Distinct VSV-based Nipah virus vaccines expressing either glycoprotein G or fusion protein F provide homologous and heterologous protection in a nonhuman primate model

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Summary

Background Nipah virus (NiV) causes recurrent outbreaks of lethal respiratory and neurological disease in Southeast Asia. The World Health Organization considers the development of an effective vaccine against NiV a priority.

Methods We produced two NiV vaccine candidates using the licensed VSV-EBOV vaccine as a backbone and tested its efficacy against lethal homologous and heterologous NiV challenge with Nipah virus Bangladesh and Nipah virus Malaysia, respectively, in the African green monkey model.

Findings The VSV-EBOV vaccine expressing NiV glycoprotein G (VSV-NiVG) induced high neutralising antibody titers and afforded complete protection from homologous and heterologous challenge. The VSV-EBOV vaccine expressing NiV fusion protein F (VSV-NiVF) induced a lower humoral response and afforded complete homologous protection, but only partial heterologous protection. Both vaccines reduced virus shedding from the upper respiratory tract, and virus replication in the lungs and central nervous system. None of the protected animals vaccinated with VSV-NiVG or VSV-NiVF showed histological lesions in the CNS, but one VSV-NiVF-vaccinated animal that was not protected developed severe meningoencephalitis.

Interpretation The VSV-NiVG vaccine offers broad protection against NiV disease.

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Introduction

Nipah virus (NiV) causes severe respiratory and neurological disease with a high case-fatality rate in infected individuals.[1] Based on its lethality, ability to transmit between people, and the lack of medical countermeasures, the World Health Organization has listed NiV as a priority pathogen for which more research is urgently needed.[2] Although no approved vaccines to prevent NiV disease and transmission currently exists, they would be essential tools to prevent NiV outbreaks or to limit their spread. Unlike the related Hendra virus in Australia, where an approved vaccine is used in horses to prevent zoonotic transmission to humans,[3] NiV is most-often transmitted directly from bats to humans, making a similar approach unfeasible and rather requiring a vaccine for use in humans. Several NiV vaccine candidates are currently in different stages of development, including live-attenuated recombinant vectors, virus-like particle and subunit vaccine approaches. All are based on the induction of neutralising antibodies against the NiV glycoprotein (G) or fusion protein (F (reviewed in[4])).

From an outbreak management perspective, the ideal NiV vaccine would be effective after only a single dose and have a short time to immunity. The

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Research in context

Evidence before this study

We performed PubMed searches for studies describing Nipah virus (NiV) vaccine candidates. We focused on studies that included a NiV challenge to test efficacy. Several NiV vaccine candidates are currently in different stages of development, including live-attenuated recombinant vectors, virus-like particle and subunit vaccine approaches. For all, efficacy seems associated with the induction of neutralising antibodies against the NiV glycoprotein (G) or fusion protein (F) of the Malaysia genotype, even though all NiV outbreaks since 1999 have been caused by the NiV Bangladesh genotype.

Added value of this study

This study assessed the efficacy of two NiV vaccine candidates based on the FDA- and EMA-licensed VSV-EBOV vaccine expressing the genotype Bangladesh G or F, against homologous and heterologous challenge. Both vaccines provided complete protection from homologous NiV, genotype Bangladesh challenge, but only the VSV-EBOV vaccine expressing NiV G afforded complete protection from heterologous NiV, genotype Malaysia challenge.

Implications of all the available evidence

The World Health Organization has listed NiV as a priority pathogen for which vaccines urgently need to be developed. Based on the existing human safety data available for the VSV-EBOV backbone, the rapid induction of protective responses by the VSV-based NiV vaccine shown previously, and the broad protection against homologous and heterologous challenge shown here, the VSV-EBOV-based vaccine expressing NiV Bangladesh G is a powerful vaccine candidate for deployment during NiV outbreaks.

recombinant vesicular stomatitis virus (VSV) vaccine platform has exactly those properties. In the FDA and EMA-approved VSV-EBOV vaccine (also known as Ervebo), the VSV glycoprotein G has been replaced with the Ebola virus (EBOV) glycoprotein GP.[5–7] During the West African Ebola epidemic, the VSV-EBOV vaccine was shown to induce protection against Ebola virus disease 10 days after administration of a single dose[8] and it has been used in several EBOV outbreaks since then to mitigate spread of the virus. VSV-EBOV has previously been established as a vaccine backbone for a NiV vaccine by insertion of the NiV G or F glycoprotein. This vaccine uses the EBOV GP for cell targeting and entry, but additionally expresses NiV G or F, resulting in the induction of neutralising antibodies and protective efficacy against NiV challenge in hamsters and nonhuman primates after a single dose.[9,10] In Syrian hamsters, this VSV-EBOV-NiV G vaccine completely protected from lethal challenge when vaccination was administered one day prior to challenge.[11] Thus, the VSV-based Nipah virus vaccine has the desired properties to become a successful medical countermeasure against NiV outbreaks. However, the VSV-EBOV backbone used in previous NiV vaccine studies was not identical to the one used in the approved VSV-EBOV vaccine, since it expresses a different EBOV GP for virus entry (derived from the Mayinga rather than Kikwit EBOV strain).

Since extensive human safety data are now available for the VSV-EBOV vaccine, we decided to reconstruct our VSV-EBOV-based NiV vaccine to match the approved vaccine vector. Moreover, since all human NiV infections since 1999 have been caused by the Bangladesh genotype of NiV,[12] we cloned the glycoprotein G and F derived from NiV, strain Bangladesh (NiV_B) rather than from NiV, genotype Malaysia (NiV_M) in this new vector. The amino acid similarity between the NiV_B and NiV_M G and F protein used is 95.7% and 98.5% respectively. Here, we tested the protective efficacy of the VSV-EBOV expressing either NiV_B G or F against lethal challenge with a homologous (NiV_B) or heterologous (NiV_M) challenge strain in African green monkeys, the gold standard model to test NiV vaccine efficacy. Protective efficacy of the vaccine expressing NiV G was 100% against homologous and heterologous challenge, whereas protective efficacy of the vaccine expressing NiV F was 100% against homologous, but 75% against heterologous challenge.

Methods

Study design

The aim of this study was to determine the protective efficacy of two vaccine candidates against homologous and heterologous NiV challenge. Twenty adult African green monkeys (11 males and 9 females; 3–6.5 kg; body condition scores 4 and 5 on a 9-point scale[13]) were used in this study; group sizes (n = 4) were determined by power analysis using previously observed survival and viral load data in published[10,14] and unpublished studies, aiming to achieve statistical significance with a confidence interval of 99% assuming 5-fold differences in viral loads between groups. African green monkeys were assigned to study groups randomly while ensuring equal distribution of sex and bodyweight (as a proxy for age). Animals were acclimated to the study room for at least 5 days prior to study start. Animals were vaccinated intramuscularly with a total dose of 10^7 PFU of VSV-$EBOV_{Kik}$ GP-NiV_B G (n = 8, hereafter VSV-NiVG), VSV-$EBOV_{Kik}$ GP-NiV_B F (n = 8, hereafter VSV-NiVF), or VSV-$EBOV_{Kik}$ GP (n = 4, hereafter VSV-EBOV) divided into two sites in the caudal thighs (Table S1). Of note, the VSV-$EBOV_{Kik}$ GP vaccine backbone matches the FDA- and EMA-approved VSV-EBOV vaccine. At several

timepoints post vaccination, serum was collected to determine the humoral response to vaccination. Twenty-eight days post vaccination, half of the animals in each vaccine cohort were challenged with a lethal dose of homologous NiV_B or heterologous NiV_M, administered as 10^5 50% tissue culture infectious dose (TCID50) intranasally (0.5 ml per nostril) and 10^5 TCID50 intratracheally (4 ml) as previously established.[14] After inoculation with NiV, animals were monitored for signs of disease and assigned a clinical score based on an IACUC-approved scoring sheet.[15] Scoring was done by the same individual throughout the study; this person was blinded to group assignment of the animals. A score (0–15) was assigned for general appearance, skin and fur, nose/mouth/eyes/head, respiration, feces and urine, food intake, and locomotor activity. Animals were euthanised by trained and experienced personnel when the total clinical score reached the critical number of 35 using an American Veterinary Medical Association-approved method. Clinical exams were performed on 0, 3, 5, 7, 10, 14, 21, 28, 35 days post inoculation (dpi). On exam days, clinical parameters such as bodyweight, body temperature, respiration rate, and oxygen saturation (SPO_2) were collected, as well as a blood sample and oral and nasal swabs. Swabs were collected in 1 ml of DMEM (Sigma) with 50 U/ml penicillin and 50 μg/ml streptomycin (Gibco) and blood was collected in EDTA and serum tubes (BD). Potential confounders such as the order of treatments and measurements, or animal/cage location could not be controlled in this experiment. Upon euthanasia on 35 dpi or when the animal reached humane endpoint, necropsies were performed and tissue samples were collected. No criteria were set for including or excluding animals or datapoints from the experiment; all animals and datapoints were included in the analysis.

Ethics and biosafety statement

All animal experiments were approved by the Institutional Animal Care and Use Committee of Rocky Mountain Laboratories, NIH (#2018–022) and carried out in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International accredited facility, according to the institution's guidelines for animal use, and followed the guidelines and basic principles in the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the Guide for the Care and Use of Laboratory Animals. African green monkeys were housed in adjacent individual primate cages allowing social interactions, in a climate-controlled room with a fixed light–dark cycle (12-hr light/12-hr dark). Animals were monitored at least twice daily throughout the experiment; this was increased to a minimum of 4 times per 24 h when severe disease started to develop. Commercial monkey chow, treats, and fruit were provided twice daily by trained personnel. Water was available ad libitum. Environmental enrichment consisted of a variety of human interaction, commercial toys, videos, and music. Humane endpoint criteria, specified and approved by the Institutional Animal Care and Use Committee (IACUC), were applied to determine when animals should be humanely euthanised.

Sample inactivation was performed according to standard operating procedures for removal of specimens from high containment approved by the Institutional Biosafety Committee.

Vaccine constructs

The plasmid pVSVXN2 (kindly provided by J. Rose, Yale University, New Haven) was modified to encode the open reading frame (ORF) for Ebola virus, Kikwit strain glycoprotein ($EBOV_{Kik}$ GP) in place of that encoding the VSV glycoprotein (G).[16] To account for antigenic variation in the NiV_B virus glycoprotein G, we determined the G amino acid consensus sequence from all available NiV_B G sequences in GenBank at the start of this project (AY9886001, FJ513078, JN808857, JN808863, JN808864). The resulting NiVB G consensus sequence was identical to those of several NiVB G sequences in GenBank (entries AY988601, JN808857, and JN808863); this consensus sequence was chemically synthesised (Invitrogen). The NiV_B F glycoprotein was amplified from NiV, strain Bangladesh/200401066. The entire open reading frames of the NiV_B G or F were then cloned into pVSVXN2ΔG-$EBOV_{Kik}$ GP downstream of $EBOV_{Kik}$ GP after duplication of the VSV intergenic regions as described elsewhere[17] (Fig. 1a). BHK-T7 cells were transfected using transit-LT1 Transfection Reagent (Mirus, Madison, WI) with individual plasmids encoding the VSV N, P, and L ORFs and the modified VSV genomic plasmids as shown in Fig. 1a and described previously.[6] Cells were incubated at 37 °C for 7 days, at which time supernatant was collected and passaged once on fresh Vero cells. Cultures were monitored daily for cytopathic effect (CPE) and supernatants were collected. Vaccine stocks were prepared, stored at −80 °C, and sequences were confirmed by NGS; no changes from the vector sequence were observed. The titer of vaccine stocks was determined using a conventional plaque assay. Briefly, 10-fold serial dilutions of the vaccine stock were prepared in DMEM. VeroE6 were inoculated in duplicate with vaccine stock dilutions and incubated for 1 h at 37 °C and 5% CO_2. Supernatant was removed from wells and replaced with MEM containing 2% FBS, 2 mM L-glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin and 0.5% agarose. 24–72 h later, wells were stained with crystal violet and plaques were counted.

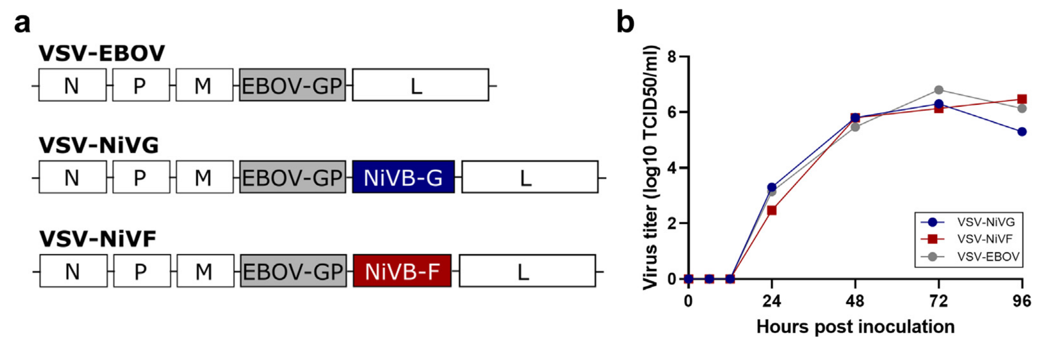

Fig. 1: **Design and in vitro growth kinetics of VSV-NiV vaccines.** a. Genome organization of the VSV vectors used in this study. The VSV vectors in which the VSV glycoprotein was replaced with those of Ebola virus and Nipah virus were generated using the VSV reverse genetics system as described in the Methods section. N nucleoprotein; P phosphoprotein; M matrix protein; EBOV-GP Ebola virus glycoprotein; NiVB-G Nipah virus, Bangladesh glycoprotein G; NiVB-F Nipah virus, Bangladesh glycoprotein F; L polymerase. b. In vitro growth kinetics of the VSV vaccines used in this study. VeroE6 cells were infected with a MOI of 0.001 with VSV-EBOV, VSV-NiVG, and VSV-NiVF in triplicate. Supernatant samples were collected at different timepoints post-infection and virus titers were determined.

Western blot analysis

NiV_B, VSV-EBOV, VSV-NiVG and VSV-NiVF were concentrated. Supernatant from VeroE6 cells infected with NiV_B, VSV-EBOV, VSV-NiVG and VSV-NiVF were harvested and cleared by centrifugation at 2000 rpm for 10 min. Supernatant was then stacked on top of 5 ml 15% Optiprep (Sigma-Aldrich) and spun in an ultracentrifuge for 1.5 h at 21,000 rpm. Pellets were resuspended in PBS. VSV-EBOV, VSV-NiVG and VSV-NiVF were further purified by spinning the resuspended pellet overnight over a 10–50% Optiprep gradient in an ultracentrifuge at 39,000 rpm. Bands were harvested and spun over an Amicon 15 ml, 50,000 kDa tube (Millipore Sigma) for 30 min at 3500 rpm to remove Optiprep from the sample. Samples were brought up to 500 μl using PBS and mixed 1:1 with SDS gel electrophoresis sample buffer containing 20% β-mercaptoethanol and heated to 99 °C for 10 min. Samples were run on a 12% mini Portean TGX stain free protein gel and transferred to a polyvinylidene difluoride membrane using the Trans-Blot Turbo 'Mixed MW' protocol (Bio-Rad Laboratories). The membrane was blocked for 2 h at room temperature in PBS with 5% powdered milk and 0.1% Tween 20 (Thermo Fisher Scientific). EBOV-GP was detected using anti-EBOV GP (ZGP 12/1.1, 1 μg/ml) and horse-radish peroxidase (HRP)-labeled secondary antibody staining using anti-mouse Immunoglobulin G (IgG; 1:10,000) (Millipore Sigma).[18] A polyclonal antiserum against NiVG was generated through the PolyExpress™ Custom Polyclonal Antibody Production Services (GenScript). Briefly, rabbits were injected with the NiVG peptide CRYDKVMPYGPSGIK, serum harvested and polyclonal antibodies purified using antigen affinity purification. The specificity of the resulting polyclonal antibody was tested using cell lysate from mock-infected cells, concentrated NiV_B and recombinant F or G protein. NiVG was detected in Western blots using a 1:250 dilution and horse-radish peroxidase (HRP)-labeled secondary antibody staining using anti-rabbit Immunoglobulin G (IgG; 1:10,000) (Jackson ImmunoResearch). The blots were imaged using the SuperSignal West Pico chemiluminescent substrate (Thermo Fisher Scientific) and a FluorChem E system (ProteinSimple).

Virus and cells

Nipah virus strain Bangladesh/200401066 (GenBank AY988601) and strain Malaysia/199901924 (GenBank AF212302) were provided by the Special Pathogens Branch of the Centers for Disease Control and Prevention, Atlanta, GA, and propagated once in Vero E6 cells in DMEM supplemented with 2% fetal bovine serum (FBS), 1 mM L-glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin (cell culture passage 2 material of both viruses was kindly provided by the CDC, Viral Special Pathogens Branch). VeroE6 cells were maintained in DMEM supplemented with 10% fetal calf serum, 1 mM L-glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin. BHK-T7 cells were grown at 37 °C and 5% CO_2 in minimum essential medium (Thermo Fisher Scientific) containing 10% tryptose phosphate broth (Thermo Fisher Scientific), 5% FBS, 2 mM L-glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin. STR profiling of cell lines has not been performed; cell lines are tested monthly for the presence of mycoplasma and have remained negative.

Replication kinetics

VeroE6 cells were grown to confluency in a 12-well plate and infected with a multiplicity of infection (MOI) of 0.001 with VSV-EBOV, VSV-NiVG, and VSV-NiVF in triplicates. After 1 h, the inoculum was removed and

replaced with DMEM supplemented with 2% FBS, penicillin/streptomycin and L-glutamine. Supernatant samples were collected at 0, 6, 12, 24, 48, 72, and 96 h post-infection and stored at –80 °C until titration. Virus titers from these samples were determined by inoculating VeroE6 cells with 10-fold serial dilutions in triplicate. After 1 h, DMEM supplemented with 2% FBS, penicillin/streptomycin and L-glutamine was added and cells were incubated at 37 °C. Cells were monitored for cytopathic effect and the TCID50 was calculated for each sample.

Quantitative PCR

RNA was extracted from swab samples in DMEM and from EDTA blood samples using the QIAamp viral RNA mini kit (Qiagen) according to the manufacturer's instructions. RNA was extracted from tissues using the RNeasy kit (Qiagen); tissues (30 mg) were homogenised in RLT buffer and RNA was extracted according to the manufacturer's instructions. 5 μl RNA was used in a one-step real-time RT-PCR targeting the N gene of NiV_B or NiV_M using the Rotor-Gene probe kit (Qiagen) according to instructions of the manufacturer using the following primer-probe set for NiV_B: forward primer 5′-GTTCAGGCCAGAGAAGCTAAATTT-3′, reverse primer 5′-CCTCTTCGTCGACATCTTGATCA-3′, probe 5′-FAM-CTGCAGGAGGTGTGCTCATCGGAGG-TAMRA-3′, and for NiV_M: forward primer 5′-GTTCAGGCTAGAGAGGCAAAATTT-3′, reverse primer 5′- CCCCTTCATCGATATCTTGATCA-3′, probe 5′-FAM-CTGCAGGAGGTGTGCTCATTGGAGG-TAMRA-3'. In each run, dilutions of synthetic RNA standards with known copy numbers were run in parallel, to calculate copy numbers in the samples. RT-qPCR assays and RNA standards specific for NiV_B or NiV_M were used depending on the virus isolate the animals were challenged with.

ELISA

IgG antibody responses were measured in an ELISA using recombinantly expressed NiV_M glycoprotein F or G. NiV-G and NiV-F were produced and purified as described previously.[19] Briefly, NiV-G Malaysia (residues E144–T602, gene accession number NC_002728) was cloned into the pHLSEC mammalian expression vector[20] and NiV-F Malaysia (residues G26–D482, gene accession number AY816748.1) was cloned into the pHLSEC vector containing a C-terminal GCNt trimerization motif.[21] The constructs were transiently expressed in human embryonic kidney (HEK) 293T cells then further purified by Ni-NTA and size exclusion chromatography. Nunc Maxisorp 96-well flat-bottom Immuno Plates (ThermoFisher) were coated with NiV F and G (50 ng in 50 μl per well, diluted in PBS) overnight at 4 °C. Subsequently, plates were blocked with 5% skim milk in PBS containing 0.05% Tween 20 (PBST) for 1.5 h at 4 °C. After 3 washes with PBST, 50 μl of diluted serum samples (starting dilution: 1:100) were added and the plates were incubated 1 h at 37 °C. Bound antibodies were detected after three washes using horseradish peroxidase (HRP)-conjugated anti-monkey IgG (KPL). Following incubation for 1 h at 37 °C, bound HRP was detected using the ABST Peroxidase substrate system (KPL). The absorbance was measured at 405 nm; sera were considered positive when absorbance was higher than three standard deviations above the mean of negative control sera.

Virus neutralization assay

Two-fold serial dilutions, starting at a 1:20 dilution, of heat-inactivated (30 min, 56 °C) sera were prepared in duplicate in DMEM containing 2% FBS, 1 mM L-glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin, after which 100 TCID50 of NiV_B or NiV_M was added. After 1 h incubation at 37 °C, this mix was added to Vero E6 cells. At 5 dpi, wells were scored for CPE. The virus neutralization titers were expressed as the reciprocal value of the highest dilution of the serum that still inhibited virus replication.

Virus titrations

Virus titration of tissue samples was performed by end-point titration in VeroE6 cells. Tissue homogenates were prepared by adding 1 ml DMEM to the weighed tissue and homogenising using a TissueLyzer II (Qiagen). Homogenates were centrifuged to clear the homogenate before inoculating cells. VeroE6 cells were inoculated with tenfold serial dilutions of swab medium or tissue homogenates. One hour after inoculation, the inoculum was removed and replaced with 100 μl DMEM supplemented with 2% fetal calf serum, 1 mM L-glutamine (Lonza), 50 U/ml penicillin and 50 μg/ml streptomycin (Gibco). Five days after inoculation, CPE was scored.

Histopathology

Histopathology and immunohistochemistry were performed on African green monkey lungs and central nervous system (CNS). After fixation for a minimum of 7 days in 10% neutral-buffered formalin and embedding in paraffin, tissue sections were stained with hematoxylin and eosin (HE). Immunohistochemistry was performed using a custom-made rabbit antiserum against Nipah virus N (GenScript) at a 1:500 dilution. For the lungs, 3 standardised sections were analysed for each of the 6 lung lobes. For the CNS, the whole formalin-fixed brain is placed in a matrix mold and eight evenly spaced coronal sections are collected and embedded. This allows for consistent and thorough histologic examination of the cerebrum, cerebellum, and brainstem. Slides were analysed and scored according to a standardised

scoring system by a veterinary pathologist blinded to group assignment of the animals.

Statistical analysis

Statistical analysis using Graphpad Prism version 8.4.3 was performed on tissue viral load data using a 2-way ANOVA using Sidak's or Tukey's multiple comparison test to account for non-normal distribution of data with small sample size and different number of samples per group due to experimental design.

Role of funders

Funders had no input in study design, data collection, data analysis, interpretation, writing of this report or decision to publish.

Results

VSV-NiVG and VSV-NiVF have replication kinetics similar to their VSV-EBOV backbone

Efficient growth of replicating vaccines in cell culture is essential for large-scale production of the vaccine. After the two vaccine candidates VSV-NiVG and VSV-NiVF (Fig. 1a) were rescued, we compared their growth kinetics to those of VSV-EBOV, the backbone of the two vaccine candidates. VeroE6 cells were inoculated with a MOI of 0.001 of the three vaccines and replication was followed over time. Although the VSV-EBOV replicates less efficiently than recombinant wild type VSV,[22] replication kinetics were remarkably similar between VSV-EBOV, VSV-NiVG and VSV-NiVF and all three viruses reached similar peak titers at 72 hpi (Fig. 1b). Thus, the presence of an additional open reading frame did not hamper replication of the VSV-NiVG and VSV-NiVF vaccines in vitro, an important aspect for vaccine production. To assess whether the additional NiV glycoproteins were incorporated into the VSV-EBOV particles, we performed Western blot analysis on purified recombinant VSV particles using a monoclonal antibody against EBOV GP and a polyclonal antibody against NiV G. All three vaccines, VSV-EBOV, VSV-NiVG and VSV-NiVF showed incorporation of EBOV GP as expected (Fig. S1). NiV G could also be detected in purified VSV-NiVG samples (Fig. S1), indicating the incorporation of NiV G into the vaccine particles. Of note, several antibodies were tested, but none were suitable for detection of NiV F in Western blot. Thus, we cannot confirm if NiV F is also incorporated into the VSV vaccine, although this appears likely based on the results with NiV G.

Humoral response to vaccination

Three groups of adult African green monkeys were vaccinated intramuscularly with 10^7 PFU of VSV-NiVG (n = 8), VSV-NiVF (n = 8) or VSV-EBOV (n = 4) (Table S1). Injection site reactions or other adverse effects were not observed in any of the animals vaccinated with the three different vaccines. At several timepoints post vaccination, sera were collected to determine the humoral response to vaccination. ELISA using recombinantly expressed NiV_M G or F were performed to determine the IgG response to vaccination. At 14 days post vaccination (i.e. 14 days before challenge), all animals vaccinated with VSV-NiVG had high IgG titers against heterologous NiV_M G that remained high until the time of challenge (0 dpi) (Fig. 2). In the animals vaccinated with VSV-NiV-F, only 6 of 8 animals had detectable IgG against NiV_M F on 14 days post vaccination (Fig. 2). All VSV-NiVF-vaccinated animals had detectable anti-F IgG by the time of challenge. No responses to NiV G or F were detected in the animals vaccinated with VSV-EBOV (Fig. 2). Anti-G and -F titers cannot be directly compared as two distinct antigens were used for the assays.

Neutralising titers were determined at the time of challenge (0 dpi) in a microneutralisation assay using NiV_B and NiV_M. All 8 animals vaccinated with VSV-NiVG had neutralising titers against the homologous NiV_B at time of challenge, while only 5 of these animals also neutralised NiV_M (Fig. 3). In contrast, only 1 of 8 VSV-NiVF-vaccinated animals had neutralising titers against NiV_B at time of challenge, while 3 of 8 had NiV_M neutralising antibodies (Fig. 3).

Protective efficacy against homologous and heterologous challenge

Twenty-eight days post vaccination, half of the animals vaccinated with VSV-NiVG, VSV-NiVF, and VSV-EBOV received a homologous challenge with a lethal dose of NiV_B. All animals vaccinated with VSV-NiVG or VSV-NiVF survived the NiV_B challenge (Fig. 4a); clinical signs were limited to a reduced appetite (Fig. 4b), possibly as the result of repeated anesthesia. The two control animals vaccinated with VSV-EBOV and challenged with NiV_B reached humane endpoints on 6 and 9 days post inoculation (dpi). The other half of vaccinated animals in each group received a heterologous challenge with a lethal dose of NiV_M. All animals vaccinated with VSV-NiVG survived this challenge (Fig. 4c), with clinical signs again limited to a reduced appetite (Fig. 4d). In the group vaccinated with VSV-NiVF and challenged with NiV_M, three animals showed only reduced appetite after challenge and survived. However, one animal in that group showed a reduced appetite until 11 dpi, then became asymptomatic until the morning of 17 dpi, when it was found obtunded and was euthanised. The two control animals vaccinated with VSV-EBOV and challenged with NiV_M reached humane endpoints on 7 and 8 dpi. Serum samples collected at time of euthanasia showed that animals vaccinated with VSV-NiVG

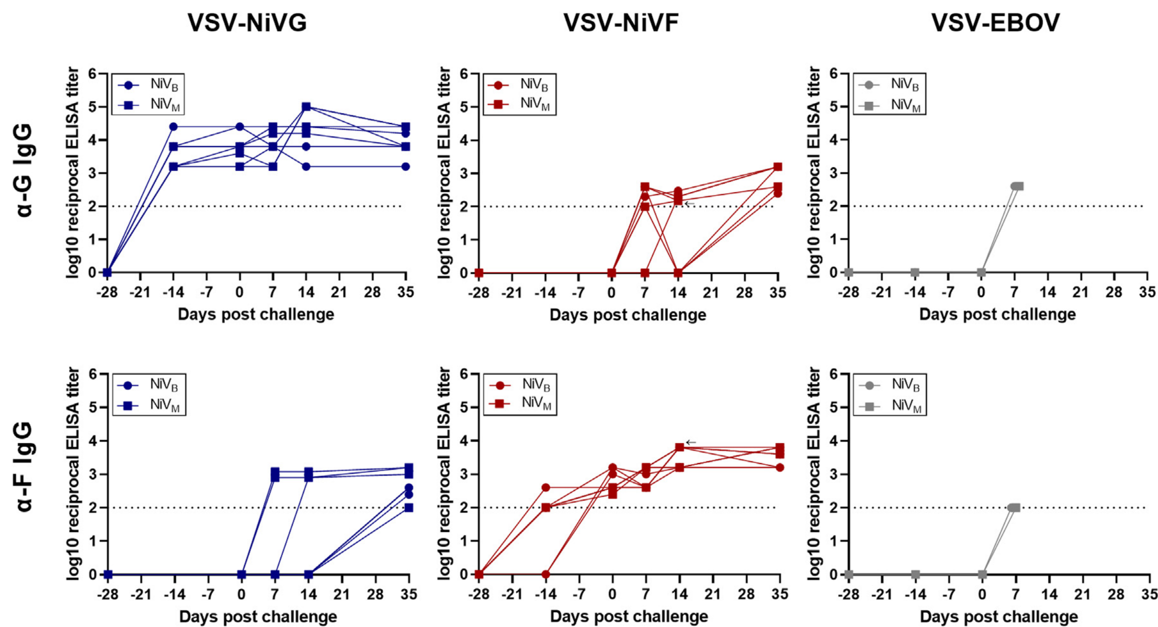

Fig. 2: **Serum IgG responses after vaccination and challenge.** Serum samples were collected from African green monkeys after vaccination with 10^7 PFU of VSV-EBOV (n = 4), VSV-NiVG (n = 8), and VSV-NiVF (n = 8), and after lethal challenge with 10^5 TCID50 intranasally and 10^5 TCID50 intratracheally of NiV$_B$ (homologous) or NiV$_M$ (heterologous). IgG responses against NiV G (top panels) and NiV F (bottom panels) were determined using an ELISA assay with recombinant NiV$_M$ G or F glycoprotein in duplicate. Legends indicate which challenge virus the animals received. Dotted lines indicated the lower limit of detection of the assay. Arrows in the VSV-NiVF panels indicate the animal euthanized on 17 rather than 35 dpi.

developed IgG against glycoprotein F in ELISA, and animals vaccinated with VSV-NiVF developed IgG against glycoprotein G (Fig. 2). Moreover, neutralising titers increased between time of challenge and time of euthanasia in almost all of the animals vaccinated with VSV-NiVG or VSV-NiVF, together indicating that vaccination did not confer sterile protection (Fig. 3).

Nipah virus replication after lethal challenge

To monitor virus replication in the vaccinated animals after challenge, we performed regular clinical exams during which blood, nose swabs and throat swabs were collected and analysed for the presence of NiV RNA. All four VSV-EBOV vaccinated control animals became viremic after challenge with NiV$_B$ or NiV$_M$, but none of the VSV-NiVG- or VSV-NiVF-vaccinated animals did, not even the VSV-NiVF vaccinated animal that was euthanised on 17 dpi (Fig. 5a). Viral RNA could be detected in nose swabs collected from all VSV-EBOV-vaccinated animals after NiV$_B$ or NiV$_M$ challenge, but in only one of the VSV-NiVG- and one of the VSV-NiVF-vaccinated animals; both these animals had received a heterologous challenge (Fig. 5a). Interestingly, one of these positive swabs was collected on 14 dpi from the VSV-NiVF-vaccinated animal that was euthanised on 17 dpi. However, a swab collected from this animal at time of euthanasia was negative. Viral RNA was also detected in throat swabs from all VSV-EBOV vaccinated animals after NiV$_B$ or NiV$_M$ challenge; viral RNA was detected in throat swabs from only three animals that received the VSV-NiVG or VSV-NiVF vaccine (Fig. 5a). Thus, vaccination greatly reduced virus shedding after homologous and heterologous NiV virus challenge.

When the animals reached humane endpoints, or at the end of the experiment on 35 dpi, animals were euthanised and tissues were collected. In the VSV-EBOV-vaccinated control animals, high NiV viral loads were detected in lungs (n = 6 tissue samples per animal) and the central nervous system (CNS; n = 3 tissue samples per animal) (Fig. 5b); with statistically significantly higher viral loads in NiV$_B$-challenged animals than in NiV$_M$-challenged animals (2-way ANOVA; lungs: $p < 0.0001$; CNS $p = 0.001$). Protected animals showed very little evidence of virus replication in the lungs; viral RNA could only be detected in a single lung lobe of two animals vaccinated with VSV-NiVG, and one lobe of one animal vaccinated with VSV-NiVF Additionally, no histological lesions or viral antigen could be detected by histology in the protected animals vaccinated with VSV-NiVG or VSV-NiVF and challenged with NiV$_B$ or NiV$_M$ (Fig. S2). Viral RNA, but no infectious virus (Table S2), could be detected in the CNS of 7 of 8 protected animals vaccinated with VSV-NiVG or VSV-NiVF and receiving a homologous challenge, and 2/7 protected animals receiving a heterologous challenge

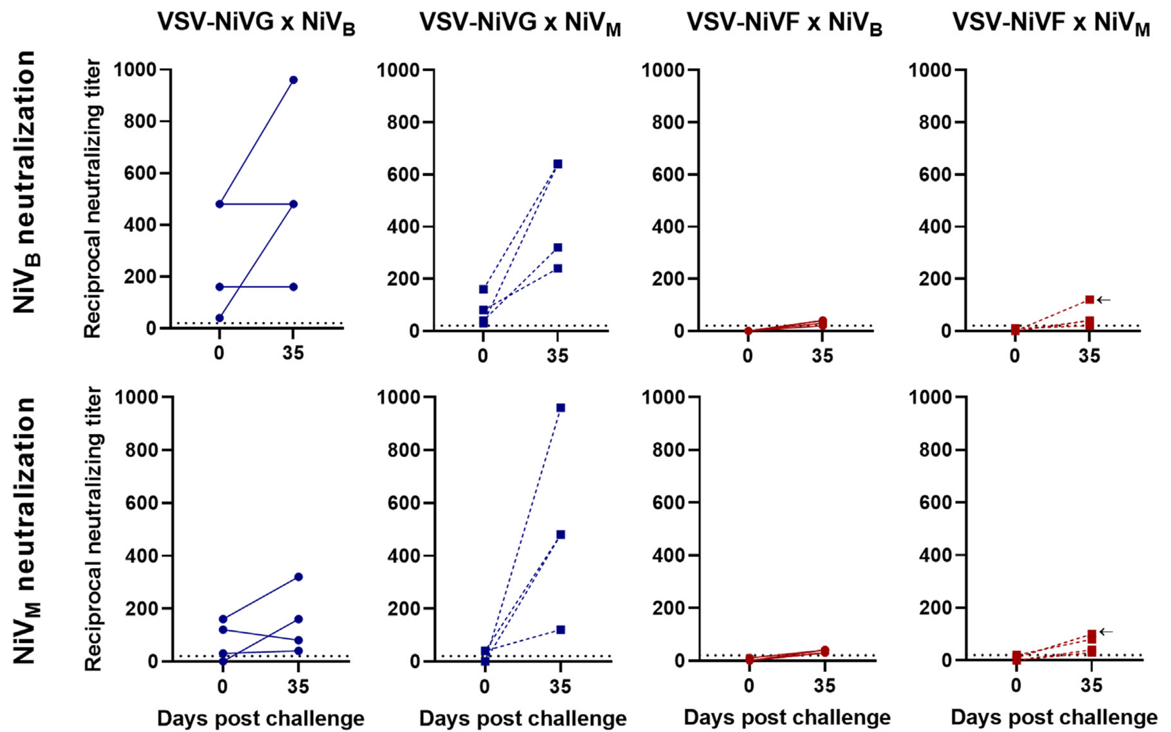

Fig. 3: **Neutralising antibody responses after vaccination and challenge.** Serum samples were collected from African green monkeys after vaccination with 10^7 PFU of VSV-NiVG (n = 8), and VSV-NiVF (n = 8), and after lethal challenge with 10^5 TCID50 intranasally and 10^5 TCID50 intratracheally of NiV_B or NiV_M. Neutralizing antibody titers were determined against homologous NiV_B (top panels) and heterologous NiV_M (bottom panels) in duplicate. Legends at the top indicate which vaccine and which challenge virus the animals received. Dotted lines indicated the lower limit of detection of the assay. Arrows in the VSV-NiVF × NiV_M panels indicate the animal euthanized on 17 rather than 35 dpi.

(Fig. 5c). The single unprotected animal vaccinated with VSV-NiVF and challenged with NiV_M had levels of viral RNA in the CNS at time of euthanasia similar to those in the VSV-EBOV-vaccinated animals challenged with NiV_M (Fig. 5d).

Absence of histopathological changes in the CNS of protected animals

To evaluate the ability of the VSV-NiVF and VSV-NiVG vaccine to protect from NiV replication in the CNS that may not cause overt clinical signs until long after NiV challenge, we thoroughly assessed the CNS of all animals histologically. Despite the presence of viral RNA, no histologic lesions were detected in the animals vaccinated with VSV-EBOV and challenged with NiV_B or NiV_M, potentially due to the early timepoint after challenge (6–9 dpi) at which these control animals were euthanised (Fig. 6a). None of the protected animals vaccinated with VSV-NiVG or VSV-NiVF showed histological abnormalities in the CNS attributed to NiV infection (Fig. 6a); nor was viral antigen detected by immunohistochemistry (Fig. S3). The animal vaccinated with VSV-NiVF, challenged with NiV_M, and euthanised with clear neurological signs on 17 dpi had severe meningoencephalitis with multifocal regions of lymphoplasmacytic perivascular cuffing throughout the cerebrum and cerebellum. There were few large foci of inflammation and necrosis within the neuroparenchyma, characterised by fibrinoid necrosis of vessels with rare fibrin thrombi, lymphoplasmacytic to rarely neutrophilic infiltrates, gliosis, malacia, and edema. NiV antigen was abundantly present in neurons within these lesions (Fig. 6b).

Discussion

Henipaviruses are considered WHO priority pathogens because of their potential to cause public health emergencies.[2] The absence of efficacious medical countermeasures makes accelerated research and development for henipaviruses a priority. Vaccines are important preventive countermeasures and multiple approaches have been utilised in preclinical studies to protect against henipaviruses. Among those are live-attenuated and replication-defective recombinant vaccine platforms based on poxviruses, VSV, adenovirus, measles virus and rabies virus, but also virus-like particles as well as subunit vaccines. The successful platforms utilised either henipavirus G or F or a combination of both

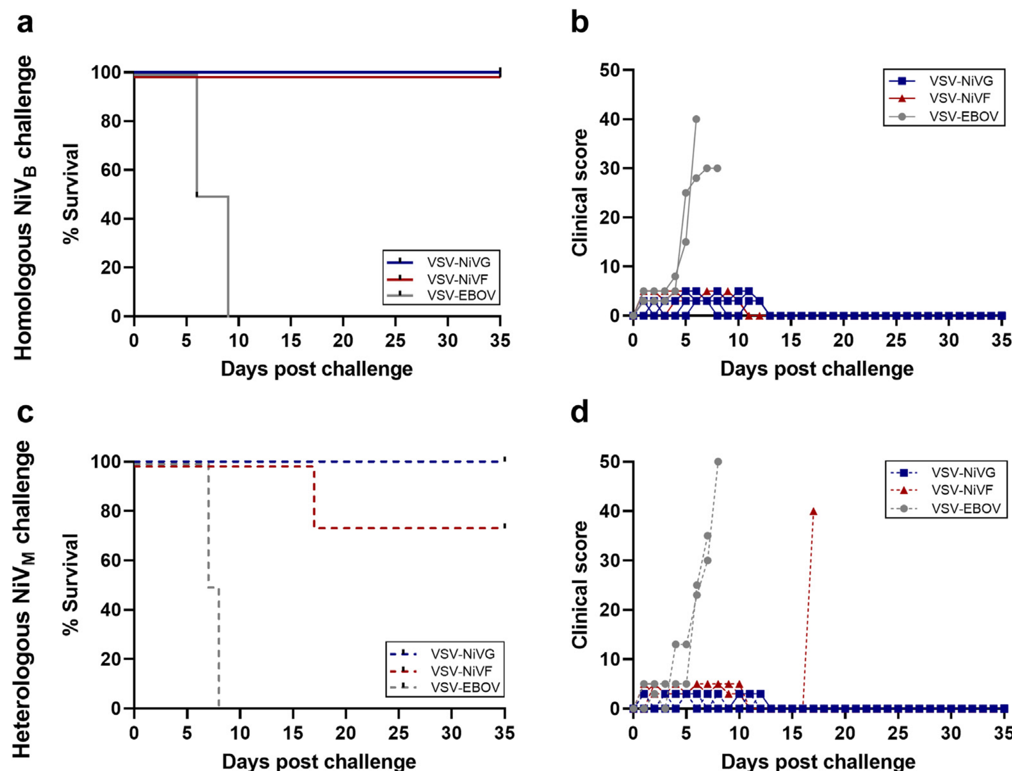

Fig. 4: **Protective efficacy of VSV-NiV vaccines against homologous and heterologous challenge.** On 28 days post vaccination with 10^7 PFU of VSV-EBOV (n = 4), VSV-NiVG (n = 8), and VSV-NiVF (n = 8), all animals received a lethal dose of 10^5 TCID50 intranasally and 10^5 TCID50 intratracheally of homologous NiV_B (a, b) or heterologous NiV_M (c, d). Survival after NiV challenge is indicated in a and c. After NiV inoculation, the animals were observed at least twice daily for clinical signs of disease and scored using a predetermined clinical scoring system (b, d).

antigens.[4] Here, we upgraded the live-attenuated recombinant VSV vaccine platform to express either NiV_B G or F. We chose the VSV-EBOV vaccine vector for its EBOV GP-mediated tropism for dendritic cells and other innate immune cells with favorable outcome for the immune response.[10] We made two important improvements to the existing VSV-based NiV vaccine.[9] We first adapted the VSV backbone vector to the licensed VSV-EBOV expressing the Kikwit strain glycoprotein to take advantage of existing clinical safety and toxicity data. We then adapted the NiV antigens to the currently circulating Bangladesh genotype. Although the presence of NiV G on the VSV-EBOV particles could potentially change the cellular tropism of the vaccine to include neurons, the neurovirulence of the VSV-NiVG vaccine was considerably lower in a standardized monkey neurovirulence test than that of the yellow fever 17DD vaccine approved for use in humans.[23]

The data presented here show that the VSV-NiVG vaccine affords complete protection of African green monkeys from homologous and heterologous NiV challenge, while the VSV-NiVF vaccine affords complete protection from homologous, but not heterologous challenge. This could be due to the lower induction of neutralising antibodies and IgG by the VSV-NiVF than the VSV-NiVG vaccine, providing a hint of what the correlates of protection for a NiV vaccine may be. Our data, however, raise an interesting question since at the time of challenge, neutralising antibody titers were below the lower limit of detection of our assay in 4 out of 8 VSV-NiVF vaccinated animals, yet 7 out of 8 animals were protected from lethal challenge. Thus, either very low levels of neutralising antibodies may be enough to provide protection, or total IgG, and perhaps even cellular responses, are more important than previously thought. This contrasts with the prevailing assumption that neutralising antibodies are the main mechanism of protection after vaccination, an assumption that is rather based on the efficacy of neutralising antibodies as antiviral treatment[24–26] than on studies aimed at establishing correlates of protection for NiV vaccines. Additionally, it was shown that rapid induction of antibody responses may be important for surviving a NiV infection.[27] However, these data were based on a single surviving (unvaccinated) animal and protection from infection after vaccination versus survival in a naïve individual may not be dependent on similar mechanisms. In pigs, it has been shown that induction of humoral and

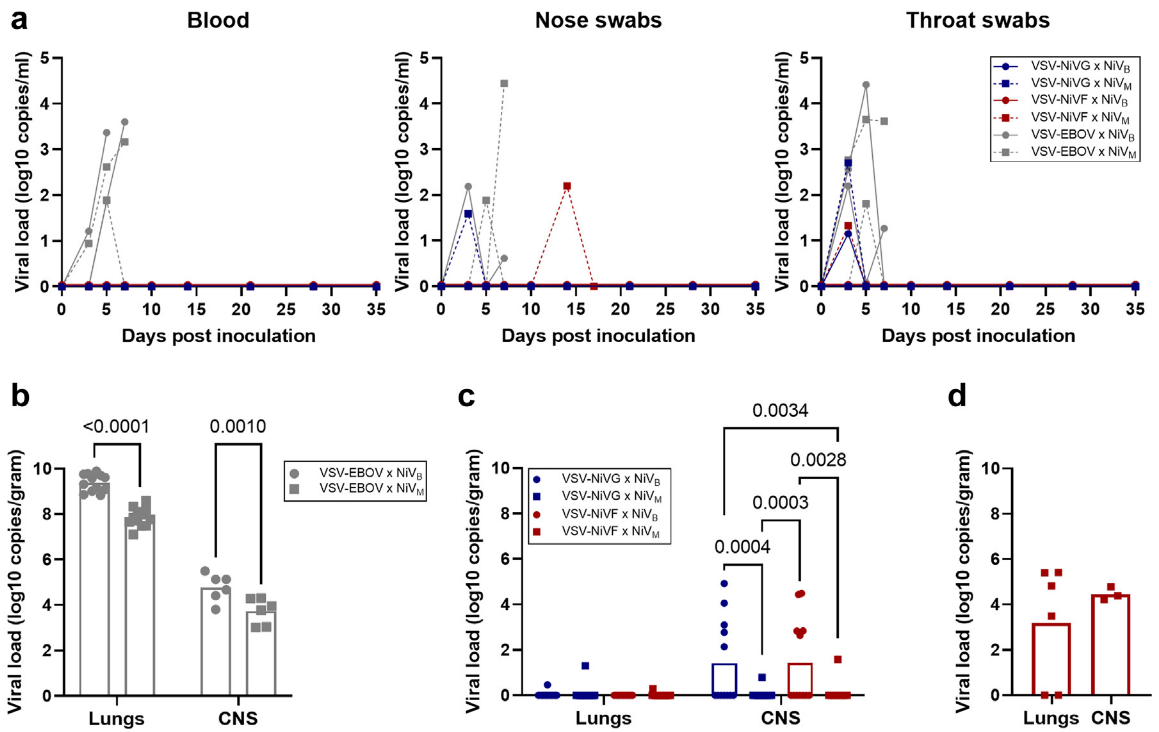

Fig. 5: **Reduced Nipah virus replication in protected animals.** a. After lethal challenge of the vaccinated African green monkeys with 10^5 TCID50 intranasally and 10^5 TCID50 intratracheally of homologous NiV_B or heterologous NiV_M, clinical exams were performed at regular intervals and whole blood, nose swabs, and throat swabs were collected and analysed for the presence of NiV RNA using qRT-PCR. When VSV-EBOV-vaccinated animals (n = 4) reached humane endpoints (b) or VSV-NiVG (n = 8) or VSV-NiVF (n = 7) vaccinated animals at the end of the experiment on 35 dpi (c), animals were euthanized, samples of lungs (6 lung lobes per animal) and CNS (3 samples per animals) were collected and analysed for the presence of NiV RNA using qRT-PCR. d. Viral loads in the lungs and CNS of the African green monkey vaccinated with VSV-NiVF and challenged with NiV_M (n = 1) that was euthanized on 17 dpi. Copy numbers were calculated by including dilutions of PCR standards with known copy numbers in each qRT-PCR run. qRT-PCR assays and standards specific for NiV_B or NiV_M were used depending on the virus isolate the animals were challenged with. Statistical analysis was performed using a 2-way ANOVA with Sidak's (b) or Tukey's (c) multiple comparisons test; statistically significant differences are indicated by p-values.

cellular immunity are both important for protection after vaccination[28]; however, due to absence of disease in this study, this is solely based on measures of virus replication rather than clinical improvement and survival. Currently, the role of cellular immune responses to NiV vaccination and infection is understudied for most vaccine approaches as well as for henipavirus infection. Unfortunately, we were unable to include analyses of the cellular response to vaccination and subsequent challenge in the present study, making it impossible to determine how cellular responses may have contributed to protection from heterologous challenge, especially in the VSV-NiVF vaccinated animals. The poxvirus platform (Modified Vaccinia Ankara) induced B and T cell responses to vaccination[29] and T cell activation has also been reported for the VSV platform.[10,30] Overall, data on cellular responses is limited and more mechanistic studies, such as depletion or passive transfer experiments, are necessary to determine the protective immune mechanisms triggered by infection and vaccination bearing in mind that vaccine platforms themselves may trigger distinct immune responses.

Although the VSV-NiVG vaccine afforded complete protection from lethal challenge, it did not afford sterile protection. However, there was a marked reduction in NiV shedding from the nose and mouth, indicating that NiV transmission from a vaccinated, infected person would be less likely to occur.

One concern with NiV vaccines is that subsequent infection with NiV could result in persistence of the virus in the CNS and that this could lead to relapse or late-onset encephalitis, as has previously been observed in a small subset of NiV disease survivors.[31] In the African green monkeys vaccinated with VSV-NiVG, viral RNA was detected in the CNS by qRT-PCR at lower levels than in unvaccinated control animals. No infectious virus was detected in the CNS, nor were histological lesions or viral antigen present. Findings were similar in the animals vaccinated with VSV-NiVF, except

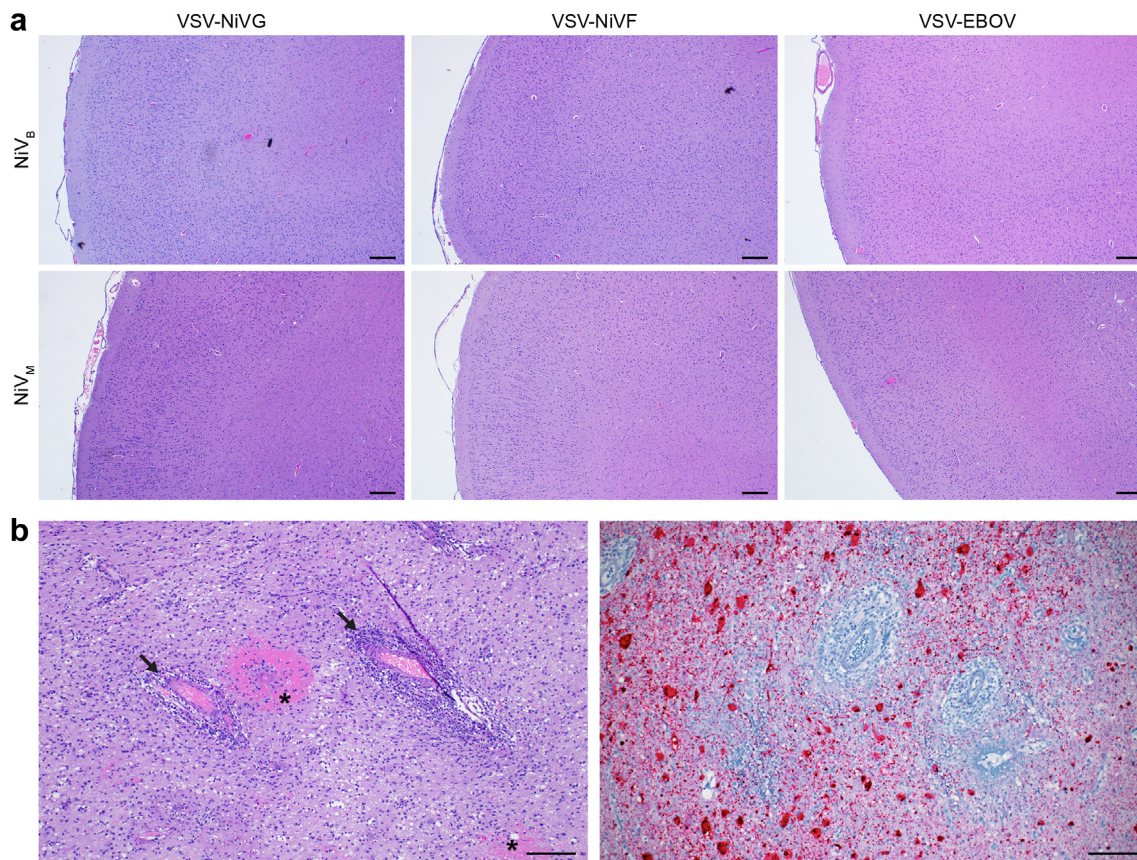

***Fig. 6:* Absence of histologic lesions in the CNS of protected animals.** At time of euthanasia, brains were collected in formalin and eight evenly spaced coronal sections were stained using hematoxylin and eosin and analysed for the presence of lesions. a. In the animals vaccinated with VSV-NiVG (n = 8; left panels) and VSV-NiVF (n = 7; middle panels) protected from lethal NiV_B (top panels) or NiV_M (bottom panels) challenge, no histologic lesions were detected that could be attributed to NiV infection; the same was true for the control animals vaccinated with VSV-EBOV (n = 4; right panels) and euthanised during acute NiV infection on 6–9 dpi. Magnification 40x, scale bar 200 μm. b. The animal vaccinated with VSV-NiVF that was not protected from NiV_M challenge (n = 1), developed fulminant inflammatory CNS lesions with lymphoplasmacytic perivascular cuffing (arrows), multifocal hemorrhages (asterisk), diffuse gliosis, and edema (left panel). Immunohistochemistry using an anti-NiV N antibody revealed marked immunoreactivity within the focus of inflammation, primarily within neurons. Magnification 100×, scale bar 100 μm.

in the one animal that was euthanised with severe meningoencephalitis; in this animal, high levels of viral RNA were detected in the CNS, and fulminant lesions with NiV antigen were present. The meaning of the detection of viral RNA in qRT-PCR is unclear at the moment and we cannot exclude persistence in the CNS in vaccinated animals. Animal models of persistent NiV infection would help to address these concerns but are unfortunately not available at this time. In the meantime, the risk of late-onset encephalitis has to be weighed against the high case-fatality rate of NiV during the acute disease stage.

One limitation of our study is that we were not able to include a recent NiV isolate from India as challenge virus in our study. However, although some of the NiV isolates from India are slightly divergent, the NiV isolates from India are more closely related to NiV_B than NiV_M,[32] so we expect the VSV-NiVG vaccine to afford protection against the currently known NiV isolates from India as well.

The VSV-NiVG vaccine has several properties that make it an ideal vaccine candidate. It affords protection after a single injection, it provides protection from homologous and heterologous challenge, and by using the licensed VSV-EBOV vector as a backbone in our vaccine, we can build on a wealth of human safety data, as well as experience with manufacturing and distribution for this vector. The VSV-EBOV vaccine also has been shown to afford protection within 10 days of vaccination[8]; although it has to be determined whether the VSV-NiVG has an equally short time to protection in nonhuman primates, Syrian hamsters vaccinated with a similar

VSV-based NiV vaccine expressing NiV_M G were protected from lethal NiV_M challenge when they were vaccinated just days before lethal challenge.[11] Recently, it was shown that a replication-deficient VSV vector expressing NiV_B G provided 100% protection against lethal NiV_B challenge 7 days after vaccination.[30]

Taken together, our data show that the VSV-NiVG is a promising vaccine candidate that offers broad protection against NiV disease; therefore, it is currently undergoing safety testing in a phase 1 clinical trial (NCT05178901).[33]

Contributors

Conceptualization, E.d.W. and H.F.; investigation, E.d.W., F.F., J.Cr., K.G., R.M.-H., B.N.W., K.M.-W., A.O., J.Ca., S.W., R.R., V.A.A., J.L., D.P.S., A.M., and H.F.; data verification: all authors listed under 'investigation' verified the individual data they contributed; E.d.W. verified all data; writing-original draft, E.d.W. and H.F.; writing, review and editing, all authors. All authors read and approved the final version of the manuscript.

Data sharing statement

All study data are available in the text and figures and will be made available upon request.

Declaration of interests

H.F. claims intellectual property regarding vesicular stomatitis virus-based vaccines for viral hemorrhagic fevers (US patent 8012489). E.d.W., A.M., and H.F. have received a royalty related to the VSV-Nipah vaccine discussed in this manuscript. All other authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2022.104405.

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