Journal of Infection 89 (2024) 106237

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Viruses and Viral Diseases

# Replication, safety and immunogenicity of the vectored Ebola vaccine rVSV-ΔG-ZEBOV-GP in a sub-Saharan African paediatric population: A randomised controlled, open-label trial in children aged 1-12 years living in Lambaréné, Gabon

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## ARTICLE INFO

*Article history:*
Accepted 29 July 2024
Available online 8 August 2024

*Keywords:*
RVSVΔG-ZEBOV-GP (ERVEBO®) vaccine
Varicella-zoster virus (VZV) vaccine (VARILIX®)
RVSVΔG vector
-rVSVΔG-ZEBOV-GP antibodies
Children aged 1–12 years Lambaréné, Gabon

## SUMMARY

*Background:* Unlike adults, children experienced stronger and longer vector replication in plasma and shedding in saliva following rVSVΔG-ZEBOV-GP vaccination. The resulting risks of immunosuppression or immune hyperactivation leading to increased Adverse Events (AEs) and altered antibody responses are concerns that have been addressed in the present manuscript.

*Methods:* Children aged 1–12 years living in Gabon received either rVSVΔG-ZEBOV-GP (ERVEBO®) vaccine or the varicella-zoster virus (VZV) vaccine (VZV). The concentration of rVSVΔG vector in blood and saliva, the occurrence of AEs up to day 28; the anti-rVSVΔG-ZEBOV-GP and anti-VZV IgG antibody titres, neutralising and avidity functions of anti-rVSVΔG-ZEBOV-GP by day 365; were assessed in serum. (PACTR202005733552021)

*Findings:* In the rVSVΔG-ZEBOV-GP group, 70% and 7% of children had > 0 copies/ml of rVSVΔG respectively in plasma by day 3 and in saliva by day 14 after vaccination, with no detection on day 28. Significantly higher but transient AEs occurred in the rVSVΔG-ZEBOV-GP group. Both vaccines induced seroconversion

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https://doi.org/10.1016/j.jinf.2024.106237

on day 28 and sustainable IgG antibody titres by day 365. Avidity and neutralisation functions of the anti-rVSVΔG-ZEBOV-GP antibodies peaked at day 28 and were maintained by day 365.
*Interpretation:* The replication and shedding do not affect the favourable risk-benefit balance of the rVSVΔG-ZEBOV-GP in children.


## Background

Ebola virus disease (EVD) is a Zoonosis discovered in 1976 in Sudan and Democratic Republic of the Congo (DRC).[1,2] The virus enters the human body through mucosal surfaces as well as breaks and abrasions in the skin, or by parenteral introduction. Human-human transmission during outbreaks occurs by direct contact with infected patients or cadavers. In addition, sexual transmission and ingestion of contaminated food may be possible sources of infection.[3] Several epidemics have already been documented around the world,[4] with the case fatality rate of 61.0% (95% confidence interval: 51.6–69.4).[5]

Five strains have been documented, including the Zaire strain that was at the root of the 2014–2016 EVD epidemic and marked as the worst in history in which there were 28,616 cases recorded across Africa, Europe, and North America and with a staggering case fatality rate of 40%.[6] This spurred urgent efforts to develop therapies and vaccines targeting the Zaire species of Ebola virus. Among these, the recombinant vesicular stomatitis virus-Zaire Ebola virus envelope glycoprotein (rVSVΔG-ZEBOV-GP) vaccine was the first used to circumvent the epidemic.

The rVSVΔG consists of deleting the glycoprotein (G) gene from the wild VSV genome and replacing it with the Zaire Ebola glycoprotein (ZEBOV GP) gene to obtain a replication-competent viral vectored vaccine (rVSVΔG-ZEBOV-GP vaccine). The rVSVΔG is a newly approved vaccine platform promising to develop vaccines against major infectious diseases like tuberculosis, non-Zaire Ebola virus, Marburg virus, Lassa fever virus, influenza virus, and therapy against some cancers.[7,8] The rVSVΔG has minimal to no risk of recombination to a wild-type VSV or reversion to virulence; the vector is unable to integrate into the host genome, effectively mitigating the risks of rVSVΔG-induced mutations and cancers. Moreover, its attenuation is associated with reduced risk of neuroinvasion and neurovirulence.[7,9] rVSVΔG-ZEBOV-GP vaccine may induce adverse events including pain and oedema at the injection site and fever, chills, fatigue, headache, myalgia, arthralgia, lymphopenia and arthritis, all of which are transient and of mild to moderate intensity. Following a single dose of the rVSVΔG-ZEBOV-GP vaccine, seroconversion rates are nearly 100%. This vaccine induces antibodies that peak in ~ 1–3 months, and after a decline, typically plateau at 1 year post-vaccination, and titres remain stable for up to 5 years.[7,10] A single dose of rVSVΔG-ZEBOV-GP induced 100% protective efficacy against laboratory-confirmed infection and reduced the risks of illness and death.[7,11,12]

The evidence on the quality, safety, immunogenicity and protective efficacy of rVSVΔG-ZEBOV-GP led to its approval by the European Medicines Agency (EMA) and U.S. Food & Drug Administration (FDA), and pre-qualification by the World Health Organisation (WHO) for its use in adults aged 18 years and above since 2019. Studies in vaccinated children aged 6–17 years in Gabon, Guinea, and Sierra Leone have shown similar safety profiles to those observed in adults,[12,13] providing the basis for a pivotal paediatric phase 2 trial that assessed the safety and immunogenicity of three candidate vaccines: rVSVΔG-ZEBOV-GP, the heterologous Ad26.-ZEBOV and MVA-BN-Filo regimen, and ChAd3-EBO-Z vaccines.[13–19] Based on data produced by these studies, including the phase 1 study in Gabon, in 2023 the FDA (July) and the EMA (August) approved the rVSVΔG-ZEBOV-GP vaccine for use in children, alongside WHO prequalification (November), thus extending its use in individuals aged 12 months and above.[20] In addition, the Strategic Advisory Group of Experts (SAGE) on Immunisation recommended off-label use of the vaccine from birth in the context of an outbreak; during the EVD Zaire-strain epidemic in 2018–2020, vaccination was extended to infants aged 6–11 months.[21,22] No clinical trials evaluating rVSVΔG-ZEBOV-GP have been conducted in infants. The rVSVΔG-ZEBOV-GP vaccine is licensed for use as ERVEBO®.

In a phase 1 clinical trial conducted in Gabonese adolescents and children vaccinated with rVSVΔG-ZEBOV-GP in 2016–2017, ongoing replication of rVSVΔG was noted at the protocol-assessed time-point of 7 days post-vaccination, with the shedding of the vaccine in saliva and urine.[13] A phase 2 trial reported on rVSVΔG shedding in a subset of paediatric participants, which showed that the greatest percentage of children with observed shedding occurred on Day 7 post-vaccination (25.0%), though shedding was shown to occur until day 28 post-vaccination.[23] The current limited data raise questions about the tolerability of the rVSV in children, especially the effects of the vaccine on the innate immune systems of children with potentially induced immunosuppression or excess activation.[24] To address this question, we designed a randomised, controlled phase 2 trial to evaluate the occurrence of adverse events and the titres and functions of antibody-induced immune responses in relation to the kinetics of the rVSVΔG viraemia (vaccinemia) in plasma following rVSVΔG-ZEBOV-GP immunisation in children aged 1–12 year(s).

## Methods

### *Trial design and participants*

The study was conducted at the Centre de Recherches Médicales de Lambaréné (CERMEL) clinical trial unit in Lambaréné, Gabon. The catchment area of CERMEL involves Lambaréné town, located 254 kilometres (Km) by road from Libreville, the capital of Gabon, and its surrounding villages within a radius of 70 km in both Southern and Northern directions. The Ebola Zaire strain is endemic, and several EVD outbreaks occurred between 1994 and 2001 in Gabon.[4]

The study was a Phase 2, randomised, controlled, open-label trial designed to generate further tolerability, safety and immunogenicity data of the rVSVΔG-ZEBOV-GP vaccine (at nominal doses of $7{\cdot}8\times10^7$ plaque-forming units [pfu]) as compared with a varicella zoster vaccine (VZV, GlaxoSmithKline, UK) in children aged 1–12 years living in Lambaréné, Gabon. A total of 120 children were recruited according to the study protocol approved by the National Ethics Committee of Gabon [0079/2019/PR/SG/CNER] and the CERMEL Institutional Ethics Committee [CEI-009/2014]. Assuming no rVSVΔG shedding in the VZV vaccinees, the sample size had a power of 90% to reject the null hypothesis of no viral shedding in the rVSVΔG-ZEBOV-GP vaccinees, considering a minimal proportion of 35% saliva shedding in children (aged 6–12 years) observed in the phase 1 study.[13] A total of 56 participants per age group were needed using Fisher's exact two-tailed test at 5% level of significance. Assuming a 10% loss to follow-up by day 28 after vaccination, 60 participants per age group were enrolled. The sample size had been calculated using a 2:1 ratio, thus 40 and 20 participants per age

group were allocated to receive the rVSVΔG-ZEBOV-GP and varicella vaccines, respectively.

Written informed consent was obtained from all parents/guardians of the participants before enrolment. Eligible participants were healthy children aged 1–12 years (inclusive) at the time of screening. Exclusion criteria included participation in a previous Ebola vaccine trial, receipt of any licensed vaccines within 30 days of planned study immunisation, or presence of any febrile illness (fever > 38 °C). Children found to have a history of varicella infection, ongoing infection with the human immunodeficiency virus, hepatitis B or C virus infection, or clinically significant medical conditions at screening were excluded. Full inclusion and exclusion criteria are listed in the study protocol (supplementary pp 15–44). The trial was registered with the Pan African Clinical Trial Registry (PACTR202005733552021) and Clincaltrials.gov (NCT05130398).

*Vaccines*

The rVSVΔG-ZEBOV-GP vaccine was supplied in individually packaged sterile single-dose vials. The vaccine concentrations in the vials were formulated as the dose with minimal potency of $7{\cdot}8\times10^7$ plaque-forming units (pfu). They were administered intramuscularly in a 1·0 ml volume without dilution. To assess the clinical significance of the higher and longer-lasting replication of rVSV in children and its shedding in the saliva of the vaccinees, VZV (a viral, live, attenuated vaccine) was chosen as a control. VZV is a lyophilised vaccine with a diluent syringe or ampoule included to prepare the live attenuated Oka strain of varicella-zoster virus. A 0·5 ml dose ($10^{3.3}$ plaque-forming units) of the reconstituted vaccine was administered.

*Study procedures*

The trial was age-stratified, with age groups including preschool (1–5 years) and school children (6–12 years) with 60 participants in each stratum. Participants were centrally randomised to receive rVSVΔG-ZEBOV-GP or VZV in a 2:1 ratio. The study statistician provided computer-generated randomisation lists in a sealed envelope to the pharmacist and investigators. Only eligible participants at the enrolment visit were randomised into the study. Vaccinated participants who left the study for any reason were not replaced. The injection site was decontaminated with alcoholic solution (70% ethanol) prior to injection. Participants received an injection of rVSVΔG-ZEBOV-GP or an injection of VZV vaccine into the outer aspect of the subject's upper arm (deltoid area) intramuscularly and subcutaneously, respectively. No local massage was performed post-injection. They were then observed for 60 min.

Vaccinees were assessed on days 1, 2/3, 7, 14, 21, 28, 56, 84, 180, and 365 after vaccination in both groups. Solicited adverse events related to the injection site and systemic reactogenicity were reviewed for 14 days after injection. Unsolicited adverse events were reviewed, and saliva samples were collected for 28 days after each injection. Blood samples for the assessment of laboratory-based safety endpoints (including a complete blood count with differential measurements of serum creatinine, alanine aminotransferase, and aspartate aminotransferase levels) were collected at screening and days 7, 56, 84, and 180 after vaccination.

Clinical investigator evaluations to detect changes in medical status and data on concomitant medication were collected throughout the study. Based on previous studies, data on symptoms of arthralgia and arthritis were solicited, as well as data on mouth ulcers, mucosal lesions, and dermatological reactions such as rash. During physical examination, joint stiffness, tenderness, pain or limitation of motion, swelling, and other arthritic signs/symptoms were thoroughly investigated. Clinical investigators graded adverse events into one of four categories: mild (does not interfere with routine activities), moderate (interferes with routine activities), severe (unable to perform routine activities), or potentially life-threatening (hospitalisation or emergency room visit).

The co-primary safety endpoints were AEs (the occurrence of solicited adverse events within 14 days after vaccination, and the occurrence of unsolicited and serious adverse events [SAE], including assessment of values of safety laboratory measures, within 28 days) and rVSV ribonucleic acid (RNA; the concentration of rVSV in blood or saliva as detected by real time polymerase chain reaction [RT-PCR] within 28 days). Secondary endpoints, exploring both safety and immunogenicity, included but were not limited to a detailed description of all SAEs; the concentration of ZEBOV-GP-specific binding antibodies by ELISA; avidity of GP-specific serum antibodies, and the detection of neutralising antibodies via pseudovirus neutralisation tests up to day 365.

*Vaccine shedding*

Detection of the rVSV was previously described.[25] Plasma and saliva samples were processed and stored in Trizol LS or RNAlater at CERMEL. The samples at RNA later were extracted automatically using QIASymphony DPS Virus/Pathogen Kit (Qiagen,937036) on QIASymphony instrument. The samples at Trizol were extracted manually in combination with an RNA Purification Kit (Thermo Fisher,12183555). The Taqman real-time PCR was performed on the LightCycler 480 II Instrument. rVSV-ZEBOV RNA was extracted from the vaccine at a concentration of $10^8$ copies/ml to carry out calibration curves. Linear regression was used to interpret the quantity of vaccinemia according to the cycle threshold (Ct) value. The lower limit of quantitation (LLOQ) corresponds to Ct value = 34.225 equivalent to $10^1$pfu/ml, and the lower limit of detection (LLOD) to Ct value = 40. The viral load was monitored from day 0 to day 28.

*Immunogenicity*

Recombinant ZEBOV-GP protein (ZEBOV-GP1,2) was constructed based on the Zaire EBOV, Mayinga subtype (GenBank accession # U231871·1) and silent mutations were introduced at editing site 1019–1025 as described previously.[26] ZEBOV-GP specific enzyme-linked immunosorbent assays (ELISAs) were performed on serum samples to determine ZEBOV-GP IgG antibody titres, as described elsewhere (SOP AP-03–35; USAMRIID ELISA).[25,27,28] ELISAs were also performed on serum samples collected at days 0, 28, 180 post-vaccination to determine varicella zoster virus IgG antibody titres. Biolayer Interferometry (BLI)-based binding kinetics assay was used to assess binding interactions of vaccine-induced serum antibodies of vaccinees and neutralising antibodies were detected using VSV pseudovirions (Supplementary p2).

*Statistical analyses*

The safety analysis was based on the intention-to-treat population (all participants randomly assigned and vaccinated). Descriptive statistics were presented as medians, ranges, and interquartile ranges (Q1; Q3) for continuous variables; categorical variables, including demographic characteristics and adverse events, were summarised using frequency and percentage. Chi-squared test was used to compare pairwise proportions. We calculated and visualised the frequency of AEs per vaccine group, and the relative risk of each AE with corresponding 95% confidence intervals, with epitools and ggplot2 R packages, respectively. Antibody responses are reported as the geometric mean titre with 95% confidence intervals. Seropositivity rates for rVSVΔG-ZEBOV-GP, VZV, and neutralisation titres were defined as the percentage of participants having a titre > 100, > 50, and ≥6, respectively, which were defined as the threshold for seropositivity. McNemar's test compared the seropositivity rates

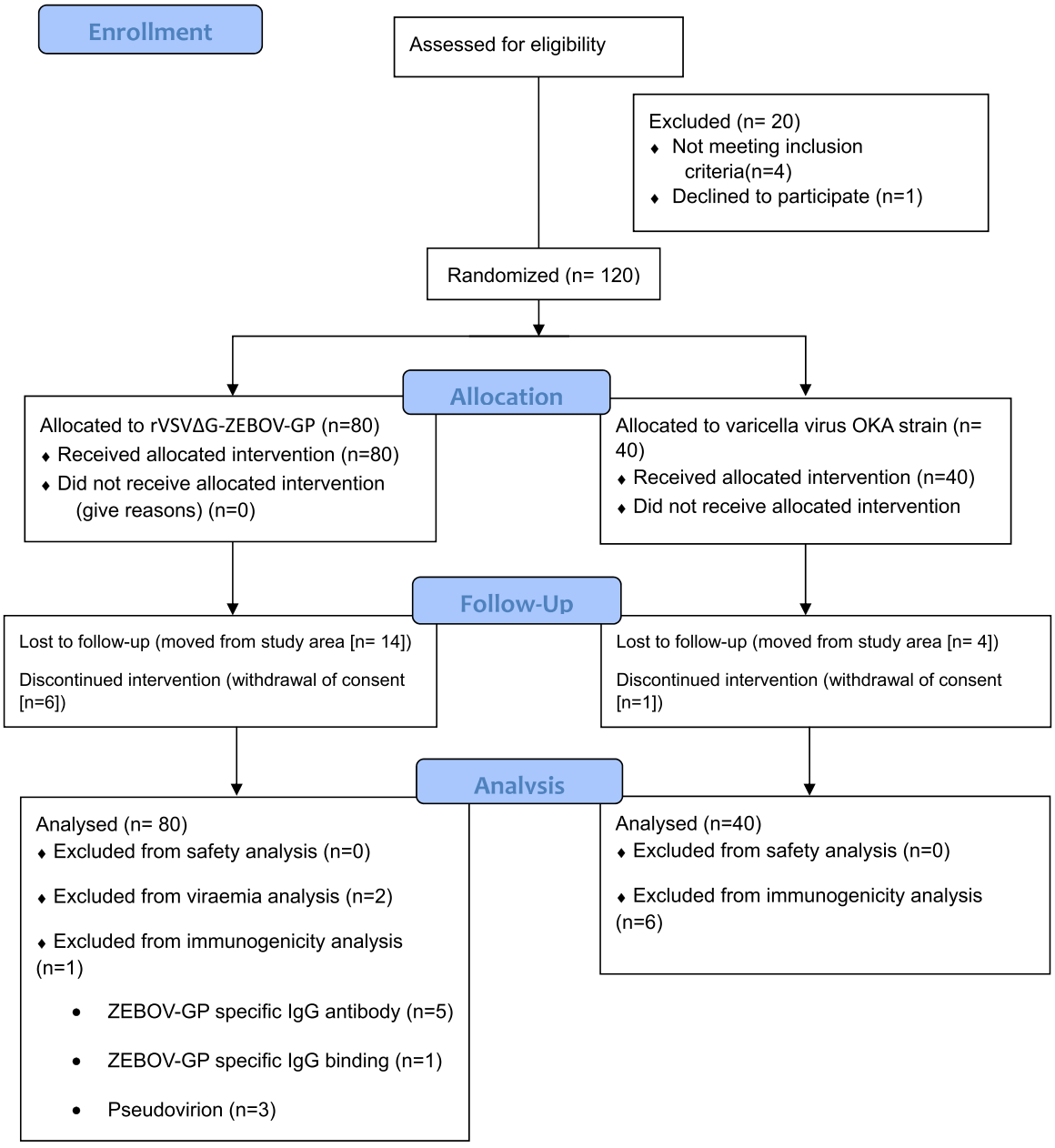


**Fig. 1.** Trial flow diagram.

between day 0 and other days. Statistical comparison between magnitudes of antibodies induced at each time-point according to rVSV vaccinemia was done using a non-parametric one-way ANOVA (Kruskal-Wallis). Wilcoxon signed-rank test was used for comparisons between two age groups of vaccinated children at each time-point and between magnitudes of antibodies induced at day 0 vs other time-points within an age group; statistical significance was denoted as (*) for $p < 0{\cdot}05$, (**) for $p < 0{\cdot}01$, (***) for $p < 0{\cdot}001$, (****) for $p < 0{\cdot}0001$. Statistical analyses were performed with the use of R software, version 4·3·2.

*Role of the funding source*

The funders had no role in study design, data collection and analysis, decision to publish, and preparation of the manuscript.

## Findings

*Study population*

From April 09, 2021 to August 11, 2021, a total of 80 (45 [56·2%] male and 35 [43·8%] female) participants were vaccinated with rVSVΔG-ZEBOV-GP and a total of 40 (21 [52·5%] male and 19 [47·5%] female) participants with the VZV vaccine in a consecutive manner (Fig. 1). All randomised participants were successfully vaccinated. The median age of participants was 3 and 8 years in the age groups 1–5 years and 6–12 years, respectively. One hundred and eight (90%) participants completed the follow-up visits that were scheduled during the 28-day window after vaccination; 25 (20·8%) participants were lost to follow-up by the conclusion of the trial. The discontinuations were due to moving out of the study area (n = 18) and withdrawal of consent (n = 7), all unrelated to the study vaccines.

**Table 1**
Participant demographic characteristics at enrolment.

| | rVSVΔG-ZEBOV-GP N = 80 [Q1; Q3] / (%/SD) | VARILRIX® N = 40 [Q1; Q3] / (%/SD) |
|---|---|---|
| Age group (years) | | |
| 1–5 | 40 (50.0%) | 20 (50.0%) |
| 6–12 | 40 (50.0%) | 20 (50.0%) |
| Median Age (years) | | |
| 1–5 | 3 [2 to 4] | 3 [2 to 4] |
| 6–12 | 8 [7 to 11] | 8 [6 to 10] |
| Sex | | |
| Male | 45 (56.2%) | 21 (52.5%) |
| Female | 35 (43.8%) | 19 (47.5%) |
| Height (cm) | 112.8 (20.4) | 111.2 (20.5) |
| Weight (kg) | 19.9 (7.5) | 19.8 (8.1) |

Additional details regarding the demographic characteristics of the participants are provided in Table 1.

*rVSVΔG-ZEBOV-GP vaccine is more reactogenic than the VZV vaccine*

There was a higher occurrence of solicited events in the rVSVΔG-ZEBOV-GP vaccine group relative to the VZV vaccine group (Fig. 2). Pain at the injection site was reported in 82·5% (66/80) of participants in the rVSVΔG-ZEBOV-GP group and 62·5% (25/40) of those in the VZV group on days one to three after vaccination; four participants reported injection site pain at day seven only in the rVSVΔG-ZEBOV-GP group, and there were no reports in either group by day 14. Other local solicited adverse events occurred less frequently. Swelling at the site of injection was observed in 12 (15%) and 2 (5%) participants in the rVSVΔG-ZEBOV-GP and VZV groups, respectively, whilst erythema was observed in 4 (5%) participants in the rVSVΔG-ZEBOV-GP group only.

Objective fever was noted in 23 of the 120 vaccinees on Day 1, all occurring in the rVSVΔG-ZEBOV-GP group: 21 (91·3%) had grade 1–2 fever (temperature range, 38·0 to 38·9 °C) and 2 (8.7%) experienced grade 3 fever (temperature range, 39·0 to 40·0 °C). One of the episodes of grade 3 fever was associated with paranoia according to the investigator, that occurred within 12 h (overnight) after vaccination with rVSVΔG-ZEBOV-GP but had subsided by 24 h after vaccination. There was no occurrence of objective fever on day 2/3 after vaccination. There were two occurrences of objective fever on day 7 in both vaccine groups (2·8%, rVSVΔG-ZEBOV-GP group; 5·1%, VZV group) and three occurrences on day 14 (4·3%, rVSVΔG-ZEBOV-GP group; 8·6%, VZV group). Another participant presented to study physicians five days after rVSVΔG-ZEBOV-GP vaccination after experiencing a grade 3 solicited event with associated mouth ulceration, tongue ulceration, hypersalivation, and mild angioedema. The participant quickly recovered following treatment with outpatient care with an oral second-generation antihistamine. All other cases of solicited adverse events, including nausea and vomiting, headache, fatigue, loss of appetite, and chills, were of mild to moderate intensity. Eight (6·67%) participants experienced arthralgia during the solicited period, 5 (8·3%) in the rVSVΔG-ZEBOV-GP group and 3 (7·5%) in the VZV group. No cases of arthritis were diagnosed.

In the 6–12-years-old group, the rVSVΔG-ZEBOV-GP group reported significantly more systemic solicited adverse events such as subjective and objective fever, nausea and vomiting, headache, fatigue, loss of appetite, and chills in comparison to their counterparts in the VZV group. There were no such associations in 1–5-year-olds, between the vaccine groups (Supplementary Table 2).

Solicited events had median times to onset of 2 days (Q1, Q3; 2·0; 2·0) and 2 days (Q1, Q3; 2·0, 9·0) in the rVSVΔG-ZEBOV-GP and VZV groups, respectively; these events quickly abated in both vaccine groups.

*All unsolicited and serious adverse events were deemed unrelated to the study of vaccines*

The most frequently reported unsolicited adverse events were cough and rhinorrhoea in 23 (19%) and 20 (17%) participants, respectively. Relative to the rVSVΔG-ZEBOV-GP group, a higher proportion of participants in the VZV group reported cough (14% vs 30%) and rhinorrhoea (15% vs 20%) overall, and in both age groups (Supplementary Table 4). There were no unsolicited adverse events judged to be related to either vaccine.

There were six SAEs in vaccinees in the 360 days after vaccination, four (5%) and two (5%) in the rVSVΔG-ZEBOV-GP and VZV vaccine groups, respectively; four (6·7%) and two (3·3%) in the 1–5-year-old and 6–12-year-old age groups, respectively; they were all determined to be unrelated to the study vaccines. Most SAEs were relatively unremarkable, three cases of malaria (malaria-endemic region) and 2 cases of lower respiratory tract infection (Supplementary Table 6). One of the SAEs was a case of tuberculosis (suspected miliary TB based on chest x-ray) that presented with haemoptysis and epistaxis five weeks after vaccination with rVSVΔG-ZEBOV-GP. Sputum examination noted bacilli sensitive to rifampicin; quadruple therapy was chosen as treatment. The participant was successfully discharged one week after hospitalisation having recovered from the presenting signs/symptoms.

*Transient leukopenia, monocytosis, and lymphocytosis in both vaccine groups*

Leukopenia was observed in 11 (13·7%) and 3 (7·5%) vaccinees in the rVSVΔG-ZEBOV-GP and the VZV vaccine group, respectively; the majority (78.6%) were in the 6–12-years age group. Monocytosis occurred in 10·8% and 12·5% of the rVSVΔG-ZEBOV-GP and the VZV vaccine recipients, respectively. The same proportion of participants, 7·5%, experienced lymphocytosis in both vaccine groups. Occurrences of monocytosis and lymphocytosis were similar in both age groups. More than 80% of the cases of abnormal values of complete blood counts were resolved by day 28, and all cases by day 84. They were all considered not clinically significant by the investigator.

Abnormal elevations of liver enzymes and creatinine values were observed in 10% of participants in each vaccine group at any visit between day 0 and day 28, also equally distributed per age group. The elevations resolved by day 28 and none were considered clinically significant laboratory changes in either group.

*rVSV replication occurred on days 1-3 and shedding at day 7*

The majority (69%) of participants in the rVSVΔG-ZEBOV-GP group had positive rVSVΔG.

replication, predominantly on day 1 (Fig. 3 and Supplementary Table 7); there was one (2·5%) viraemic participant in the VZV group (at their day 1 visit). A higher median concentration of rVSVΔG-ZEBOV-GP was observed in plasma from participants in the 1–5-year-old age group (27·94 [12·96 - 56·81] copies/ml in 1–5-year-olds vs 14·37 [0 - 32·84] copies/ml in 6–12-year-olds on day 1 post-vaccination). The participants in the VZV group were in the 6–12-year-old age group (21·02 copies/ml). When comparing participants with quantifiable vaccinemia (PCR result above the limit of quantification [lower limit corresponding to Ct value = 34·2]) with those with detectable vaccinemia (PCR positive but below the limit of quantification), participants in the latter group reported occurrence of fatigue in 17 of 54 (31%), whilst participants in the former group reported no occurrence of fatigue (Table 2). Vaccinemia was not associated with an increased frequency of adverse events nor higher antibody responses within these participants compared to those

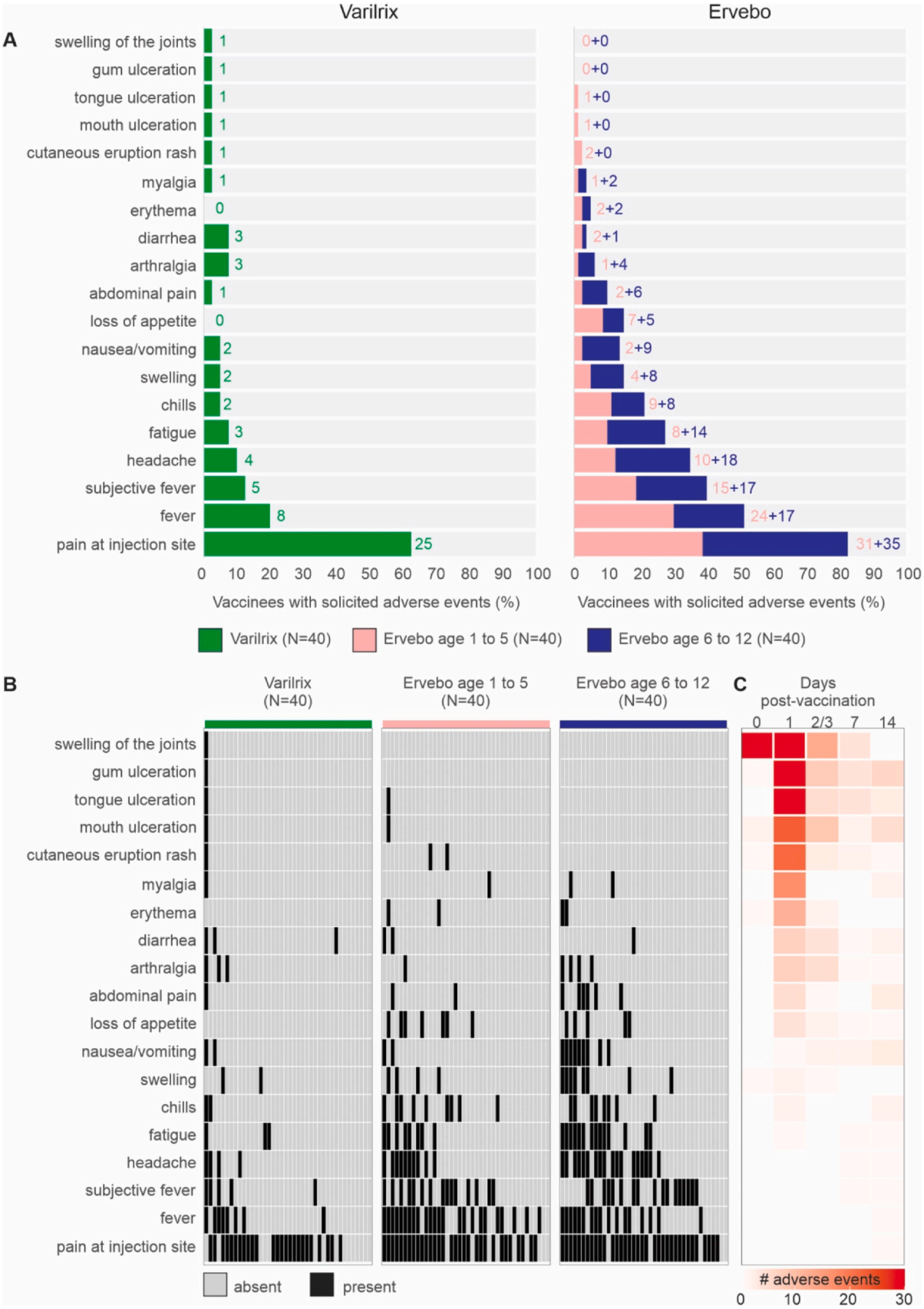


**Fig. 2.** Occurrence of Solicited Adverse Events. (A) Frequency of adverse events (AEs) in Varilrix vaccinees (represented by green bars) and in Ervebo vaccinees (represented by pink bars for vaccinees aged between 1 and 5 years, and blue bars for vaccinees aged between 6 and 12 years). The numbers indicate the absolute count of AEs that occurred between 0- and 14-days post-vaccination. (B) Occurrence of AEs per vaccinee. AEs are displayed in rows, with each column representing vaccinees from each group. The colours grey and black represent the absence or occurrence, respectively, of an AE during the first 14 days post-vaccination. (C) Number of AEs per time point. The colour intensity is proportional to the number of AE occurrences, considering all vaccinees.

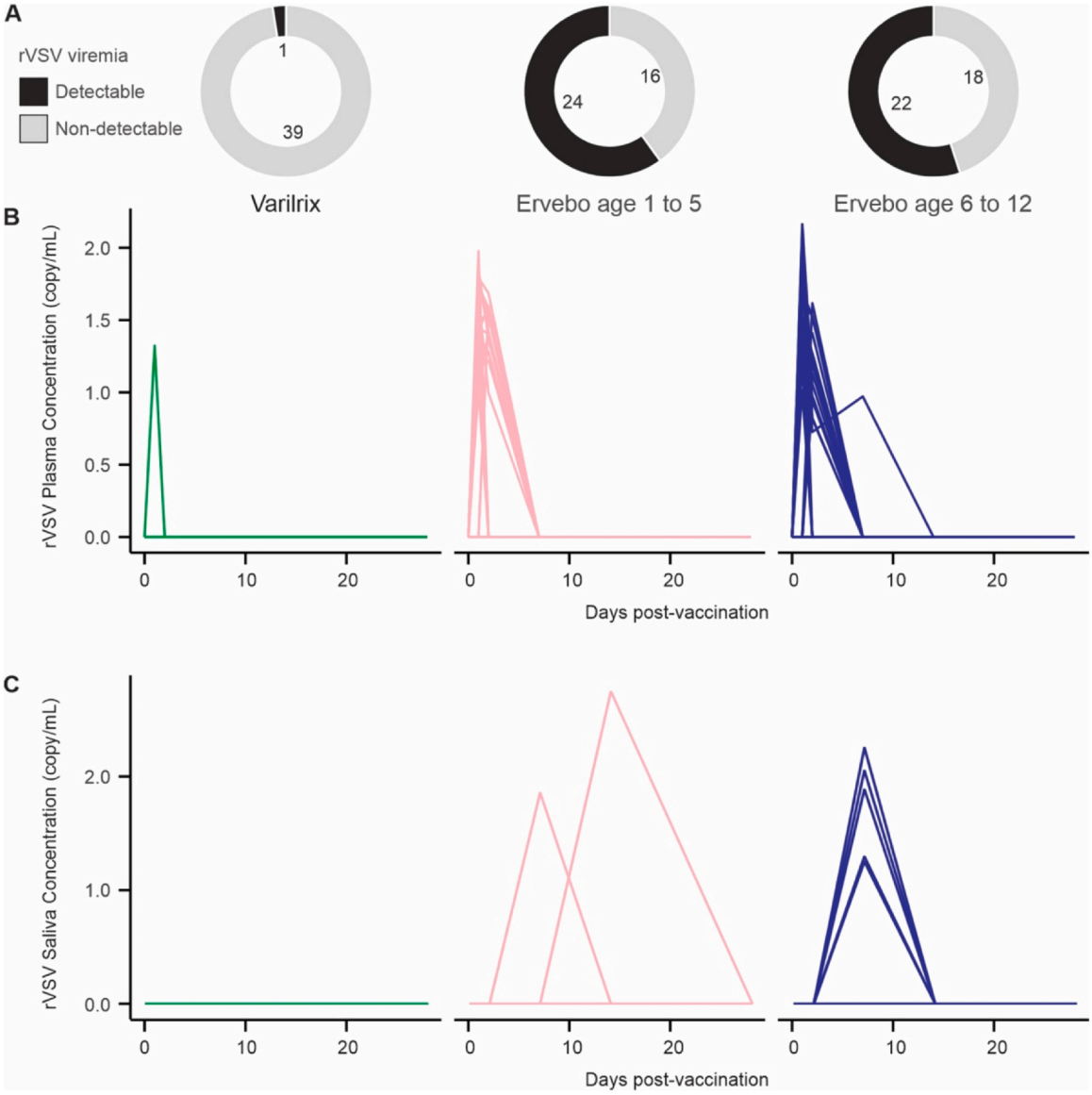


**Fig. 3.** Plasma Recombinant Vesicular Stomatitis Virus (rVSV) viremia and shedding in vaccinees. (A) Frequency of vaccinees with detectable viremia, represented by black bars, across different vaccine- and age-groups. The number of vaccinees is indicated within the pie charts. The name of each group is displayed at the bottom of the respective pie chart. (B) Levels of rVSV in the plasma of vaccinees, with each line representing an individual vaccinee. The name of each group is displayed at the top of the respective graph. There was no evidence of viremia by day 28. (C) No detection of rVSV in saliva of participants by day 28.

**Table 2**
Occurrence of solicited adverse events in quantifiable vs detectable viraemic participants.

| Variable | Detectable (N = 42) | Non-Detectable (N = 26) | Quantifiable (N = 13) | p-value |
|---|---|---|---|---|
| Subjective Fever | 17 (40.5%) | 7 (26.9%) | 9 (69.2%) | 0.040 |
| Objective Fever | 22 (52.4%) | 16 (61.5%) | 3 (23.1%) | 0.073 |
| Nausea/Vomiting | 7 (16.7%) | 3 (11.5%) | 1 (7.69%) | 0.746 |
| Diarrhoea | 2 (4.76%) | 0 | 1 (7.69%) | 0.277 |
| Headache | 19 (45.2%) | 7 (26.9%) | 2 (15.4%) | 0.109 |
| Fatigue | 17 (40.5%) | 5 (19.2%) | 0 | 0.007 |
| Myalgia | 1 (2.38%) | 2 (7.69%) | 0 | 0.571 |
| Arthralgia | 3 (7.14%) | 2 (7.69%) | 0 | 0.849 |
| Abdominal Pain | 3 (7.14%) | 2 (7.69%) | 3 (23.1%) | 0.258 |
| Chills | 10 (23.8%) | 5 (19.2%) | 2 (15.4%) | 0.812 |
| Mouth Ulceration | 1 (2.38%) | 0 | 0 | 1.000 |
| Tongue Ulceration | 1 (2.38%) | 0 | 0 | 1.000 |
| Cutaneous Eruption/Rash | 1 (2.38%) | 1 (3.85%) | 0 | 1.000 |
| Injection site Pain | 36 (85.7%) | 18 (69.2%) | 13 (100%) | 0.037 |
| Erythema | 4 (9.52%) | 0 | 0 | 0.281 |
| Swelling at injection site | 8 (19.0%) | 4 (15.4%) | 0 | 0.250 |

**Table 3**
Endpoint geometric mean titres and seropositivity rates of vaccinated paediatric cohort measured by ZEBOV-GP specific IgG antibody ELISA.

| | Day 0 | Day 28 | Day 180 | Day 365 |
|---|---|---|---|---|
| **1–5-year-old age group** | | | | |
| *N* | 37 | 31 | 32 | 28 |
| Mean endpoint titre (95% CI) | 65.23 (44.4–86.7) | 557.6 (376.9–738.2) | 331.2 (273.3–419.2) | 293.8 (146.1–441.5) |
| Seropositivity, *N* (%) | 3 (8) | 27 (87) | 27 (84) | 23 (82) |
| Change in mean endpoint titre vs D0 (p-value) | x | < 0.0001 | < 0.0001 | < 0.0001 |
| Change in seropositivity (vs D0) (p-value) | x | < 0.0001 | < 0.0001 | < 0.0001 |
| **6–12-year-old age group** | | | | |
| *N* | 38 | 36 | 34 | 27 |
| Mean endpoint titre (95% CI) | 53.92 (45.98–61.87) | 1466 (988.9–1944) | 539.8 (386.1–693.6) | 444.8 (308.9–580.8) |
| Seropositivity, *N* (%) | 1 (3) | 35 (97) | 34 (100) | 26 (96) |
| Change in mean endpoint titre vs D0 (p-value) | x | < 0.0001 | < 0.0001 | < 0.0001 |
| Change in seropositivity (vs D0) (p-value) | x | < 0.0001 | < 0.0001 | < 0.0001 |
| 1–5-year-olds vs 6–12-year-olds (p-value) | ns | < 0.0001 | 0.0083 | 0.00247 |

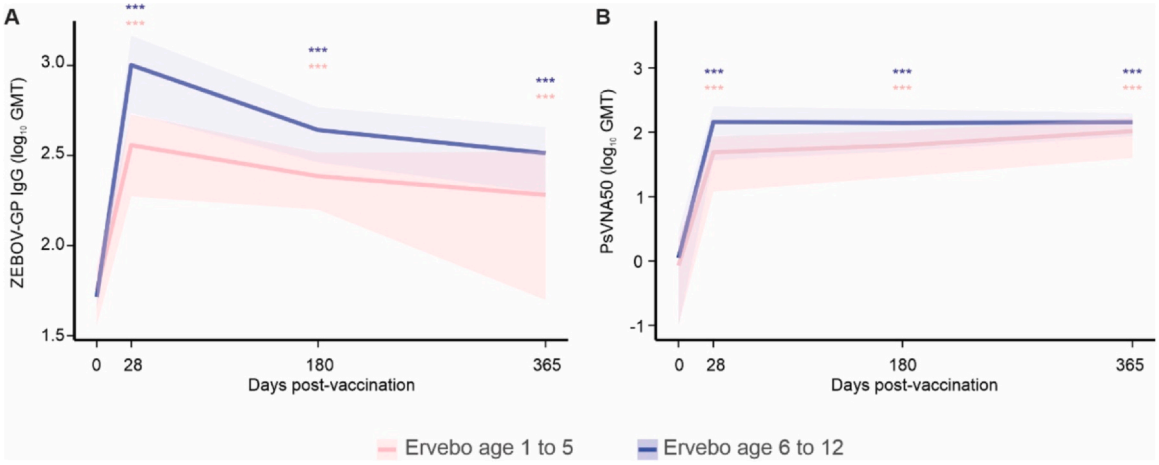


**Fig. 4.** Endpoint titres of ZEBOV-GP specific IgG antibody responses in vaccinated paediatric cohort. Serum samples from the vaccinated paediatric cohort were assessed for ZEBOV-GP specific IgG antibodies using ZEBOV-GP specific IgG ELISA (A), and PsVNA50 pseudovirions (B). The line represents the geometric mean titres (GMT), and the shaded area indicates the 95% confidence interval. Asterisks denote a significant difference between baseline and each time point for Ervebo vaccinees aged between 1 and 5 years (pink) and Ervebo vaccinees aged between 6 and 12 years (blue) (*p < 0.05, **p < 0.01, ***p < 0.001).

negative by PCR (Supplementary pp 10–13); fatigue was reported in 5/26 (19%) of those that were PCR negative.

Seven participants in total were positive for rVSVΔG in saliva samples (Supplementary Figure 2), two and five in the age groups 1–5 and 6–12 years, respectively. Viral RNA was detected in participants' saliva at days 7 and 14. All participant plasma and saliva specimens were negative by day 28.

### *rVSV-ZEBOV elicits rapid and durable ZEBOV-GP specific IgG antibody responses that remain elevated up to 1-year post-vaccination*

Baseline ZEBOV-GP specific IgG antibody levels were generally below the seropositivity threshold. ZEBOV-GP-specific IgG titres were observed four weeks post-vaccination, with seropositivity rates of 87% in the 1–5-year-old group and 97% in the 6–12-year-old group (Table 3). ZEBOV-GP specific IgG antibody titres in the 1–5-year age group rose significantly by day 28 post-vaccination and remained statistically significantly unchanged at day 180 and day 365. However, ZEBOV-GP specific IgG antibody titres in the 6–12-year age group statistically significantly increased at day 28, followed by a decline by day 180 and remained stable until one-year post-vaccination (Fig. 4). Importantly, ZEBOV-GP specific IgG antibody titres show a statistically significant increase compared to the baseline at all time points post-vaccination in both age groups.

### *VZV elicits Varicella Zoster virus-specific IgG antibody responses that remain elevated up to 6months post-vaccination*

Baseline VZV-specific IgG antibody levels were generally below seropositivity threshold, albeit seropositive baseline IgG antibody levels were detected in 5 of 34 (15%) individuals in the VZV arm (1 individual in the cohort of 1–5-year age group and 4 individuals in the 6–12-year-old age group); VZV-specific IgG antibody titres were observed four weeks post-vaccination, with seropositivity rates of 62% (8/13) in the 1–5-year-old group and 71% (12/17) in the 6–12-year-old group. VZV-specific IgG antibody titres in both age groups rose significantly by day 28 post-vaccination, with a geometrical mean titre/GMT (standard deviation) of 90·7 (4·3) and 116·8 (4·6) in the 1–5-year-old and 6–12-year-old age groups, respectively, followed by a decline, but remained statistically significantly unchanged at day 180, with a GMT value of 70·5 (3·2) and 70·2 (3·9), respectively (Supplementary Table 8). Importantly, VZV-specific IgG antibody titres showed a statistically significant increase compared to the baseline at both time points post-vaccination in both age groups.

### *Avidity and neutralisation functions of the rVSV-ZEBOV-GP-induced antibodies*

On-rates of rVSV-ZEBOV-GP induced IgG antibodies were $10^4$-$10^5$ $M^{-1}S^{-1}$ and $10^6$-$10^7$ $M^{-1}S^{-1}$, in 1–5- and 6–12-years old children,

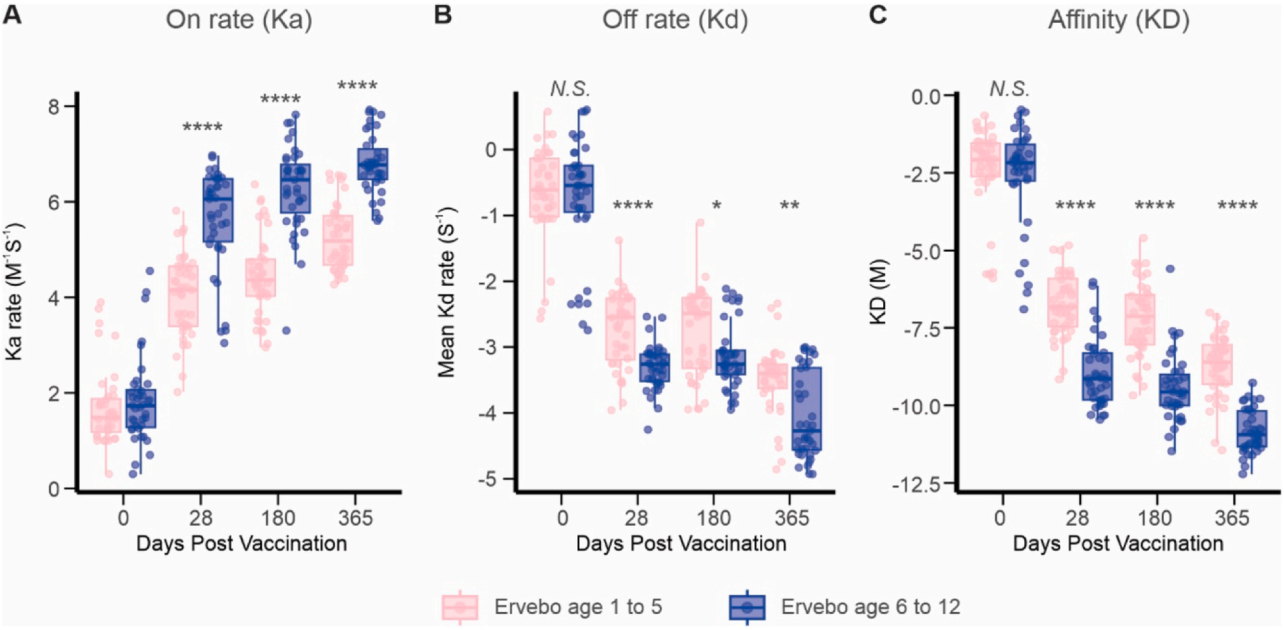


**Fig. 5.** Binding kinetics of ZEBOV-specific IgG antibodies in vaccinated paediatric cohort. On-rates, Ka (A), off-rates, Kd (B) and equilibrium dissociation constant, KD (C) of ZEBOV-GP-specific IgG antibodies in vaccinees in the Lambaréné paediatric cohort were measured using a BLI-based avidity assay. Vaccinees were grouped into two age groups of 1–5 (in pink; n = 40) and 6–12 (in blue; n = 39) years. Statistical significance was denoted as *for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$.

respectively. The on-rates increased statistically significantly by day 28 and continued a slight increase within the same ranges up to day 365 post-vaccination in both age groups (Fig. 5A). The Off-rates were $10^{-2}$-$10^{-3}$ $S^{-1}$ and $10^{-3}$-$10^{-4}$ $S^{-1}$, in 1–5- and 6–12-years old children, respectively, on days 28 and 180. At day 365, the off-rates dropped to $10^{-3}$-$10^{-4}$ $S^{-1,}$ and $10^{-4}$-$10^{-5}$ $S^{-1}$, in 1–5- and 6–12-years old children, respectively (Fig. 5B). The avidity of the rVSVΔG-ZEBOV-induced IgG antibodies was 0.01 nM and 1 nM at day 28 and peaked at 1 nm and 100 nM at day 365, in children aged 1–5 and 6–12 years old, respectively (Fig. 5C).

Using an Ebola Pseudovirion neutralisation assay, 90% (28/31) and 100% (37/37) of children seroconverted at day 28 in the 1–5 and 6–12 years old age groups, respectively. In the 6–12-year-old age group children, the neutralising antibodies (Nabs) titres peaked on day 28 with a mean GMT of 140 while the peak was delayed to 100 at day 365 in the 1–5-year-old age group (Fig. 6A) There were statistically significant positive correlations between ZEBOV-GP specific IgG antibodies and NAbs on day 28, day 180, and day 365 post-vaccination (Fig. 6B).

## Discussion

The rVSVΔG-ZEBOV-GP vaccine has been recently authorised to prevent EVDs in children aged ≥ one year by the FDA and the EMA based on its favourable safety profile. However, intense replication and shedding of the vector rVSVΔG specific to children may induce specific safety signals or affect immune responses adversely. We designed a phase 2 clinical trial in Lambaréné, Gabon, to assess the clinical significance of the rVSVΔG replication and shedding. We also investigated the effects of the replication on the magnitude of ZEBOV-GP specific IgG antibody response and its avidity and neutralisation functions in children aged 1–12 years up to one-year post-vaccination.

In our trial, the control group received the varicella-zoster vaccine, a live attenuated vaccine to compare the safety profiles and the patterns of immune responses with rVSVΔG-ZEBOV-GP assuming that comparable safety and immunogenicity profiles refer to the effective control of rVSVΔG-ZEBOV-GP vaccine in the children. rVSVΔG was detected in the plasma of study participants soon after vaccination with no indication of prolonged replication, similar to previous findings and conforming to the rVSVΔG-ZEBOV-GP vaccine's intrinsic properties. The absence of prolonged replication suggests appropriate immune control of the rVSVΔG-ZEBOV-GP-induced replication.[13,25,29–31]

In this trial, which was sampled to detect rVSVΔG shedding, the proportion of children experiencing shedding in saliva was lower (12.5% in 6–12-year-olds and 5% in 1–5-year-olds) compared to that reported as an exploratory endpoint from two previous studies (~30%). This finding may reflect the expected proportion of shedding when extrapolating to the children's population. However, technical considerations like difficulty in saliva sampling in young children, vaccine dose or batch effect or differential sensitivity of the laboratory assays cannot be ruled out.[23] We found no correlation of the concentration of the rVSVΔG in plasma with the frequency of solicited adverse events, nor with the titres of IgG antibodies and avidity and neutralisation, reinforcing the idea of appropriate control of the rVSVΔG replication in children and consequently adequate vaccine tolerability for the paediatric population.

The increased risk of solicited adverse events in the rVSVΔG-ZEBOV-GP recipients refers to the expected and stronger reactogenicity induced by a replication-competent viral vector platform. However, these adverse events were of mild to moderate intensity, and all were transient with a shorter duration than the VZV vaccine recipients. The seroconversion rates were 25% higher for rVSVΔG-ZEBOV-induced IgG antibody titres than VZV-specific IgG antibody titres from day 28 up to one-year post-vaccination, suggesting an ability of rVSVΔG to improve vaccine-induced immune responses. We found high avidity of the rVSVΔG-ZEBOV-induced IgG antibody in both age groups up to day 365, with notably higher levels in the older children, suggesting strong antibody–antigen binding, clonal selection and effector functions. Serum samples of > 95% of children elicited NAb titres that reduced or stopped the infectivity of Ebola pseudovirion after vaccination. Together, these findings demonstrate that the rVSV-ZEBOV-GP vaccine effectively engendered high titres of antibodies with strong effector properties in children.

The detection of rVSV-ZEBOV-GP vaccinemia in a VZV-vaccinated participant was unexpected, with possible causes of this finding

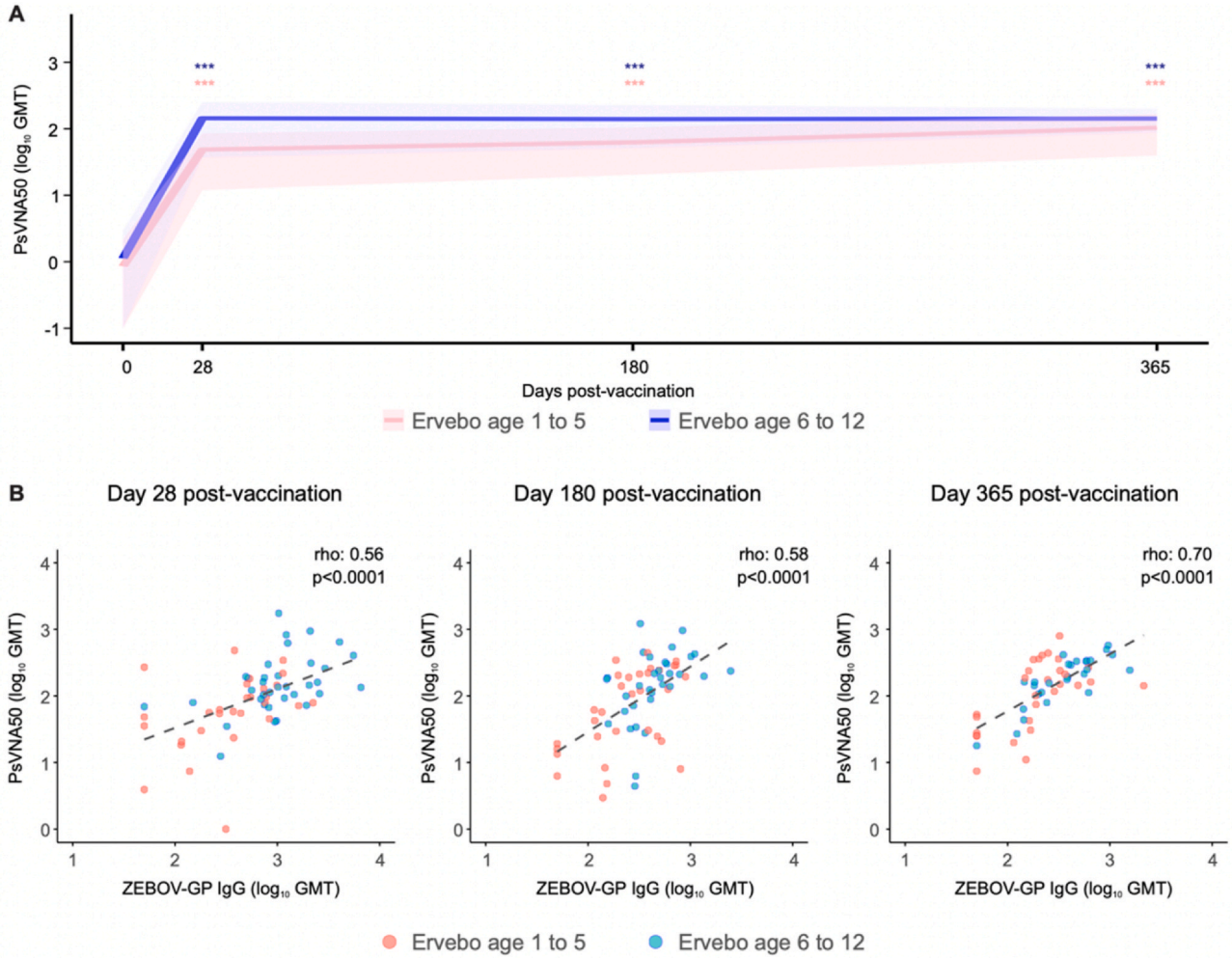


**Fig. 6.** ZEBOV-GP specific IgG neutralising antibody responses in vaccinated paediatric cohort. (A) Serum samples from the vaccinated paediatric cohort were assessed for ZEBOV-GP specific IgG antibodies using PsVNA50 pseudovirions. The line represents the geometric mean titres (GMT), and the shaded area indicates the 95% confidence interval. Asterisks denote a significant difference between baseline and each time point for Ervebo vaccinees aged between 1 and 5 years (pink) and Ervebo vaccinees aged between 6 and 12 years (blue) (*p < 0.05, **p < 0.01, ***p < 0.001). (B) Correlation analyses between ZEBOV-GP specific IgG antibody responses using PsVNA50 pseudovirions vs ZEBOV-GP specific IgG ELISA. There were significant positive correlations between pseudovirions neutralisation titres and ELISA titres at each time point for Ervebo vaccinees aged between 1 and 5 years (pink) and Ervebo vaccinees aged between 6 and 12 years (blue).

including contamination or vaccine exchange, as the horizontal transmission of rVSV in animal models has thus far proved negative; further investigation into possible vaccine transmission as a result of shedding is ongoing and may contribute to advance the discussion about the public health impact of the shedding of the rVSVΔG-ZEBOV-GP.

The adverse event rates and the immunogenicity profiles observed in this trial were similar to those observed in the previous trial in Gabon and in PREVAC and ring vaccination studies.[12,19]

The trend of antibody response data from previous rVSVΔG-ZEBOV-GP vaccine studies suggests that children vaccinated with rVSVΔG-ZEBOV-GP develop immune responses comparable to adults.[13,19,23] The safety and immunogenicity profiles of the rVSVΔG-ZEBOV-GP vaccine in our study confirm recent FDA/EMA/WHO recommendations for its use in children's populations, specifically those living in a Central African region vulnerable to EVD outbreaks. In addition, the single-dose rVSVΔG-ZEBOV-GP regime elicits adequate responses in children living in Lambaréné, Gabon. Previous exploration of rVSVΔG-ZEBOV-GP vaccination regimes did not show an advantage of a dose boost over a single-dose regimen.[19]

A limitation of our study might be the lack of blinding, meaning awareness of the randomisation arm might have influenced the judgement and assessments of the clinical investigators. The lack of blinding was influenced by the difference in vaccine administration between the study vaccines (intramuscular vs subcutaneous). The similarities observed between our safety analysis results and those from previous trials suggest that this was not the case.[32] rVSVΔG-ZEBOV-GP antibody titres were not measured in the control group, representing a lack of this comparison between intervention and control groups. However, this limitation did not affect the value of the findings from the intervention group.

In conclusion, EVD is an ever-present danger with a genuine risk of worldwide dissemination, and there is a need for efficacious vaccines for all populations, but most especially in outbreak hotspots. Multiple randomised, controlled clinical trials, including the present, have consistently reported that rVSVΔG-ZEBOV-GP at the licensed dose is safe, tolerable, and immunogenic in children.

## Funding

This trial is funded by the Innovative Medicines Initiative 2 (IMI 2) Joint Undertaking (grant number: 116068). The Biomedicine and Social Sciences research team of CERMEL is funded by the European

and Developing Countries Clinical Trials Partnership (EDCTP-TMA-SF-1946-VARSAF).

## Author contributions

STA, PGK, and CAS designed the trial with the contribution of the VSV-EBOPLUS team members. DM and the VSV-EBOPLUS team members raised funds and supported the trial. AA, FPM, ALK, BFO, and STA implemented the trial. AA, FPM, ALK, BFO, STA, KK, RK, SSN, SR, TPV, OE, and AMH CAS generated clinical and laboratory data. JCBO, SM, and HIN analysed centralised data. AA, STA, KK, RK, SSN, SR, TPV, OE, AMH, CAS and PGK interpreted the data. AA and AST drafted the manuscript. All authors reviewed and approved the manuscript.

## Data Availability

The datasets generated and/or analysed during the current study are available from the corresponding author upon request.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

The authors thank the trial participants and their families.

We thank the trial implementers and coordinators: Pamel Minsoko Angoissa, Fallowne Mbadinga, Armel Mbouna, and Dani Nzinga.

The authors would like to thank the Proteomics Core facility at the University of Gothenburg, Gothenburg, Sweden, for producing and purifying ZEBOV-GP.

## Appendix A

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## Appendix B. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2024.106237.

## References

1. Deng IM, Duku O, Gillo AL. *Ebola haemorrhagic fever in Sudan, 1976. Bull World Health Organ* 1978;**56**:247.
2. Burke J, Declerq R, Ghysebrechts G. *Ebola haemorrhagic fever in Zaire, 1976. Bull World Health Organ* 1978;**56**:271.
3. Feldmann H, Geisbert TW. *Ebola haemorrhagic fever. Lancet* 2011;**377**:849.
4. Outbreak History | Ebola | CDC. ⟨https://www.cdc.gov/ebola/outbreaks/?CDC_AAref_Val=https://www.cdc.gov/vhf/ebola/history/chronology.html⟩ [Accessed June 24, 2024].
5. Izudi J, Bajunirwe F. *Case fatality rate for Ebola disease, 1976–2022: a meta-analysis of global data. J Infect Public Health* 2024;**17**:25–34.
6. Bell BP, Damon IK, Jernigan DB, Kenyon TA, Nichol ST, O'Connor JP, et al. *Overview, control strategies, and lessons learned in the CDC response to the 2014–2016 Ebola Epidemic. MMWR Suppl* 2019;**65**:4–11.
7. Monath TP, Fast PE, Modjarrad K, Clarke DK, Martin BK, Fusco J, et al. *rVSVDELTAG-ZEBOV-GP (also designated V920) recombinant vesicular stomatitis virus pseudotyped with Ebola Zaire Glycoprotein: standardized template with key considerations for a risk/benefit assessment. Vaccin X* 2019;**1**:100009. https://doi.org/10.1016/j.jvacx.2019.100009
8. Stojdl DF, Lichty BD, TenOever BR, Paterson JM, Power AT, Knowles S, et al. *VSV strains with defects in their ability to shutdown innate immunity are potent systemic anti-cancer agents. Cancer Cell* 2003;**4**:263–75.
9. Fathi A, Dahlke C, Addo MM. *Recombinant vesicular stomatitis virus vector vaccines for WHO blueprint priority pathogens. Hum Vaccin Immunother* 2019;**15**:2269–85.
10. Huttner A, Agnandji ST, Engler O, Hooper JW, Steve K, Keersten R, et al. *Antibody responses to recombinant vesicular stomatitis virus-Zaire Ebolavirus vaccination for Ebola virus disease across doses and continents: 5-year durability. Clin Microbiol Infect* 2023;**29**:1587–94.
11. Rupani N, Ngole ME, Lee JA, Aluisio AR, Gainey M, Perera S, et al. *Effect of recombinant vesicular stomatitis virus-Zaire Ebola virus vaccination on Ebola virus disease illness and death, Democratic Republic of the Congo. Emerg Infect Dis* 2022;**28**:1180–8.
12. Henao-Restrepo AM, Camacho A, Longini IM, Watson CH, Edmunds WJ, Egger M, et al. *Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ça Suffit!). Lancet* 2017;**389**:505–18.
13. Agnandji ST, Fernandes JF, Bache EB, Obiang Mba RM, Brosnahan JS, Kabwende L, et al. *Safety and immunogenicity of rVSVΔG-ZEBOV-GP Ebola vaccine in adults and children in Lambaréné, Gabon: a phase I randomised trial. PLoS Med* 2017;**14**:e1002402. https://doi.org/10.1371/JOURNAL.PMED.1002402
14. Afolabi MO, Ishola D, Manno D, Keshinro B, Bockstal V, Rogers B, et al. *Safety and immunogenicity of the two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in children in Sierra Leone: a randomised, double-blind, controlled trial. Lancet Infect Dis* 2022;**22**:110.
15. Tapia MD, Sow SO, Mbaye KD, Thiongane A, Ndiaye BP, Ndour CT, et al. *Safety, reactogenicity, and immunogenicity of a chimpanzee adenovirus vectored Ebola vaccine in children in Africa: a randomised, observer-blind, placebo-controlled, phase 2 trial. Lancet Infect Dis* 2020;**20**:719–30.
16. Manno D, Bangura A, Baiden F, Kamara AB, Ayieko P, Kallon J, et al. *Safety and immunogenicity of an Ad26.ZEBOV booster dose in children previously vaccinated with the two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen: an open-label, non-randomised, phase 2 trial. Lancet Infect Dis* 2022;**23**:352–60.
17. Nyombayire J, Ingabire R, Magod B, Mazzei A, Mazarati JB, Noben J, et al. *Monitoring of adverse events in recipients of the 2-dose Ebola vaccine regimen of Ad26.ZEBOV followed by MVA-BN-Filo in the UMURINZI Ebola Vaccination Campaign. J Infect Dis* 2023;**227**:268–77.
18. Anywaine Z, Barry H, Anzala O, Mutua G, Sirima SB, Eholie S, et al. *Safety and immunogenicity of 2-dose heterologous Ad26.ZEBOV, MVA-BN-Filo Ebola vaccination in children and adolescents in Africa: a randomised, placebo-controlled, multicentre Phase II clinical trial. PLoS Med* 2022;**19**:e1003865.
19. PREVAC Study Team, Kieh M, Richert L, Beavogui AH, Grund B, Leigh B, et al. *Randomized trial of vaccines for Zaire Ebola virus disease. N Engl J Med* 2022;**387**:2411–24.
20. U.S. FDA Approves Merck's ERVEBO® (Ebola Zaire Vaccine, Live) for Use in Children 12 Months of Age and Older – Merck.com. ⟨https://www.merck.com/news/u-s-fda-approves-mercks-ervebo-ebola-zaire-vaccine-live-for-use-in-children-12-months-of-age-and-older/⟩ [Accessed Oct 5, 2023].
21. Ebola virus vaccines. ⟨https://www.who.int/groups/global-advisory-committee-on-vaccine-safety/topics/ebola-virus-vaccines⟩ [Accessed June 25, 2024].
22. Extraordinary meeting of the Strategic Advisory Group of Experts on Immunization on Ebola vaccination, May 2024: conclusions and recommendations Final draft Full meeting report to be published in the WHO Weekly Epidemiological. ⟨www.who.int/immunization/interim_ebola_recommendations_feb_2019.pdf⟩ [Accessed July 1, 2024].
23. Lee AW, Liu K, Lhomme E, Blie J, McCullough J, Onorato MT, et al. *Immunogenicity and vaccine shedding after 1 or 2 doses of rVSVΔG-ZEBOV-GP Ebola Vaccine (ERVEBO®): results from a phase 2, randomized, placebo-controlled trial in children and adults. Clin Infect Dis* 2023;**78**:870–9. https://doi.org/10.1093/CID/CIAD693
24. Chen RT, Carbery B, Mac L, Berns KI, Chapman L, Condit RC, et al. *The Brighton Collaboration Viral Vector Vaccines Safety Working Group (V3SWG). Vaccine* 2015;**33**:73–5.
25. Agnandji ST, Huttner A, Zinser ME, Njuguna P, Dahlke C, Fernandes JF, et al. *Phase 1 trials of rVSV Ebola vaccine in Africa and Europe. N Engl J Med* 2016;**374**:1647–60.
26. Mohan GS, Li W, Ye L, Compans RW, Yang C. *Antigenic subversion: a novel mechanism of host immune evasion by Ebola virus. PLoS Pathog* 2012;**8**:e1003065.
27. Huttner A, Dayer JA, Yerly S, Combescure C, Auderset F, Desmeules J, et al. *The effect of dose on the safety and immunogenicity of the VSV Ebola candidate vaccine: a randomised double-blind, placebo-controlled phase 1/2 trial. Lancet Infect Dis* 2015;**15**:1156–66.
28. Rudge TL, Sankovich KA, Niemuth NA, Anderson MS, Badorrek CS, Skomrock ND, et al. *Development, qualification, and validation of the Filovirus Animal Nonclinical Group anti-Ebola virus glycoprotein immunoglobulin G enzyme-linked immunosorbent assay for human serum samples. PLoS One* 2019;**14**:e0215457.

29. Kennedy SB, Bolay F, Kieh M, Grandits G, Badio M, Ballou R, et al. *Phase 2 placebo-controlled trial of two vaccines to prevent Ebola in Liberia. N Engl J Med* 2017;**377**:1438–47.
30. Huttner A, Combescure C, Grillet S, Haks MC, Quinten E, Modoux C, et al. *A dose-dependent plasma signature of the safety and immunogenicity of the rVSV-Ebola vaccine in Europe and Africa. Sci Transl Med* 2017;**9**:eaaj1701.
31. Martinez-Murillo PA, Huttner A, Lemeille S, Medaglini D, Ottenhoff THM, Harandi AM, et al. *Refined innate plasma signature after rVSVΔG-ZEBOV-GP immunization is shared among adult cohorts in Europe and North America. Front Immunol* 2024;**14**. https://doi.org/10.3389/FIMMU.2023.1279003
32. Heppner DG, Kemp TL, Martin BK, Ramsey WJ, Nichols R, Dasen EJ, et al. *Safety and immunogenicity of the rVSVΔG-ZEBOV-GP Ebola virus vaccine candidate in healthy adults: a phase 1b randomised, multicentre, double-blind, placebo-controlled, dose-response study. Lancet Infect Dis* 2017;**17**:854–66.