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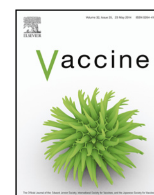

Comparative efficacy of two next-generation Rift Valley fever vaccines

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ABSTRACT

Rift Valley fever virus (RVFV) is a re-emerging zoonotic bunyavirus of the genus *Phlebovirus*. A natural isolate containing a large attenuating deletion in the small (S) genome segment previously yielded a highly effective vaccine virus, named Clone 13. The deletion in the S segment abrogates expression of the NSs protein, which is the major virulence factor of the virus. To develop a vaccine of even higher safety, a virus named R566 was created by natural laboratory reassortment. The R566 virus combines the S segment of the Clone 13 virus with additional attenuating mutations on the other two genome segments M and L, derived from the previously created MP-12 vaccine virus. To achieve the same objective, a nonspreading RVFV (NSR-Gn) was created by reverse-genetics, which not only lacks the NSs gene but also the complete M genome segment. We have now compared the vaccine efficacies of these two next-generation vaccines and included the Clone 13 vaccine as a control for optimal efficacy. Groups of eight lambs were vaccinated once and challenged three weeks later. All mock-vaccinated lambs developed high fever and viremia and three lambs did not survive the infection. As expected, lambs vaccinated with Clone 13 were protected from viremia and clinical signs. Two lambs vaccinated with R566 developed mild fever after challenge infection, which was associated with low levels of viral RNA in the blood, whereas vaccination with the NSR-Gn vaccine completely prevented viremia and clinical signs.

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1. Introduction

Rift Valley fever virus (RVFV) is a mosquito-borne *Phlebovirus* of the *Bunyaviridae* family that causes recurrent outbreaks among ruminants, particularly sheep. The virus is endemic to the African continent, the Arabian Peninsula and several islands off the eastern coast of southern Africa. Abortion storms and high mortalities among newborn animals are characteristic features of RVFV outbreaks, although losses among adult animals can also be considerable. Transmission of the virus among ruminants occurs via mosquito vectors, whereas humans can be infected via either mosquito bite or via contact with bodily fluids released during the slaughtering of diseased animals. Infections in humans generally manifest as mild, transient disease, but a small percentage of patients develop serious complications, such as ocular impediments, hemorrhagic fever or encephalitis. Considering the zoonotic nature of RVFV, vaccination of livestock will benefit both animal and human health [1].

The first veterinary RVFV vaccine was developed by Smithburn in 1949 [2]. After serial intracerebral passage in mice, a mutant virus was isolated with strongly reduced hepatotropism. Although the resulting Smithburn strain is attenuated, its residual virulence is well recognized [3,4]. To create a virus with a more attenuated profile, Caplen and co-workers cultivated the virulent RVFV strain ZH548, isolated during the Egyptian outbreak of 1977, in the presence of the mutagen 5-fluorouracil [5]. This procedure yielded the mutagenized MP-12 strain which was derived from the parental virulent strain after 12 successive passages in the presence of the mutagen. Later studies demonstrated that the MP-12 virus contains attenuating mutations in each of the three genome segments [6].

The safety and efficacy of MP-12 were evaluated in several extensive animal trials. These experiments suggested that the

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vaccine can be safely applied in lambs, calves and ewes after the first trimester of gestation [7–11]. In a recent study, vaccination of ewes during the first trimester of gestation with either MP-12 or a corresponding recombinant virus did not cause untoward effects in the ewes, although one of four ewes of each group was found to carry a dead fetus at the end of the experiment [9]. However, it has remained unclear whether these fetal mortalities were caused by the vaccine viruses. Nevertheless, efforts are continuing to further attenuate the MP-12 virus by reverse-genetics [9,12].

In 1995, a natural nonpathogenic RVFV isolate was described, named Clone 13, which contains a large (70%) internal deletion in the NSs gene of the small (S) genome segment [13]. Studies on the Clone 13 virus demonstrated that the NSs protein suppresses type-I interferon induction [14,15], which was later attributed to its interaction with SAP30 of the repressor complex [16]. Subsequent studies demonstrated that the NSs protein is multifunctional by (i) acting as a general inhibitor of transcription by sequestering p44 and XPB [17], causing degradation of p62 [18] as well as of the other TFIIH subunits like p52, p34 and cdk7 [17]; (ii) promoting the degradation of PKR [19,20], (iii) modifying gene expression to impair the hosts coagulation cascade [21] and (iv) interacting with gamma satellite pericentromeric sequences, provoking abnormal nuclei during cell division, a function of NSs possibly responsible for RVFV-mediated teratogenesis [22]. In addition, NSs was shown to be responsible for cell cycle arrest at either G0/G1 or S phase, as well as DNA damage response via the ATM protease [23,24]. These extensive studies have demonstrated that the NSs protein is the primary virulence factor of the virus and thereby revealed the molecular basis of the avirulent phenotype of Clone 13. The Clone 13 virus was shown to be highly effective and safe in gestating sheep [25] and calves [26] and was marketed in 2010 by the Onderstepoort Biological Products company in South Africa.

Despite the high safety profile of the Clone 13 virus, efforts are continuing to create vaccines with an even stronger safety signature. To this end, scientists of Institut Pasteur combined the attenuating mutations on the L and M segments of MP-12 with the S segment of the Clone 13 virus. The resulting reassortant virus, named R566, contains attenuating mutations on each of the three genome segments [27].

In an alternative strategy to develop a vaccine that optimally combines efficacy and safety, a novel vaccine based on a nonspreading RVFV, named NSR-Gn, was recently developed at CVI-WUR [28]. NSR-Gn particles are capable of infecting cells of the vaccinated animal, thereby triggering innate and adaptive immune responsive very similar to those elicited by live-attenuated vaccine viruses. The particles are however optimally safe, since they are incapable of producing progeny virions, due to the absence of the M genome segment. The NSR-Gn vaccine was recently demonstrated to confer sterile immunity in lambs after a single vaccination [28]. In the current study, the vaccine efficacies of R566 and NSR-Gn were compared, using Clone 13 vaccination as a control for optimal efficacy.

2. Materials and methods

2.1. Ethics statement

All animal experiments were conducted in accordance with the Dutch Law on Animal Experiments (Wod, ID number BWBR0003081) and approved by the Animal Ethics Committee of CVI-Lelystad. To minimize suffering of the animals during our vaccination challenge experiment, lambs were humanely euthanized when they could no longer be stimulated to drink, feed or stand.

2.2. Preparation of the vaccines

The Clone 13 [13] and the NSR-Gn [28] vaccines were previously reported in literature. The R566 vaccine virus was created as follows: Vero cells were grown as described [29] and co-infected with MP-12 and Clone 13 at a multiplicity of infection of 1 and 0.1, respectively. Viruses released in the culture medium were collected at 48 h post infection when a complete cytopathic effect was observed as described [30]. The viruses were titrated by plaque assay using the standard method described earlier [31]. Plaques were picked, amplified in Vero cells and screened for NSs expression by immunostaining with NSs-specific antibodies [17]. The origins of the L, M and S segments were determined by RT-PCR amplification and sequencing as described [30]. Each selected reassortant was sub-cloned three times by plaque assay and the sequences verified at the first and third cycle.

The R566 and Clone 13 vaccine strains were cultured on Vero cells grown in 490 cm^2 roller bottles. The culture medium consisted of a combination of Glasgow's and Eagles modified minimal essential medium (MEM) supplemented with fetal bovine serum and antibiotics. Vaccines were diluted in culture medium to achieve titers appropriate for vaccination.

2.3. Preparation of the challenge virus

The molecularly cloned 35/74 (rec35/74) virus was derived from the sequence of strain 35/74 and was titrated on baby hamster kidney (BHK) cells as tissue culture infective dose 50 ($TCID_{50}$) using the Spearman–Kärber algorithm [32,33]. The virus was handled under biosafety level-3 laboratory conditions in class-III biosafety cabinets.

2.4. Vaccination and challenge

Thirty-two lambs of the Romane (INRA 401) breed, 12–14 weeks of age at the moment of vaccination were purchased from Institut National de la Recherche Agronomique (INRA, Nouzilly, France). Lambs were randomized according to gender and age into 4 groups of 8 individuals each and submitted to general health inspection before the start of the study. The Clone 13 vaccine was administered subcutaneously in the right axilla at a dose of 10^5 pfu in a volume of 1 ml. The R566 vaccine was administered subcutaneously in the right axilla with a dose of 10^6 pfu in a volume of 2 ml. The NSR-Gn vaccine was administered via the intramuscular route (neck muscle) at a dose of 10^6 $TCID_{50}$ in a volume of 1 ml. Control lambs were inoculated with 2 ml phosphate buffered saline (PBS, GIBCO™, Carlsbad, CA, USA) subcutaneously in the right axilla and 1 ml of PBS in the neck muscle.

Three weeks after vaccination, all lambs were sedated by intramuscular administration of medetomidine (40 μg/kg medetomidine hydrochloride, Sedator, Eurovet, The Netherlands) and subsequently challenged by administrating 1 ml culture medium containing 10^5 $TCID_{50}$ of molecularly cloned RVFV strain 35/74 in the *vena jugularis*. The rescue of the challenge virus was previously reported [34]. For vaccination and challenge, 18 gauge, 25 mm needles were used. Blood samples for the preparation of sera were collected starting on day 6 before vaccination (DPV −6), on the day of vaccination (DPV 0) and subsequently every week until the end of the experiment. EDTA blood samples were collected on DPV −6, 0 and subsequently daily from DPV 21 (the day of challenge) to DPV 32, and on DPV 35 and 42. Body weights were determined on DPV -6 and weekly starting on DPV 0. Rectal body temperatures were determined daily. The lambs that survived the challenge were euthanized 3 weeks after challenge (DPV 42) by intravenous administration of 50 mg/kg sodium pentobarbital (Euthasol®, ASTfarma,

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