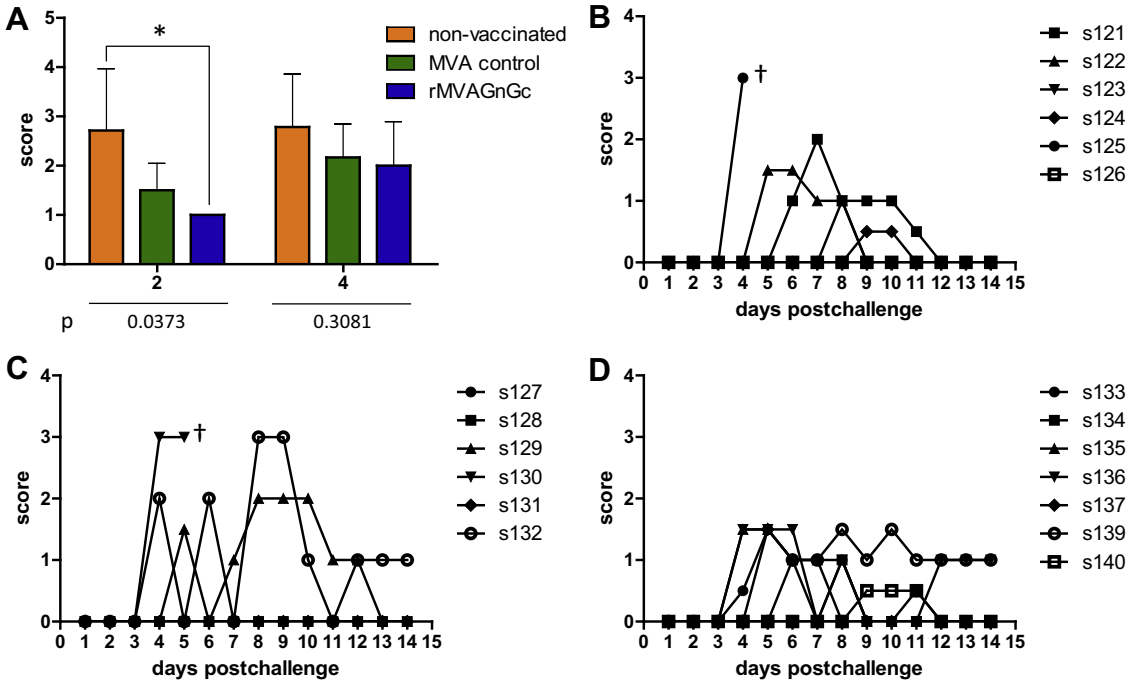

Fig. 1. Clinical evaluation in challenged sheep. (A) Early clinical signs (mucosal hyperemia) recorded at days 2 and 4 post challenge (dpc). Severity was categorized according to the relative intensity of irritation (1: mild, 2: moderate, 3: strong, 4: very strong). The graph bars depicts median with interquartile range from each group of sheep. The *p* values are indicated for each day post challenge. Asterisk denotes statistical significance ($p < 0.05$). (B–D) Late clinical signs scored for each individual sheep from the rMVA-GnGc group (B), MVA control group (C) and the non vaccinated group (D), according to the following categorization: mild to strong conjunctivitis (0.5–1.5), if bilateral (+0.5); inappetence or apathetic behaviour (+1); respiratory distress (+1).

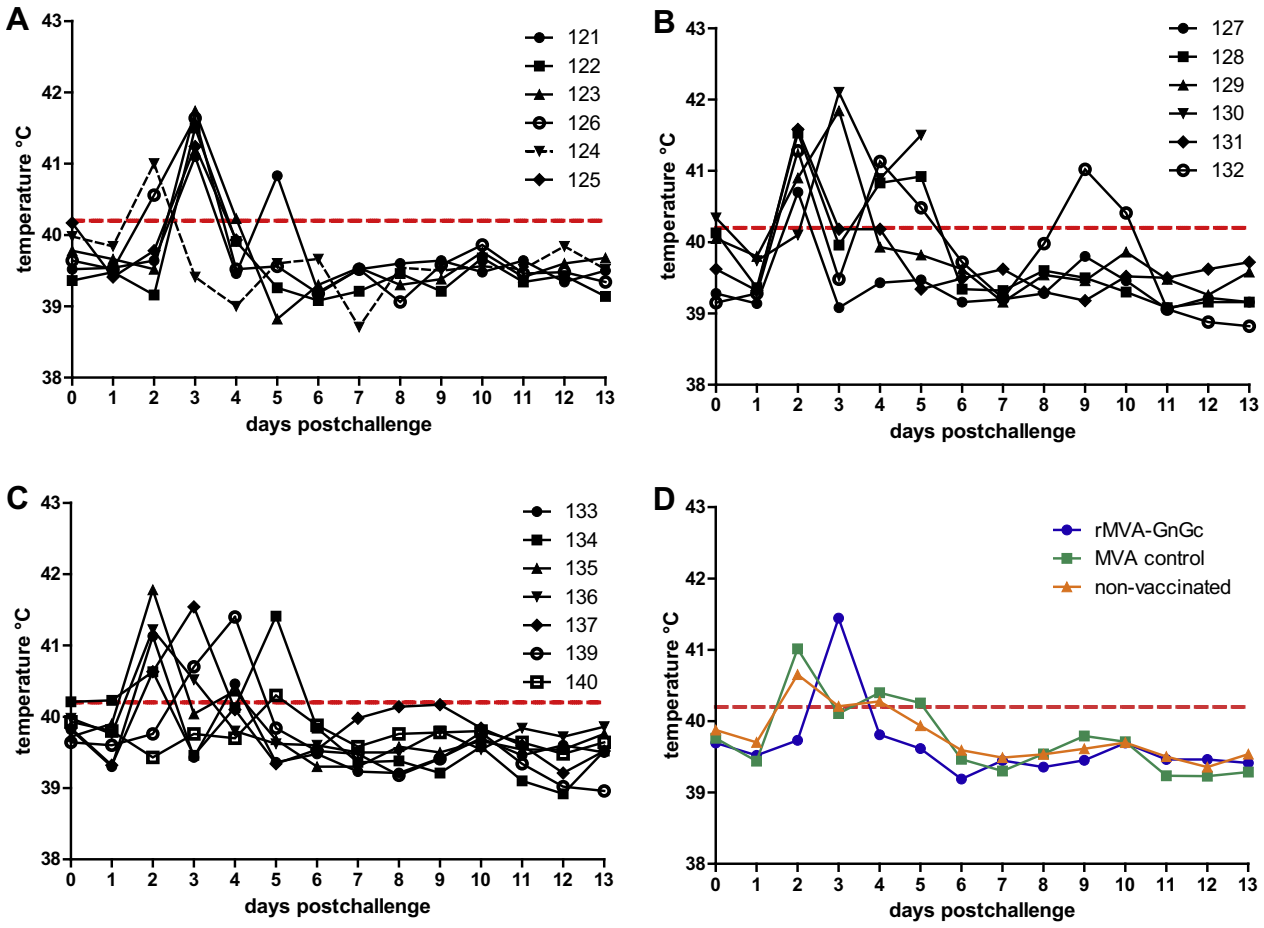

Fig. 2. Pyrexia in challenged sheep. The graphs A, B and C display individual rectal temperatures sampled at similar times for sheep from the rMVA-GnGc group (A), MVA control group (B) and the non vaccinated group (C). Mean temperatures for each group are displayed in (D) Dotted line indicates threshold of fever.

challenge in most of the lambs from all groups. At day 5 post challenge two sheep from both the saline control and the rMVA-GnGc groups were positive for viral RNA detection in blood. However no virus was isolated at this time point in both groups. Interestingly, four lambs from the MVA control group were positive at day 5 post challenge and live virus was isolated from one of them. This lamb was found dead at day 5 post challenge (#130).

Overall, nasal and oral viral shedding post-challenge was more pronounced in both control groups than in the rMVA-GnGc group. At least one lamb in each of the control groups was positive for RVFV RNA in nasal swabs at day 7 post challenge, whereas in the rMVA-GnGc group only two lambs, the one that received an incomplete dose (#124) and the one that died shortly after infection (#125), were positive at day 3 post challenge by RT-qPCR. Virus was isolated from controls at day 5 post challenge and only in one lamb at day 3 in the rMVA-GnGc group. In contrast to what was observed for the viraemia, more virus was detected in nasal swabs in the group receiving saline. With respect to oral swabs viral RNA was still detected at day 5 post challenge in some lambs from both control groups but not in the rMVA-GnGc group. In addition virus could be isolated at day 5 in one lamb, and even at day 7 pc, viral RNA was detected in one of the animals from the MVA control group.

3.3. Antibody detection

The level of antibodies raised against the RVFV viral nucleoprotein N was quantified by a commercial competition ELISA (Fig. 3). As early as 5 days post challenge (19 days post immunization) all lambs from the rMVA-GnGc group had seroconverted. In contrast, only four lambs from both control groups had comparable anti-N antibody titers in serum. Complete seroconversion in lambs from control groups occurred later, between 6 and 9 days post challenge (20 and 23 days post immunization respectively). A similar acceleration of the antibody responses was observed for RVFV neutralizing antibodies (Fig. 4). While no pre-challenge (day 14 post immunization) RVFV neutralizing antibodies were detected in any of the control groups, one lamb from the rMVA-GnGc group showed low but detectable titers of neutralizing antibodies. Moreover, by day 5 and 9 post challenge (19 and 23 days post immunization, respectively) the titers of RVFV neutralizing antibodies differed significantly ($p = 0.0160$ and $p = 0.0021$, respectively), with the vaccine group showing the highest titers and faster induction of antibodies (Fig. 4).

4. Discussion

The aim of this work was to evaluate the performance of a modified vaccinia virus Ankara expressing the RVFV glycoproteins (rMVA-GnGc) as a potential single shot vaccine candidate against RVF in a sheep model of infection. Recent studies have evaluated the protective efficacy of other poxviral vectors such as capripoxvirus and lumpy skin disease virus (LSDV) in sheep (Soi et al., 2010) (Wallace et al., 2006). Two doses of these vaccines, expressing the viral glycoproteins, provided protection against fever and viremia after virulent RVFV challenge. In mouse models these vectors

Table 1
Quantitation of viral RNA and virus isolation in blood, nasal and oral swabs of lambs.

Sample	Blood												Nasal swab						Oral swab					
dpc[d]	1		3		5		7		9		13		3		5		7		3		5		7	
Lamb #	RNA[e]	VI[f]	RNA	VI	RNA	VI	RNA	VI	RNA	VI	RNA	VI	RNA	VI	RNA	VI	RNA	VI	RNA	VI	RNA	VI	RNA	VI
121[a]	neg		4.7	3.7	neg		neg		neg		neg		neg		neg		neg		neg		neg		neg	
122[a]	neg		4.8	3.5	neg		neg		neg		neg		neg		neg		neg		neg		neg		neg	
123[a]	neg		4.4	neg	2.0	neg	neg		neg		neg		neg		neg		neg		neg		neg		neg	
124[a]	neg		3.7	1.8	1.9	neg	neg		neg		neg		2.2	–	neg		neg		neg		neg		neg	
125[a]	neg		6.9	⩾4.7									3.4	2.3					2.1	neg				
126[a]	neg		4.2	3	neg		neg		neg		neg		neg		neg		neg		neg		neg		neg	
127[b]	neg		2.7	1.8	neg		neg		neg		neg		neg		–		neg		neg		neg		neg	
128[b]	neg		5.8	4.2	1.9	neg	neg		neg		neg		3.7	neg	3.6	1.6	2.2		neg		1.8	neg	neg	
129[b]	neg		7.8	⩾4.7	4.2	neg	2.7	neg	2.1	neg	neg		3.0	neg	3.0	1.6	4.1		2.0	neg	2.2	neg	neg	
130[b]	neg		7.3	4.4	3.7	1.8							4.2	neg	3.2	neg			1.8	neg	2.4	1.8		
131[b]	neg		2.9	1.8	neg		neg		neg		2.1	neg	neg		neg		neg		neg		neg		neg	
132[b]	neg		6.8	4.9	2.5	neg	2.7	neg	neg		1.6	neg	3.9	2.3	3.0	1.9	neg		neg		neg		2.6	neg
133[c]	neg		neg		neg		neg		neg		neg		2.2	2.1	neg		neg		neg		neg		neg	
134[c]	neg		neg	neg	neg		neg		neg		neg		neg		neg		neg		neg		neg		neg	
135[c]	neg		4.9	3.0	neg		neg		neg		neg		2.2	1.8	2.5	1.6	neg		neg		neg		neg	
136[c]	neg		neg		neg		neg		neg		neg		2.3	2.1	neg		neg		1.7	–	3.5	neg	neg	
137[c]	neg		5.5	4.5	neg		neg		neg		neg		4.1	1.8	2.7	1.6	3.1		2.5	–	2.0	neg	neg	
139[c]	neg		3.2	neg	1.6	neg	neg		neg		neg		neg	neg	neg		neg		neg		neg		neg	
140[c]	neg		2.1	1.8	4.1	neg	neg		neg		neg		neg	neg	neg		neg		neg		neg		neg	

[a] rMVAGnGc group.
[b] MVA control group.
[c] Non vaccinated control group.
[d] Days post challenge.
[e] Detection of viral RNA (values in $\log_{10}$ viral GEC/ml).
[f] Virus isolation (Infectious dose in $\log_{10}$ $TCID_{50}$/ml); neg: negative result.

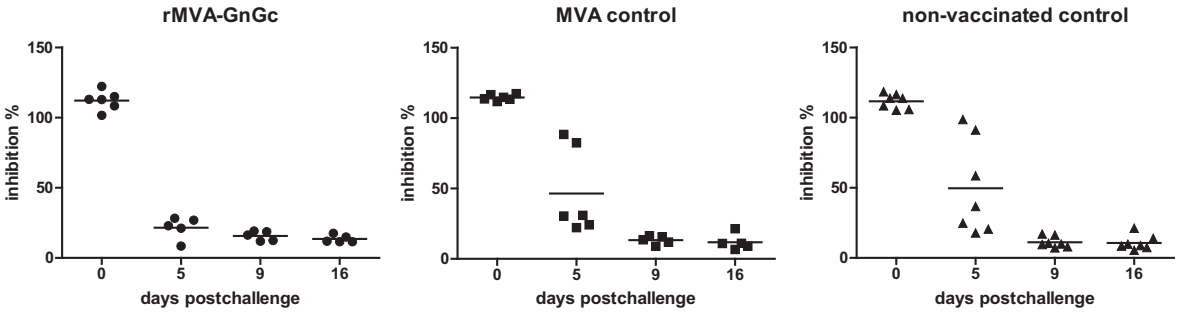

Fig. 3. Detection of anti-nucleoprotein antibodies after viral challenge. The figure shows individual seep serum tested in a competition ELISA. Serum samples were assayed for the presence of anti N antibodies at 0, 5, 9 and 16 days after virus challenge (corresponding to 14, 19, 23 and 30 days post immunization).

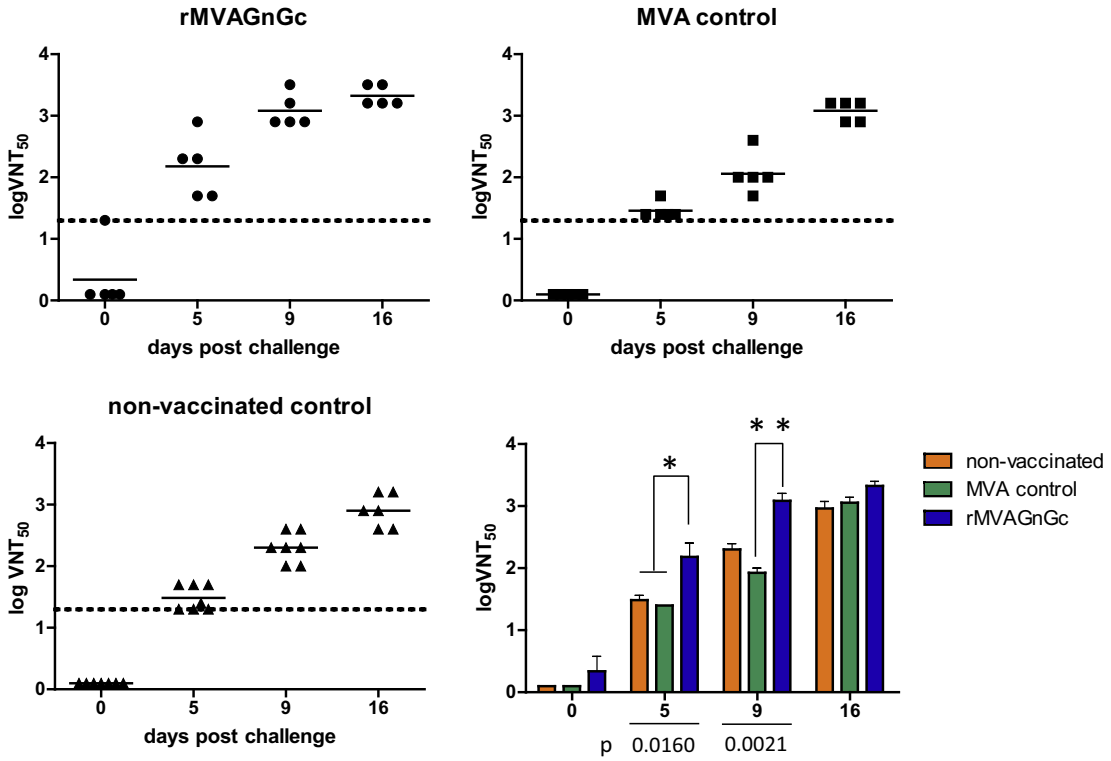

Fig. 4. Neutralizing antibody kinetics. 50% end point titers were measured at 0, 5, 9 and 16 days after virus challenge (corresponding to 14, 19, 23 and 30 days post immunization). Scattered plots with mean values are depicted. Bars indicate mean ± SD. Dotted line indicates the sensitivity threshold of the VNT assay. Significant ($p < 0.05$) p values are indicated for determined days post challenge. Asterisks denote statistical significance ($^{*}p < 0.05$; $^{**}p < 0.01$).

provided incomplete or no protection when administered in a single dose format. This fact was also noted for a recombinant vaccinia virus (VACv) expressing the RVFV glycoproteins (Papin et al., 2011).

Our previous data in mouse models (Lopez-Gil et al., 2013) has shown that a single inoculation of the rMVA-GnGc vaccine was sufficient to protect mice from a lethal challenge. Moreover, this protection was achieved with no apparent or very mild clinical display and, most importantly, in the absence of detectable viremia. Therefore, the promising data obtained in the mouse model prompted us to test the level of protection of a single administration of rMVA-GnGc in sheep, one of the natural targets of RVFV. From the point of view of field veterinary vaccines, single dose vaccination is a preferred choice over multiple administrations, taking in account economical and practical reasons, particularly in developing countries. Recommendations for new RVFV vaccine developments include single dose delivery as an advantage over repeated administrations, thus minimizing the risks of transmission by needle injections in endemic RVFV areas (Kortekaas et al., 2011).

We used an ovine model of infection that had been previously characterized (Busquets et al., 2010). In this study the subcutaneous challenge of lambs with 10^5 $TCID_{50}$ of the 56/74 RVFV strain caused two fatalities and clear clinical signs were observed in all lambs. Intriguingly, the only two lambs that died after the challenge belonged to the groups inoculated with MVA virus (sheep from rMVA-GnGc and rMVA-GFP groups) but none of the lambs challenged in the saline group died. Perhaps the mechanisms of anti-vector immunity rendered both lambs more susceptible to disease or, alternatively, these two lambs were affected by other non adverted pathologies that could have enhanced the virulence of the challenge. Despite the death of one of the six sheep in the rMVA-GnGc group, some partially protective effects could be attributable to the immunization with rMVA-GnGc.

The most obvious consequence of vaccination was a 24 h delay in the onset of fever and early clinical signs such as mucosal irritation. This observation was serendipitously supported by the earlier onset of fever of a sheep (#124) which received an incomplete dose of this vaccine. In addition, virus detection experiments also

confirmed the partially protective effects of this vaccine. Even though at 3 days post-challenge fever peaked in the rMVA-GnGc group (mean pyrexia peaked at day 2 post challenge in both control groups) both the RNAemia and viremia levels found at this time point among the groups did not reach sufficient statistical significance (*P* values of 0.1399 and 0.1948, respectively). It is noteworthy that the levels of viremia and viral shedding were measured in samples collected at day three post challenge, one day after the peak of fever observed for both controls but simultaneous to the peak of fever in lambs from the rMVA-GnGc group. Since it has been shown that the peak of fever usually occurs simultaneously with the peak of viremia (Busquets et al., 2010), perhaps differences in virus titers at day 2 pc would have been more significant than at day 3 pc.

Interestingly, more differences were found between the groups with respect to viral shedding. In the saline group infectious virus was isolated from nasal swabs at day 3 post challenge in 4 of 7 lambs and virus isolation was still possible at later times after the challenge (up to seven days post challenge) both in nasal and oral swabs. In contrast, virus shedding was detected only at day 3 post challenge in the rMVA-GnGc group among lambs that died after challenge.

Though neutralizing antibodies were barely detectable in only one out of five lambs just before the challenge (2 weeks after vaccination), a clear priming effect could be observed in the rMVA-GnGc group since neutralizing antibodies after challenge developed faster and to higher titers than in both control groups. Therefore, it is plausible that during the first days after challenge a certain level of neutralizing antibodies was reached, able to reduce further virus replication and spreading. Additionally, it was observed that vaccinated lambs showed accelerated anti-N antibody responses even though they were vaccinated with an MVA vector expressing only RVFV glycoproteins. This fact could suggest a synergistic effect of antibodies whereby a stronger neutralizing antibody response directed against the RVFV glycoproteins accelerates the processing of nucleoprotein antigen from neutralized virus particles or virus infected cells thus facilitating the development of anti-N antibodies. Alternatively, an enhanced replication of the virus in the vaccinated animals would make more N antigen available for immune recognition. Though very unlikely that this fact could be effectively translated for field diagnosis, the different kinetics of the antibody response against a non-vaccine antigen (in this case the immunogenic nucleoprotein N) would help to distinguish vaccinated from non-vaccinated animals after experimental virus exposure.

Our results suggest that a single subcutaneous immunization of lambs with rMVA-GnGc does not confer full protection from disease; however it appears able to delay the onset and severity of clinical signs as well as the duration of illness. Moreover, viremia and virus shedding appears to be delayed and reduced in extent. Therefore, further optimization of rMVA-GnGc vaccination regimen in lambs is needed, for instance by varying the route of administration, using adjuvants or by increasing the amount of recombinant virus delivered. Since MVA is not capable of replication in sheep the amount of input virus may be a key determinant for the magnitude of the immune response mounted by the host. MVA vaccines delivered in previous trials against other pathogens in veterinary species such as calves, and/or ponies were administered with at least one or two booster doses (Antonis et al., 2007; Breathnach et al., 2006; Chiam et al., 2009). Experiments are underway to test the protection elicited with a booster dose of rMVA-GnGc in sheep as well as for potentiating the immune response elicited by a single administration of this experimental vaccine. In conclusion, the rMVA-GnGc vaccination regimen presented in this study achieved only limited protection against clinical disease in lambs; however it was able to reduce the extent of viremia and virus shedding warranting further optimization trials.

Conflict of interest

Authors declare having no competing interests.

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