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Research Article

Duration of Immunogenicity of the Recombinant Rift Valley Fever Vaccine MP-12 del-NSm21/384 in Sheep

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ABSTRACT

Rift Valley Fever Virus (RVFV) is a mosquito-transmitted *Bunyavirus* capable of causing high morbidity and mortality among humans and domestic ruminants. Vaccination of domestic ruminants with vaccines that provide safe, rapid and long-term protection after a single vaccination is an effective strategy for protecting animals against Rift Valley Fever (RVF) disease. The aim of this study was to determine the duration and titer of antibody elicited by the RVF MP-12 del-NSm21/384 vaccine in sheep. Serum samples were collected from the animals before and after vaccination at various intervals up to one-year post vaccination to determine the duration of the antibody response using a virus-neutralizing test. All vaccinated sheep remained healthy, and RVFV antibody was first detected at two weeks post-vaccination in 37.5% of vaccinated sheep and 100% at three weeks pv. Neutralizing titers reached an average of 2.6 (equivalent dilution 1/400) at 2 months post vaccination and were maintained above 1.5 (equivalent dilution 1/35) for one year, thus providing evidence that a single vaccination elicited long lasting antibody in the animals. These antibody titers were at a level shown to be protective for sheep in a challenge study performed with virulent RVF ZH-501, further supporting the use of this vaccine virus strain as a candidate for long term protection of animals against virulent RVFV infection.

Keywords: Immunogenicity; RVF MP-12 del-Nsm21/384; Vaccine; Sheep; Rift Valley Fever (RVF); Rift Valley Fever Virus (RVFV)

INTRODUCTION

Rift Valley Fever (RVF) is an economically important zoonotic disease affecting humans and animals, characterized by a high rate of abortion and mortality in new-born sheep, goats, and cattle, as well as a transient febrile illness in humans with occasional encephalitis and haemorrhagic fever [1,2]. The disease is caused by the RVF Virus (RVFV) that belongs to the *Bunyavirales* order, *Phenuiviridae* family and *Phlebovirus* genus [3]. The disease was first reported in Kenya in 1931 among sheep and humans and subsequently detected in most African countries [4,5]. RVFV spread to the Middle East in 2000 resulting in hundreds of human and animal casualties with risk of introduction to other regions [6,7]. Several vaccine candidates have been developed to prevent RVF disease [8,9]. Live attenuated vaccines have been used more frequently for controlling the disease in enzootic/endemic zones. Though effective for preventing RVF, the Smith burn vaccine has side effects, including abortion and fetal malformation in pregnant animals [10-13]. RVFV, Clone-13 is a live attenuated vaccine with the NSs gene deleted. It is temperature sensitive, raising the risk associated with using this vaccine in tropical countries without maintenance of the cold chain and an experimental study showed that the vaccine caused teratogenic effects among pregnant sheep [14-17]. Also, most vaccine candidate vaccines are not DIVA compatible (differentiating infected from vaccinated animals), which is a recommended requirement for vaccines to be used in both endemic and non-endemic countries allowing compliance with mandatory international trade restrictions during active RVF outbreaks [18,19]. Experimental studies demonstrated that the recombinant MP-12 del-NSm21/384 vaccine was immunogenic in target domestic animal species including sheep, goats and

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calves and was non-abortagenic but was the suspected cause of malformations in lambs born by vaccinated pregnant ewes [20-24].

The effectiveness of live RVFV vaccines is dependent on the neutralization potential of the induced antibody and the duration of the immune response of vaccinated animals. Many studies have demonstrated the protective capability of MP-12 and MP-12-del-NSm 21/384 but duration of immunity has not been as well studied. Morrill and Peters showed that Rhesus monkeys (Macaca mulatta) vaccinated 6 years prior with MP-12 remained protected against aerosol challenge by virulent RVFV ZH-501 while Pittman et al. reported that human volunteers exhibited target levels of neutralizing anti-RVFV antibody 5 years post vaccination with MP-12 [25,26]. These data suggested that the MP-12 strain has desired protective antibody duration, and therefore, the objective of this study was to determine the duration of antibody elicited in sheep by the MP-12-del-NSm 21/384 vaccine candidate.

MATERIALS AND METHODS

Vaccine

The RVF MP-12-del-NSm 21/384 vaccine virus was replicated in Vero cells propagated in Dulbecco Modified Eagles Media (DMEM) supplemented with Fetal Bovine Serum (FBS), and infected at a Multiplicity of Infection (MOI) of 0.01. The harvested virus suspension was mixed with a stabilizer consisting of 4% peptone, 8% sucrose and 2% glutamate, then freeze-dried as described previously [23].

Animals

The study was carried out in accordance with international guidelines for the care and handling of experimental animals as described in a protocol approved by the MCI Internal "Ethic Committee for Animal Experiment". Sixteen sheep of Sardi breed, aged 4-6 months were negative for RVFV IgG antibody by an Enzyme-Linked Immunosorbent Assay (ELISA), and individually identified by ear tags and acclimatized in the holding facility for 2 weeks before use in the experiment.

Vaccination and monitoring

Sheep were divided into two groups of eight: sheep in Group I (GI) were vaccinated subcutaneously with 1 ml of a $10^{4.0}$ Tissue Culture Infectious Dose 50% ($TCID_{50}$) of the freeze-dried MP-12-del-NSm 21/384 vaccine reconstituted in Phosphate Buffered Saline (PBS). Sheep in Group II (GII) were used as controls and vaccinated with PBS only. Sheep of the two groups were housed together. Sheep were examined daily for clinical signs, i.e. weakness, apathy and general health. Rectal temperatures and any local inflammation at the injection site were recorded daily during 14 days post-vaccination (pv) to evaluate safety. Serum samples were collected by jugular venipuncture at days 7, 14, 21, 28, 42 pv, and once each month from 2 to 12 months pv.

Virus neutralization

The immune response of vaccinated animals was determined by testing sera samples using a Virus Neutralization Test (VNT) as described in the OIE Terrestrial Manual [27]. The test is based on neutralization of the serial 1:3 dilutions of heat inactivated sera with a fixed dose of the virus (100 $TCID_{50}$). The sera virus mixture was incubated one hour and inoculated onto monolayers Vero cells then observed for the absence or present of Cytopathic Effect (CPE) once daily for 5 days. To verify the dose of virus used in the VNT, a mixture of equal volumes of the virus dose and Dulbecco's Modified Eagle's Medium (DMEM) was incubated for one hour and tested for CPE in Vero cells. The neutralizing antibody titer was calculated in accordance with Reed and Muench method [28].

RESULTS

All sheep remained healthy and maintained normal body parameters and did not show any clinical signs after vaccination throughout the study such as haemorrhage, diarrhea, nasal and ocular discharge. Body temperature of vaccinated and unvaccinated animals remained within normal limits (38.3 and 39°C). No local inflammation was recorded in vaccinated sheep at the injection site.

All vaccinated sheep developed neutralising antibody. At day (D) 14 pv, RVFV specific antibody was detected in 3/8 (37.5%) of the animals with neutralising antibody titers of 0.8 log (dilution 1/7). At D21 pv, all animals seroconverted and had antibody titers average 1.6 log (dilution 1/40) (Figures 1 and 2). The maximum titer value was 2.6 log (dilution 1/400), obtained at 2 months pv. Vaccinated sheep showed a decrease in neutralizing antibody titer later through one-year pv with a value of 1.5 log (dilution 1/35) at 12 months pv. In unvaccinated control sheep, no RVFV antibody was detected during the observation period (Figure 1).

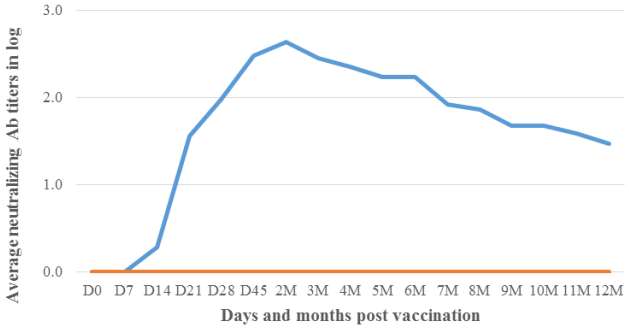

Figure 1: Average RVFV arMP-12DNSm21/384 vaccine neutralizing antibody titers for sheep vaccinated and unvaccinated sheep.
Note: (—) Vaccinated; (—) Unvaccinated

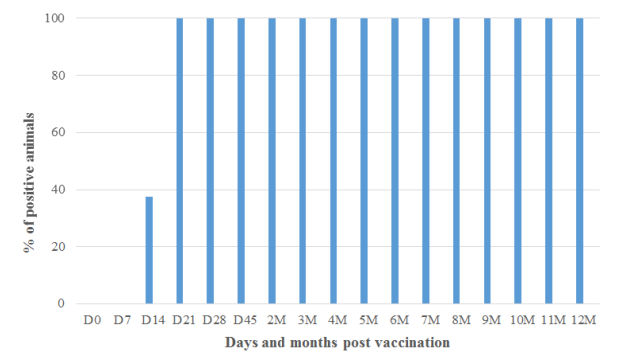

Figure 2: Percentage of RVFV arMP-12DNSm21/384 vaccine neutralizing antibody positive for vaccinated sheep during 12 months post-vaccination.

DISCUSSION

RVF is an infectious disease capable of causing a devastating impact on the health of livestock and humans resulting in economic loss to the livestock industry. Vaccination is the most efficient tool to

control the spread of RVFV and successful vaccination programs depend on a proper selection of the vaccine as well as careful handling practices [9,29,30]. Commercial vaccines currently available in the market have safety limitations in pregnant animals and the immune serum from vaccinated animals cannot be differentiated serologically between infected and vaccinated animals. The recombinant vaccine with nucleotides deleted in the non-structural M viral RNA segments provides a negative biomarker for DIVA and the prevention of reversion to virulence of the vaccine virus [31]. The recombinant RVFV MP-12-del-NSm 21/384 vaccine was also reported to be safe in pregnant animals and elicited strong immune response in livestock [24,32]. However, the vaccine was suspected of causing malformed lambs born to vaccinated pregnant sheep during the early stage of pregnancy with the RVFV MP-12-del-NSm 21/384 vaccine [24]. While the vaccine may not be recommended for use in pregnant animals, the other promising features of the vaccine were considered worthy of conducting this study to investigate the duration of the immune response in sheep, which is an important criterion for evaluating the effectiveness of the vaccine and planning vaccination protocols for each animal species.

The outbreak of RVF is normally associated with episodes of heavy rains and flooding of areas that result in huge mosquito populations. The disease occurs with a long or short cycle depending on the regions, climatic factors, and ecological variables [33-35]. Some authors, however; reported that mosquitoes in some regions maintain RVFV, regardless of any ongoing RVF outbreak [18,36]. In both situations, immunity duration knowledge is a key factor to establish a vaccination protocol assuring continuous protection of animals, mainly before new outbreaks start [37].

Sheep are the species of domestic animals most susceptible to RVFV infection showing hyperthermia, symptoms, and abortion [38]. In the present study, the recombinant RVFV MP-12-del-NSm 21/384 vaccine was tested in sheep to assess the duration of the immune response using a VNT. Weingartl showed that sheep vaccinated with this vaccine were protected against a virulent RVFV challenge with strain ZH-501 [22]. Therefore, a virulent virus challenge test was not considered necessary for this study. Others have shown that neutralizing antibodies have a good correlation with protection [15]. Dungu et al. reported that RVF vaccinated cattle, with a neutralizing antibody titer of 1/16 were protected after challenge [14]. In another study, von Teichman et al. demonstrated that vaccinated cattle with antibody titer of 1/32 were protected at challenge and did not show any clinical signs of RVF [15]. In addition, Soi et al. vaccinated sheep that developed neutralizing antibodies between 1/8 and 1/16 and were all protected against RVFV challenge. In this experiment, vaccinated sheep had neutralizing antibody titer that remained higher than 1.5 log (dilution 1/70), and therefore, indicating that the antibody titers conferred protection that lasted for at least one year.

Very few experiments have been performed to determine the duration of the immune response elicited by candidate RVFV vaccines by serological monitoring up to one-year after vaccination. Atwa et al. reported that sheep vaccinated with inactivated RVF vaccine and monitored up to 48 weeks had neutralizing antibody declining at 10 months ranging from 1.8 to 0.92 log titers [39]. In our study, antibody titers were maintained above 1.5 for one year, which are comparable to the titers reported for sheep after vaccination with RVF Clone-13 live vaccine [16].

Antibody titers in sheep were higher in our study (1/400) than those reported in goats (1/160) vaccinated with the same RVFV MP-12-del-NSm 21/384 vaccine with a dose of 10^5 PFU/ml and monitored for 3 months pv [40]. However, our antibody titers are similar to those reported by Weingartl et al, and Boumart et al. with the same vaccine in sheep monitored for one-month pv [22,23]. The antibody response in this study was slightly lower than titers observed in ewes vaccinated with the same RVFV MP-12-del-NSm 21/384 vaccine and monitored for 2 months pv [32]. The variation in antibody titers may reflect differences in the age, health status, and other variables associated with vaccinated sheep that could affect their ability to elicit immune responses to RVFV infection [40,41]. To our knowledge, this is the first study that investigated the duration of the antibody response of sheep over a twelve-month period following vaccination with a single dose of the RVFV MP-12-del-NSm21/384 vaccine.

CONCLUSION

The results of this study revealed that the recombinant RVFV MP-12-del-NSm 21/384 vaccine elicited the production of antibody titers to levels that could possibly afford protection to sheep with a single dose and without inducing adverse reactions pv. Vaccination with a single annual dose before the rainy season can prevent RVF disease among livestock in RVFV endemic/enzootic regions of Africa and the Middle East for long periods of time, perhaps the average life of the animal if used as a food source.

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Authorship

The following authors, Z. Bamouh, Z. Boumart, M. Elhayane, K. Omari Tadlaoui, O. Fassi Fihri and M.EL Harrak contributed substantially to the conception and design of the study, the collection and analysis and interpretation of data and the preparation of the manuscript, and D.M. Watts and G.E. Bettinger contributed to the preparation of the manuscript and interpretation of the data for the work, and all authors participated in the revision of the manuscript, and agreed to be accountable for all aspects of the work and approved the final version of this manuscript. All authors reviewed and complied with the journal policies detailed in the guide for authors.

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