TESTING A PROPHYLACTIC VACCINE REGIMEN AGAINST EBOLA VIRUS DISEASE IN SIERRA LEONE: VACCINE SAFETY, IMMUNOGENICITY AND FACTORS AFFECTING IMMUNOGENICITY

Daniela Manno

A thesis submitted in accordance with the requirements for the degree of Doctor of Philosophy of the University of London

May 2024

Department of Clinical Research
Faculty of Infectious and Tropical Diseases
London School of Hygiene & Tropical Medicine
University of London

The studies presented in the published papers were funded by the Innovative Medicines Initiative (IMI) 2 Joint Undertaking under grant agreement No 115854 (EBOVAC1). My contract is currently funded by the EBOVAC3 project (grant agreement No ITCRZO0210). No funding was received for student fees.

Declaration

I, Daniela Manno, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

Background

In response to the 2014-16 Ebola virus disease (EVD) epidemic in West Africa, the EBOVAC1 consortium fast-tracked the clinical development of a two-dose heterologous vaccine regimen comprising the experimental Ad26.ZEBOV and MVA-BN-Filo vaccines against Ebola virus (EBOV).

Aims and Methodology

This analytic commentary for a PhD by prior publication aims to synthesise and critically appraise the published results of four studies conducted under the EBOVAC1 project in Sierra Leone, a country severely affected by the West African EVD epidemic. These studies included a cross-sectional seroprevalence study, a randomised controlled trial (VAC52150EBL3001), an open-label trial (VAC52150EBL2011) and a cohort study, and were all conducted in Kambia district in the northwest of the country.

The main overarching objectives of these studies were to assess:

- The seroprevalence of EBOV Glycoprotein (GP) Immunoglobulin G (IgG) antibodies in a population affected by the 2014-16 EVD epidemic.
- The safety and immunogenicity of an Ad26.ZEBOV, MVA-BN-Filo vaccine regimen with a 56-day interval between doses in participants from a population affected by the EVD epidemic.
- The safety and immunogenicity of an Ad26.ZEBOV booster in participants who had been previously vaccinated with the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen.
- The effect of malaria on the immune response to the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen.

Results

Approximately 8% of Sierra Leonean adults and children who enrolled in the seroprevalence study and who reported never having had signs or symptoms of EVD

had serologic responses to EBOV GP above a seropositivity threshold. The Ad26.ZEBOV, MVA-BN-Filo vaccine regimen, with a 56-day interval between doses, was well tolerated and induced a humoral immune response that persisted for at least two years in adults and three years in children. Booster vaccination with Ad26.ZEBOV in previously vaccinated adults and children was safe and induced a robust anamnestic response within seven days. Malaria infection did not affect the binding antibody response 21 days after the second dose of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen.

Conclusions and impact of the presented work

The results of the EBOVAC1 seroprevalence study suggest that EBOV can potentially transmit undetected with some infections occurring asymptomatically or with milder symptoms. The results of the clinical trials support the use of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen for EVD prophylaxis in adults and children 1-17 years of age, with the option of providing an additional Ad26.ZEBOV booster to previously vaccinated people at imminent risk of EVD, such as at the start of an EVD outbreak. Some of these findings informed the marketing authorisation under exceptional circumstances granted to the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen in the European Union in July 2020. This vaccine regimen is suitable for EVD prophylaxis in areas where malaria is highly endemic and where the vaccine may be most needed in the future.

Acknowledgements

The accomplishment of this thesis would have not been possible without the support, guidance and collaboration of many colleagues, collaborators, research participants, friends and family.

I extend my sincerest appreciation to the EBOVAC Salone trial participants and their families, the community members and representatives of Kambia town and Rokupr, the Kambia Government Hospital and the Ministry of Health & Sanitation of Sierra Leone.

I am deeply grateful to colleagues and collaborators from the London School of Hygiene & Tropical Medicine (LSHTM), the College of Medicine and Allied Health Sciences of the University of Sierra Leone (COMAHS), GOAL and Janssen, who contributed to the studies described in this thesis. In particular, I would like to give special thanks to Dr Bailah Leigh, who was the principal investigator of the EBOVAC studies in Sierra Leone.

I would like to express my deepest gratitude to my PhD supervisors, Professor Deborah Watson-Jones and Professor Brian Greenwood, for their support and guidance during the conduct of the EBOVAC studies and for allowing me to accomplish this PhD by prior publication. Thank you for sharing your knowledge with me and for supporting my professional development. Working with you has been a great learning experience.

I am also extremely grateful to the Head of the Doctoral College Dr Sam Alsford and Dr Alex Mold for their precious support and guidance during the PhD application process and the writing of the analytic commentary.

I am grateful to the Innovative Medicines Initiative (IMI) for funding the EBOVAC1 project and my post.

I would like to thank my friends in London, Italy and around the world, for their support and encouragement during the years.

Finally, I would like to thank my husband Jonathan and my family in Italy for their support. I am particularly grateful to my mother and my grandparents for their love and encouragement and for teaching me that everything can be achieved with resilience and patience.

I would like to dedicate this thesis to my son Samuel. Being his mum has been the best thing that happened to me and made me a better person and a better scientist. I hope this will inspire him to pursue his dreams and happiness despite any challenges.

Funding

The studies presented in the published papers were funded by IMI 2 Joint Undertaking under grant agreement No 115854 (EBOVAC1). This Joint Undertaking received support from the European Union's Horizon 2020 research and innovation programme and the European Federation of Pharmaceutical Industries and Association (EFPIA). My contract is currently funded by the EBOVAC3 project (grant agreement No ITCRZO0210). No funding was received for student fees.

Table of Contents

Abstract **3**

Acknowledgements **5**

Funding **6**

List of Tables **8**

List of Figures **8**

List of acronyms and abbreviations **9**

SECTION 1: ANALYTICAL COMMENTARY **13**

Introduction **13**

Chapter 1: Background **14**

1.1 Ebola disease 14

1.2 EVD epidemic in West Africa (2014-2016) 18

1.3 EVD outbreak response 20

1.4 Vaccines against EVD 21

1.5 The EBOVAC1 project 24

Chapter 2: Seroprevalence of IgG antibodies against EBOV in Sierra Leone **25**

2.1 Seroepidemiology in Ebola vaccine research 25

2.2 Available evidence on EBOV seroprevalence 26

2.3 The EBOVAC1 seroprevalence study 30

2.3.1 Study strengths and limitations 32

2.3.2 Study contribution to knowledge 35

Chapter 3: Safety and immunogenicity of the Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen in Sierra Leone **36**

3.1 The EBOVAC-Salone trial (VAC52150EBL3001) 38

3.2 The EBOVAC booster study in children (VAC52150EBL2011) 44

3.4 Contribution to knowledge of the EBOVAC-Salone trial and the EBOVAC booster study in children 48

Chapter 4: The effect of malaria on the immune response to the two-dose Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen **49**

4.1 The EBOVAC malaria study 50

4.1.2 Study strengths and limitations 53

4.1.3 Study contribution to knowledge 54

Chapter 5: Discussion **55**
SECTION 2: RESEARCH PUBLICATIONS **61**
Paper 1: Ebola Virus Glycoprotein IgG Seroprevalence in Community Previously Affected by Ebola, Sierra Leone **61**
Paper 2: Safety and long-term immunogenicity of the two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in adults in Sierra Leone: a combined open-label, non-randomised stage 1, and a randomised, double-blind, controlled stage 2 trial **84**
Paper 3: 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 **122**
Paper 4: 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 **193**
APPENDIX 1 – Studies reporting immunogenicity and safety data of vaccines against Ebola disease, reported by vaccine and study type **219**
APPENDIX 2 – Ebolavirus seroprevalence studies published from 01 January 2017 to 11 November 2023, ordered by country, study population and year of sample collection **235**
APPENDIX 3 – EBOV GP-specific binding antibody response in different clinical trials of the Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen, with a 56-day interval between doses, listed by geographical location and age of participants **241**
References **242**

List of Tables

Table 1 - Ebola disease outbreaks since 1976 (updated 6 September 2023) 15

List of Figures

Figure 1 - Ebola virus 14
Figure 2 - Ebola virus particle, with structures of the major proteins 16
Figure 3 - Ebolavirus ecology and transmission 17

Figure 4 - EBOVAC-Salone trial and seroprevalence study sites in Sierra Leone 30
Figure 5 - Ad26.ZEBOV, MVA-BN-Filo vaccine regimen 37
Figure 6 - EBOVAC-Salone study design 38
Figure 7 - EBOVAC-Salone study: anti-EBOV GP binding antibody response in adults 41
Figure 8 - EBOVAC-Salone study: anti-EBOV GP binding antibody response in children 41
Figure 9 - EBOVAC-Salone study: anti-EBOV GP neutralising antibody response in adults 42
Figure 10 - EBOVAC-Salone study: anti-EBOV GP neutralising antibody response in children 43
Figure 11 - EBOVAC booster study in children: anti-EBOV GP binding antibody response 46
Figure 12 - EBOVAC malaria study conceptual framework 52

List of acronyms and abbreviations

Ad5	Adenovirus type 5
Ad26	Adenovirus type 26
Ad26 VNA	Ad26-specific virus neutralisation assay
AMA-1	Apical membrane antigen 1 of *Plasmodium falciparum*
AE	Adverse Event
AR	Adverse Reaction
BDBV	Bundibugyo virus (species *Bundibugyo ebolavirus*)
BOMV	Bombali virus (species *Bombali ebolavirus*)
CC	Correlation Coefficient
CDC	Centers for Disease Control and Prevention
ChAd3	Chimpanzee adenovirus 3
COMAHS	College of Medicine and Allied Health Sciences, Sierra Leone
CSS	Cross-sectional study
DBS	Dried blood spots
DRC	Democratic Republic of Congo

EBODAC	Ebola Vaccine Deployment, Acceptance and Compliance
EBOV	Ebola virus (species *Zaire ebolavirus*)
EFPIA	European Federation of Pharmaceutical Industries and Association
ELISA	Enzyme-Linked Immunosorbent Assay
EMA	European Medicines Agency
Etramp5.Ag1	Early transcribed membrane protein of *Plasmodium falciparum*
Eu	ELISA unit
EU	European Union
EVD	Ebola virus disease
FANG	Filovirus Animal Non-Clinical Group
FDA	USA Food and Drug Administration
FLW	Front-line worker
GEXP18	Gametocyte exported protein of *Plasmodium falciparum*
GLURP.R2	Glutamate-rich protein R2 region of *Plasmodium falciparum*
GMC	Geometric mean concentration
GMR	GMC ratio
GMT	Geometric mean titre
GP	Glycoprotein
GPΔMuc	Mucin-like domain-deleted GP
HCW	Healthcare worker
HIV	Human immunodeficiency virus
IgG	Immunoglobulin G
IM	Intramuscular
IMI	Innovative Medicines Initiative
Inf.U.	Infectious units
INSERM	The Institut National de la Santé et de la Recherche Médicale, France
LIPS	Luciferase Immunoprecipitation System
LLOQ	Lower limit of quantification
LSHTM	London School of Hygiene & Tropical Medicine
MARV	Marburg virus
MenACWY	Meningococcal quadrivalent (serogroups A, C, W135, and Y) conjugate vaccine

MFI	Median fluorescence intensity
MSF	Médecins sans Frontières
MSP-1.19	Merozoite surface protein 1.19 of *Plasmodium falciparum*
MVA	Modified vaccinia Ankara
nAbs	Neutralising antibodies
NGO	Non-Governmental Organization
NHP	Non-human primate
NOD	Normalised optical density
NP	Nucleoprotein
OD	Optical density
OR	Odds ratio
P. falciparum	*Plasmodium falciparum*
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PFU	Plaque-forming units
PPE	Personal protective equipment
PRNT	Plaque reduction neutralisation test
psVNA	Pseudovirion neutralisation assay
pu	Particle units
qSAT	Quantitative suspension array technology
RESTV	Reston virus (species *Reston ebolavirus*)
RCT	Randomised controlled trial
RDT	Rapid diagnostic test
Rh2.2030	Reticulocyte-binding protein homologue of *P. falciparum*
RNA	Ribonucleic acid
RR	Risk ratio
SAE	Serious adverse event
SAGE	WHO Strategic Advisory Group of Experts
SD	Standard deviation
SUDV	Sudan ebolavirus (species *Sudan ebolavirus*)
TAFV	Taï Forest virus (species *Taï Forest ebolavirus*)

TCID50	Median tissue culture infective dose
UK	United Kingdom
USA	United States of America
USD	United States dollar
VE	Vaccine efficacy
vp	Viral particles
VSV	Vesicular stomatitis virus
WHO	World Health Organization

SECTION 1: ANALYTICAL COMMENTARY

Introduction

Between 2014 and 2016, an unprecedented epidemic of Ebola virus disease (EVD) affected West Africa. The epidemic was caused by Ebola virus (EBOV), species *Zaire ebolavirus*.[1] The disease brought death and disruption to Guinea, Sierra Leone and Liberia, reached neighbouring countries such as Nigeria, Mali and Senegal, and even caused sporadic cases in Europe and North America. No prophylactic vaccine or specific treatment for EVD was available at this time and the epidemic control efforts relied primarily on epidemiological measures such as case identification and isolation, contact tracing and surveillance, safe burial practices, raising community awareness of transmission risks and restricting travels and mass gatherings.[1]

In response to the epidemic, the development of several vaccine candidates was accelerated. The EBOVAC1 consortium, coordinated by the London School of Hygiene & Tropical Medicine (LSHTM), aimed to fast-track the clinical development of a two-dose heterologous prophylactic vaccine regimen comprising the experimental Ad26.ZEBOV and MVA-BN-Filo vaccines, developed by Janssen Vaccines & Prevention B.V. and Bavarian Nordic, respectively. This vaccine regimen had been shown to protect non-human primates (NHPs) against EVD and, if proven safe and effective in humans, could have offered an additional tool to control the ongoing epidemic.

This analytic commentary for a PhD by prior publication aims to synthesise and critically appraise the published results of four studies conducted under the EBOVAC1 project in Kambia district in the northwest of Sierra Leone. These studies included a cross-sectional seroprevalence study, a randomised controlled trial (RCT; VAC52150EBL3001), an open-label trial (VAC52150EBL2011) and a malaria cohort study. The main overarching objectives of these studies were to assess:

- The seroprevalence of EBOV Glycoprotein (GP) Immunoglobulin G (IgG) antibodies in a population affected by the 2014-16 EVD epidemic.

- The safety and immunogenicity of an Ad26.ZEBOV, MVA-BN-Filo vaccine regimen, with a 56-day interval between doses, in participants from a population affected by the EVD epidemic.
- The safety and immunogenicity of an Ad26.ZEBOV booster in participants who had been previously vaccinated with the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen.
- The effect of malaria on the immune response to the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen.

This commentary will discuss the results of these studies in light of current knowledge and highlight the original contribution that these studies have made to the search for an effective and safe prophylactic vaccine against EVD.

Chapter 1: Background

1.1 Ebola disease

Ebola disease is a severe, often fatal, infection in humans and NHPs caused by viruses of the genus *Orthoebolavirus*: Ebola virus or EBOV (species *Zaire ebolavirus*; Figure 1), Sudan virus or SUDV (species *Sudan ebolavirus*), Taï Forest virus or TAFV (species *Taï Forest ebolavirus*) and Bundibugyo virus or BDBV (species *Bundibugyo ebolavirus*).

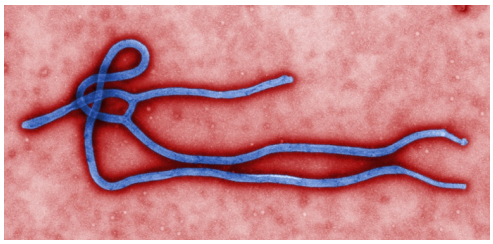


Figure 1 - Ebola virus
Source: CDC/Cynthia Goldsmith
https://upload.wikimedia.org/wikipedia/commons/e/e6/Ebola_virus_virion.jpg

The genus includes two other species: Reston virus or RESTV (species *Reston ebolavirus*), which has been associated with disease in pigs and NHPs but not in humans and Bombali virus or BOMV (species *Bombali ebolavirus*), which was identified in bats in 2018 but is not known to cause disease in either animals or humans.[2,3] Historically, EBOV, which causes EVD, has been responsible for most Ebola disease outbreaks and cases (Table 1).

Table 1 - Ebola disease outbreaks since 1976 (updated 6 September 2023)

Source: CDC, https://www.cdc.gov/vhf/ebola/history/distribution-map.html

Country	Cases	Deaths	Species	Year
Uganda	164	55	*Sudan ebolavirus* (SUDV)	2022
DRC	1	1	*Zaire ebolavirus* (EBOV)	2022
DRC	5	5	*Zaire ebolavirus* (EBOV)	2022
DRC	11	9	*Zaire ebolavirus* (EBOV)	2021
DRC	12	6	*Zaire ebolavirus* (EBOV)	2021
Guinea	23	12	*Zaire ebolavirus* (EBOV)	2021
DRC	130	55	*Zaire ebolavirus* (EBOV)	2020
DRC	3470	2287	*Zaire ebolavirus* (EBOV)	2018-2020
DRC	54	33	*Zaire ebolavirus* (EBOV)	2018
DRC	8	4	*Zaire ebolavirus* (EBOV)	2017
DRC	66	49	*Zaire ebolavirus* (EBOV)	2014
Guinea, Sierra Leone, Liberia and other countries	28,646	11,323	*Zaire ebolavirus* (EBOV)	2014-2016
Uganda	6*	3*	*Sudan ebolavirus* (SUDV)	2012
DRC	36*	13*	*Bundibugyo ebolavirus* (BDBV)	2012
Uganda	11*	4*	*Sudan ebolavirus* (SUDV)	2012
Uganda	1	1	*Sudan ebolavirus* (SUDV)	2011
DRC	32	15	*Zaire ebolavirus* (EBOV)	2008
Uganda	149	37	*Bundibugyo ebolavirus* (BDBV)	2007
DRC	264	187	*Zaire ebolavirus* (EBOV)	2007
Sudan (now South Sudan)	17	7	*Sudan ebolavirus* (SUDV)	2004
Republic of Congo	35	29	*Zaire ebolavirus* (EBOV)	2003
Republic of Congo	143	128	*Zaire ebolavirus* (EBOV)	2002
Republic of Congo	57	43	*Zaire ebolavirus* (EBOV)	2001
Gabon	65	53	*Zaire ebolavirus* (EBOV)	2001
Uganda	425	224	*Sudan ebolavirus* (SUDV)	2000
South Africa	2	1	*Zaire ebolavirus* (EBOV)	1996
Gabon	60	45	*Zaire ebolavirus* (EBOV)	1996
Gabon	37	21	*Zaire ebolavirus* (EBOV)	1996
Zaire (now DRC)	315	250	*Zaire ebolavirus* (EBOV)	1995
Côte d'Ivoire (Ivory Coast)	1	0	*Taï Forest ebolavirus* (TAFV)	1994
Gabon	52	31	*Zaire ebolavirus* (EBOV)	1994
Sudan (now South Sudan)	34	22	*Sudan ebolavirus* (SUDV)	1979
Zaire (now DRC)	1	1	*Zaire ebolavirus* (EBOV)	1977
Sudan (now South Sudan)	284	151	*Sudan ebolavirus* (SUDV)	1976
Zaire (now DRC)	318	280	*Zaire ebolavirus* (EBOV)	1976

*Numbers reflect laboratory confirmed cases only.

EBOV consists of a filamentous particle with a non-segmented, single-stranded, negative-sense ribonucleic acid (RNA) genome (Figure 2).

The virion is composed of an envelope studded with a transmembrane GP, a layer of matrix proteins and a cylindrical nucleocapsid, which contains the genome. The GP, which is the only protein on the virus surface, plays a critical role in viral adhesion and entry into the host cell and is, therefore, a main target for vaccine and therapeutic development.[3]

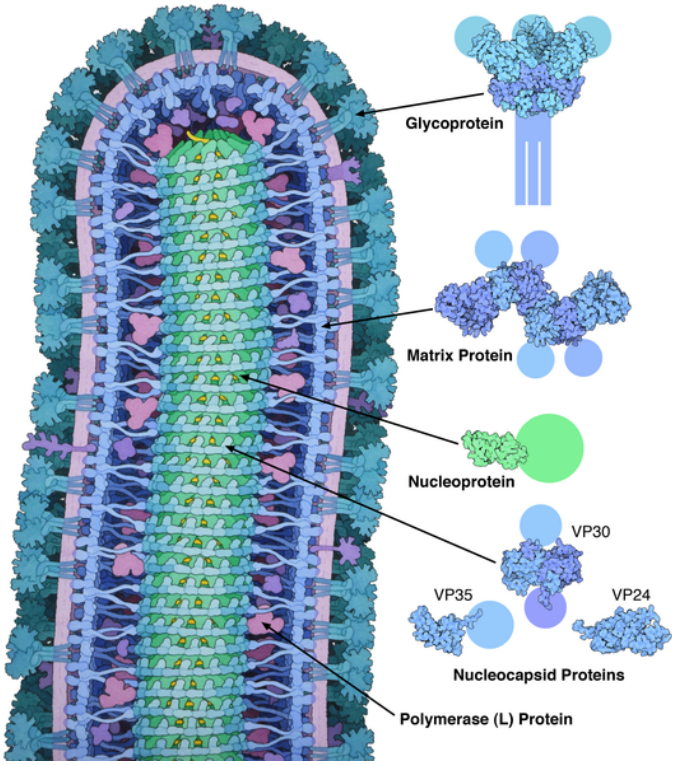


Figure 2 - Ebola virus particle, with structures of the major proteins
Source: David Goodsell
https://upload.wikimedia.org/wikipedia/commons/8/87/178-EbolaVirusProteins_EbolaProteins.png

EVD has an incubation period ranging from two to 21 days, with an average of eight to 10 days.[4] The disease typically manifests as a non-specific febrile illness with 'dry' symptoms, such as fever, headache, fatigue, myalgia and abdominal pain, and progress with 'wet' symptoms, such as diarrhoea and vomiting with substantial fluid loss and hypovolemia.[3,4] Case fatality ranges from 25% to 90%.[5] Death is usually due to tissue hypoperfusion and multiorgan failure.[3] Bleeding manifestations (i.e. bleeding from the gums, petechiae, subconjunctival haemorrhage and blood in vomit and stool) have been observed in less than half of the admitted cases in recent outbreaks and, when present, were a late finding in fatal cases.[3] For this reason, the disease is no longer referred to as 'Ebola haemorrhagic fever' as it was initially identified.[3]

EVD is a zoonotic disease (Figure 3). EBOV and other ebolaviruses circulate in wildlife and are believed to have their natural reservoir in bats.[2] One-fifth of the ebolavirus genome has been isolated from an oral swab of a greater long-fingered bat

(*Miniopterus inflatus*) captured in 2016 near an abandoned Liberian mineshaft, but the full genome has not yet been isolated in an animal host.[6]

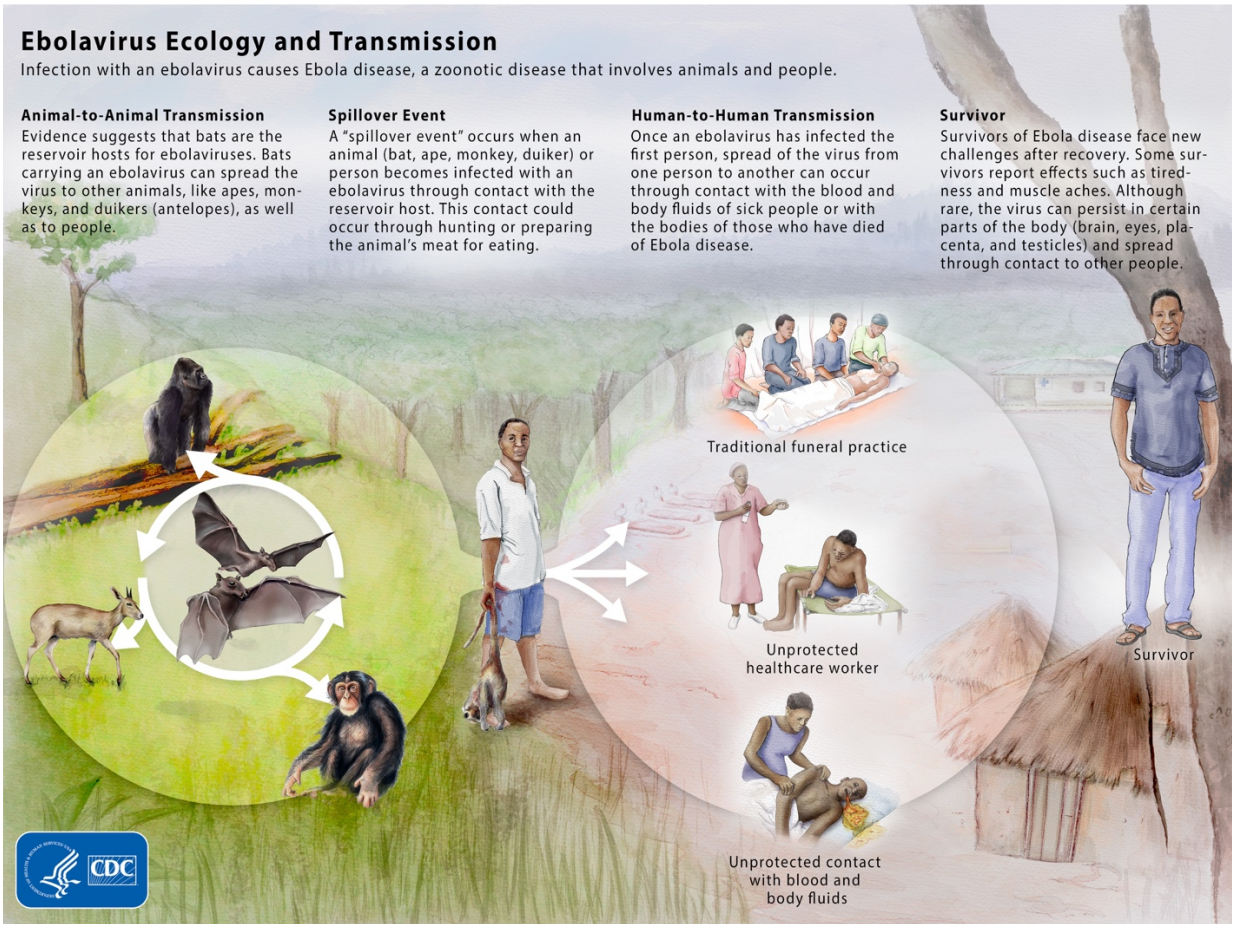


Figure 3 - Ebolavirus ecology and transmission

Source: CDC

https://www.cdc.gov/vhf/ebola/images/ebola_ecology_800px.jpg

In the past, outbreaks have been triggered by a single zoonotic transmission, called a 'spillover event', from the reservoir host to other animals, including NHPs, and humans (Figure 3). Humans can contract the infection from infected animals during activities such as hunting, bushmeat preparation or consumption, or accidental exposure to sick or dead animals and their body fluids. The infection is then spread from human to human following direct contact with the body or body fluids of a person who is sick with or has died from EVD, or following contact with contaminated objects and surfaces. Caring for someone with EVD without protection or participating in traditional funeral practices of someone who has died from EVD, which involves unprotected contact with the deceased body and belongings, are activities with a high risk of transmission.[3] EBOV has also been found to persist in the

body fluids of survivors, including semen and breast milk, with a risk of further transmission.[3] The persistence in semen of male survivors has been documented for months after recovery and sporadic cases of sexual transmission have been reported.[7-9] Transmission through breastfeeding has also been suspected in two cases.[10,11]

Ebola disease is extremely rare. Since EBOV's discovery in 1976 during an outbreak near the Ebola River in what is now the Democratic Republic of Congo (DRC), there have been 32 known Ebola disease outbreaks with a cumulative total of fewer than 40,000 reported cases (Table 1).[12] However, once underway, outbreaks have the potential to spread to a large number of people with devastating consequences for the affected populations, as seen in West Africa in 2014-16.[1]

The occurrence of outbreaks has been linked to environmental changes, such as deforestation with increased host-human interactions,[13] and socioeconomic factors, such as poverty and population density.[14] Mathematical modelling considering the underlying drivers of Ebola disease risk predicts a 1.75 to 3.2-fold increase in the rate of animal-human ebolavirus spillover in Africa by 2070.[14] Although transmission from survivors seems uncommon, as large outbreaks produce a large population of survivors, there is the possibility that future outbreaks may be also caused by the reintroduction of the virus from persistently infected survivors.[15] Both the 2021 outbreaks in Guinea and DRC (Table 1) have been genetically linked to survivors infected during previous outbreaks that took place five and two years earlier, respectively.[7,16]

1.2 EVD epidemic in West Africa (2014-2016)

The largest EVD outbreak in history originated in the village of Meliandou, in the forested rural region of south-eastern Guinea.[17,18] The index case was a two-year-old boy who was believed to have contracted the infection while playing in a hollow tree hosting a colony of insectivorous free-tailed bats (*Mops condylurus*).[17] The boy became ill on 26 December 2013 and died after two days.[17,19] He transmitted the infection to his sister and pregnant mother, who both died.[18] The mother suffered a spontaneous abortion on the night of her death, during which she was cared for by

family members, local healthcare volunteers and a healthcare worker (HCW).[18] All contacts subsequently developed EVD and some died.[18] The disease started to spread among people who attended the funeral of some of the victims causing further transmission to neighbouring villages, the Guinean capital, Conakry, and Kangama, a village across the border with Sierra Leone.[18] Some cases were also cared for in the district hospitals and this contributed to further transmission of the disease outside the initially affected area.[18]

On 10 March 2014, health officials from the two Guinean towns of Guéckédou and Macenta reported clusters of an unknown disease with a high fatality rate, characterised by fever, severe diarrhoea and vomiting to the Guinean Ministry of Health, and two days later, to Médecins sans Frontières (MSF).[19,20] The disease was confirmed to be caused by EBOV and, on 23 March 2014, the World Health Organization (WHO) announced that an EVD outbreak was occurring in Guinea.[1]

The outbreak rapidly spread in Guinea, Sierra Leone and Liberia and, by July 2014, had reached the capitals of the three countries, which became the epicentres of intense EBOV transmission.[1] The three countries had never experienced an EVD outbreak before and were poorly prepared at every level, from early detection of cases to organising and implementing an adequate response.[21]

On 8 August 2014, WHO declared the epidemic a 'Public Health Emergency of International Concern'.[1] Guinea, Liberia and Sierra Leone remained the most affected countries but sporadic cases were recorded also in Italy, Mali, Nigeria, Senegal, Spain, the United Kingdom (UK) and the United States of America (USA).[1] Secondary infections from imported cases occurred in Italy, Mali, Nigeria and the USA, mainly in healthcare settings.[1] Multiple organisations, including the WHO, the United States Centers for Disease Control and Prevention (CDC), Ministries of Health of the affected countries, several Non-Governmental Organizations (NGOs), such as MSF and government personnel from other countries (e.g. the British Army in Sierra Leone) were involved in the intense effort to end the epidemic.[1,22]

In June 2016, two and a half years after the first case was discovered, the epidemic was declared over with 28,652 reported cases and 11,325 deaths.[1] Both figures are

likely to be underestimated, considering that the extent of underreporting was between 17% and 300% depending on the area.[23-25]

The epidemic had a devastating social and economic cost, which is estimated at over 50 billion United States dollars (USD).[26] The most significant cost was attributable to the lives lost due to EVD, including losses within the healthcare workforce, and to non-Ebola-related diseases that were neglected during the epidemic.[26] On the other hand, the unprecedented extent of the EVD epidemic allowed the gathering of new knowledge about the disease and promoted research into diagnostics, vaccines and treatment, which enabled the planning and implementation of an effective response in subsequent Ebola disease outbreaks.

1.3 EVD outbreak response

The principal objectives of an EVD outbreak response are to identify and isolate cases as early as possible to provide adequate care and to prevent further spread in the community.[3] Considering that EVD initially presents with nonspecific symptoms, which are common to other infectious illnesses, the identification of cases requires a clear case definition, epidemiological linkage and laboratory confirmation.[3]

The care of EVD patients is currently based on the following components: EBOV-specific monoclonal antibodies against the virus surface GP, supportive care, and treatment of concurrent infections, if present.[27] Two EBOV-specific monoclonal antibodies were approved by the USA Food and Drug Administration (FDA) in 2020 for the treatment of EVD: Inmazeb™ and Ebanga™.[27] Both were shown to increase survival in EVD patients enrolled in an RCT conducted during the 2018-20 outbreak in DRC.[28] Supportive care aimed at maintaining or restoring normal physiology has also been shown to significantly improve chances of survival when provided early.[27]

Another essential component of an EVD outbreak response is timely contact tracing, quarantine and monitoring. This allows early identification of EVD cases in subsequent chains of transmission and vaccination of asymptomatic contacts and contacts of contacts, an approach known as ‘ring vaccination’.[3,12]

Community awareness of transmission risk factors and compliance with disease control measures are also paramount. Ebola transmission in the community can be successfully controlled only when people cease caring for the sick at home and engaging in traditional funeral practices.[3] In settings where there is high community mistrust and poor compliance with outbreak control measures, EVD outbreaks can spread uncontrolled for months or years.[12]

Given the high risk of nosocomial transmission and that losses in the healthcare workforce contribute substantially to the burden of EVD outbreaks, the protection of HCWs is crucial.[3,26] However, the full personal protective equipment (PPE) currently used during outbreaks can only be worn for a limited time in tropical conditions due to the risk of heat stress, while prophylactic vaccination offers a more practical way to protect HCWs and front-line workers (FLWs).[3]

1.4 Vaccines against EVD

Before the 2014-16 EVD epidemic in West Africa, there were no approved prophylactic vaccines against EVD.[3] Several vaccines were in pre-clinical stages of development and only EBOV DNA and adenovirus-based vaccines had been tested in phase 1 clinical trials.[29] The unprecedented scale of the West African EVD epidemic accelerated the development of several vaccines and there is currently a varied portfolio of products at different stages of clinical development.[29]

To provide an up-to-date overview of these vaccines, I have updated a literature search conducted on 20 February 2020 for one of the papers included in my PhD portfolio.[30] A new PubMed search was run on 23 March 2023 (ebola AND [vaccin* OR immunis* OR immuniz*] AND [trial* OR study OR campaign], no language restrictions), which identified 433 new publications. Following title/abstract screening, I found 19 additional articles, which, added to the previously identified list, resulted in a total of 59 publications (including two systematic reviews)[31,32] reporting immunogenicity and/or safety data from 47 clinical trials or their ancillary studies,[30,33-83] two vaccination campaigns,[84,85] and three cohort studies in subjects not enrolled in clinical trials.[86-88] I also consulted the WHO webpage, a WHO overview

dated 19 Aug 2019 and other sources providing information on Ebola vaccines.[89-92] The PubMed search was rerun on 7 November 2023 and identified five new publications since March 2023 reporting immunogenicity and safety results from three new clinical trials,[93-95] or additional results from previously published studies.[96,97] A summary of the identified vaccines follows below and a list of the studies by vaccine and study type is provided in Appendix 1.

To date, the most developed Ebola vaccines are rVSV-ZEBOV and the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen, which have both obtained broad regulatory approval for use against EVD.

The rVSV-ZEBOV vaccine consists of a recombinant, replication-competent vesicular stomatitis virus (VSV)-vectored vaccine expressing the surface GP of EBOV Kikwit 1995 strain.[92] In preclinical studies, rVSV-ZEBOV showed no toxicities and was able to protect rodents and NHPs from lethal EBOV infection.[98] Phase 1, 2 and 3 clinical trials were conducted in Europe, Africa and North America (Appendix 1) and showed that the vaccine was well tolerated.[31] The most common adverse events (AEs) after vaccination included injection site pain, fever, arthralgia, myalgia, fatigue and headache of mild to moderate intensity.[31] In a phase 1 trial in Switzerland, post-vaccination arthritis occurred in 22% of participants;[34] however, such a high percentage was not observed in other studies.[34,37,43] In a ring vaccination trial conducted in Guinea during the 2014-16 EVD epidemic, three serious adverse events (SAEs; i.e. febrile reaction, anaphylaxis and an influenza-like illness), were considered related or possibly related to the vaccine; all three SAEs resolved without sequelae.[42] rVSV-ZEBOV elicited immune responses persisting for at least five years and had an estimated 100% vaccine efficacy (VE) against EVD from 10 days after administration in the ring vaccination trial in Guinea.[39,41,42,97] Since the results on efficacy against EVD became available,[41] rVSV-ZEBOV was deployed as part of the outbreak control response under expanded access in 2016 in Guinea, during the 2018-20 outbreak in DRC (where a 97.5% VE was estimated) and during subsequent outbreaks.[85,88] rVSV-ZEBOV has received conditional marketing authorization in the European Union (EU) and WHO prequalification and was approved for use in adults in the USA and in

several African countries (commercial name: Ervebo).[99-102] This vaccine has also recently been approved for use in children 12 months or older in the USA.[103]

The Ad26.ZEBOV, MVA-BN-Filo vaccine regimen is a two-dose heterologous regimen comprising the monovalent, recombinant, replication-incompetent, adenovirus type 26 (Ad26) vector-based vaccine, encoding the EBOV GP of the Mayinga variant (Ad26.ZEBOV) as dose 1, and the recombinant, non-replicating, modified vaccinia Ankara (MVA) vector-based vaccine, encoding GPs of the EBOV Mayinga variant, the SUDV Gulu variant, and the Marburg virus (MARV) Musoke variant, and the nucleoprotein (NP) from the TAFV (MVA-BN-Filo) as dose 2, administered 56 days apart.[30] This vaccine regimen, which was shown to protect vaccinated NHPs against an EBOV challenge,[104] had a good safety profile in both adults and children in phase 1 and 2 trials conducted in Europe, North America and Africa.[30,59-70,93] The most commonly reported AEs were injection site pain, headache, myalgia, fatigue, and arthralgia. No vaccine-related SAEs were reported in these trials. However, in a vaccination campaign conducted in Rwanda from 2019 to 2021, 17 SAEs, all in children aged 2-8 years, were considered Ad26.ZEBOV-related: 10 febrile convulsions with or without gastroenteritis and seven cases with fever and/or gastroenteritis.[84] The incidence of fever during the campaign decreased after routine administration of acetaminophen at the time of administration of Ad26.ZEBOV.[84]

The Ad26.ZEBOV, MVA-BN-Filo vaccine regimen with a 56-day interval between doses induced binding and neutralising antibody responses and cellular immune responses 21 days after dose 2.[30,59-70] Binding antibody responses were shown to last for at least three years.[69] An Ad26.ZEBOV booster given two or three years after dose 1 was able to provide a rapid and strong increase in binding antibody response in both adults and children.[30,69] This vaccine regimen has received conditional marketing authorisation in the EU and several African countries and WHO pre-qualification for prophylactic use in adults and children aged one year or older (commercial name: Zabdeno, Mvabea).[105-107] The vaccine regimen was also recommended by the WHO Strategic Advisory Group of Experts (SAGE) for vaccination of lower-risk populations during the 2018-20 outbreak in DRC,[108] and

was used in the previously mentioned mass vaccination campaign in Rwanda.[84] SAGE also recommended its use in pregnant and breastfeeding women at risk of EBOV infection in an outbreak setting.[109]

Other vaccine candidates with good safety and immunogenicity data from phase 1 and 2 clinical studies include an adenovirus type 5 (Ad5)-vectored vaccine expressing the GP of the 2014 EBOV Makona variant (Ad5-EBOV), a two-dose heterologous vaccination regimen with live-attenuated recombinant VSV and Ad5-vectored vaccines expressing the EBOV GP (Makona strain) administered at a 21-day interval (GamEvac-Combi), and a chimpanzee adenovirus 3 (ChAd3)-vectored vaccine expressing the wild-type EBOV GP from the Mayinga strain (ChAd3-EBO-Z).[51-58] Ad5-EBOV and GamEvac-Combi are licensed for emergency use in the countries of manufacture, China and Russia, respectively, while the ChAd3-EBO-Z is not licensed.[90,91] Information on other candidate vaccines that have completed phase 1 studies with promising safety and immunogenicity results can be found in Appendix 1.

1.5 The EBOVAC1 project

In December 2014, in response to the EVD epidemic in West Africa, the EBOVAC1 consortium was awarded a large grant (€ 58,336,885) by the Innovative Medicines Initiative (IMI) to fast-track the development and licensure of a two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo vaccine regimen.[110] The consortium was coordinated by the LSHTM and included the University of Oxford, the National Institute for Health and Medical Research (INSERM) in France, the University of Sierra Leone and the pharmaceutical company Janssen Vaccines & Prevention BV.

The main objectives of the EBOVAC1 project were to implement:

- A phase 1 trial in Europe to establish preliminary safety and immunogenicity data (first-in-human study) and to evaluate the effect of varying sequences and intervals between doses and the durability of immune responses.

- Phase 1 trials in African populations unaffected by the epidemic to confirm the data of the first-in-human study.
- A phase 2 or 3 trial in a country affected by the epidemic (Sierra Leone) to evaluate VE, if possible, and collect additional safety and immunogenicity data to bridge with NHP data.

In addition to the clinical trials, the EBOVAC1 project included Ebola seroprevalence and malaria ancillary studies in Sierra Leone, mathematical modelling, immunology studies, social science studies and communication activities. EBOVAC1 was complemented by other projects, such as EBOVAC2 led by INSERM, which coordinated phase 2 trials with the same vaccine regimen in Europe and Africa, EBODAC (Ebola Vaccine Deployment, Acceptance and Compliance), which supported community and stakeholders trust building around participation into the EBOVAC vaccine trials and EBOMAN, which aimed to find strategies to accelerate vaccine manufacturing.[111] EBOVAC1 ended on 30 November 2021 and was followed by another project (EBOVAC3) also funded by IMI, which is still ongoing.[110] The data collected by the EBOVAC1 and EBOVAC2 projects supported the decision of the European Commission to grant marketing authorisations for the Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen in the EU.[106]

Chapter 2: Seroprevalence of IgG antibodies against EBOV in Sierra Leone

2.1 Seroepidemiology in Ebola vaccine research

Seroepidemiology, which measures the prevalence of antibodies (or less frequently, antigens) in serum or other fluids to study the distribution and determinants of infections in a population, is considered a powerful tool to guide the design and monitoring of vaccination programmes.[112] It is also commonly undertaken as a preliminary step to vaccine trials in order to understand the pre-existing immunity in

the population where the trial is to be conducted and, therefore, to provide a better interpretation of the trial results.

This approach was particularly relevant for the EBOVAC1 vaccine trial in Sierra Leone (EBOVAC-Salone, NCT02509494). The study was implemented in an area of the country (Kambia district) affected by widespread and prolonged EBOV transmission during the 2014-16 EVD epidemic.[30,68,113] It was likely that a proportion of the population had already been exposed to EBOV and this could have affected the evaluation of the immunogenicity of the vaccine regimen. To address this issue, EVD survivors were excluded from the trial. However, there was increasing evidence that some EBOV infections were asymptomatic or presented with milder symptoms.[114,115] Therefore, it was important to know if there were people in the trial population who had been exposed to EBOV without developing the classical symptoms of EVD and to quantify their prevalence.

The study of asymptomatic or mildly symptomatic infections also had a public health importance in Sierra Leone beyond the implementation of the vaccine trial. Although these infections were considered unlikely to transmit the virus, such a possibility had not been completely ruled out. EBOV had been detected in semen, vaginal secretions and breast milk in convalescent EVD survivors at the stage of recovery when they had no or minimal symptoms,[116] and the transmission from convalescent patients had been considered responsible for the reappearance of EVD in Liberia after more than three months without symptomatic cases.[117] Asymptomatic or mildly symptomatic infections could have also conferred protective immunity, which needed to be considered when forecasting EVD incidence rates during the epidemic and when planning outbreak control strategies, including a vaccination campaign if an effective vaccine had become available.[118]

2.2 Available evidence on EBOV seroprevalence

Previous research on the seroprevalence of ebolaviruses had produced heterogeneous findings. Ebolavirus IgG antibodies had been found in populations that had never experienced documented Ebola disease outbreaks, such as the Central

African Republic, or Cameroon, and in individuals with no history of EVD in areas with EBOV transmission.[119] A systematic review by Bower & Glynn based on 51 studies from 1961 to 2016, reported that in areas without known EVD cases at the time when the studies were conducted, ebolavirus IgG seroprevalence in the general population ranged from 0% to 24%, while in outbreak areas, among people without reported contact with EVD cases, it ranged from 1% to 17%.[119] The proportion of asymptomatic infection among contacts of EVD cases was estimated as 3.3% (95% CI 2.4-4.4) in the meta-analysis. Another systematic review summarising household transmission studies conducted between 1976 and 2015 estimated that the proportion of asymptomatic infection among EBOV-infected individuals was 27.1% (95% CI 15.0-40.0).[120]

For this analytic commentary, I have conducted a literature search for papers published after the systematic review by Bower & Glynn.[119] A PubMed search was run on 23 June 2023 using the same search string used by Bower & Glynn: Ebola AND (asymptom* OR antibod* OR IgG OR immun* OR ELISA OR serol*) NOT vacc* NOT immuniz* AND (Humans[Mesh]). The search was restricted to articles published after the systematic review search end date (1 January 2017) with no language restrictions and identified 757 records. To increase the chance of finding relevant articles, I also ran an additional search on PubMed with the search string "Ebolavirus AND (seroprevalence OR serol* OR serosur*)" for any paper published since 1961, no language restrictions, which identified 165 records. Following title/abstract screening for papers reporting data on ebolavirus seroprevalence in individuals without previous EVD diagnosis or in the general population, I found 24 new studies not already considered in the systematic review by Bower & Glynn, and one systematic review.[121] Two additional papers were identified from the reference lists of the above papers and one paper was found when the two search strategies were re-run on 11 November 2023, bringing the total number of new studies to 27. These are listed in Appendix 2.[18,122-144]

The newly identified papers confirm most of the observations already discussed by Bower & Glynn.[119] Studies are heterogeneous in regards to studied populations and

their exposure levels to EVD cases or possible sources of environmental exposures to EBOV (i.e. contact with wild animals) and were undertaken in different geographical areas and at different time points relative to reported outbreaks. They also used a wide variety of tests and cut-offs to determine seropositivity, which makes it challenging to combine or compare their results. Several studies used an Enzyme-Linked Immunosorbent Assay (ELISA), such as the commercially available Alpha Diagnostics International assay,[122-128] or an ELISA developed in-house,[129-132] or provided by a specific laboratory (i.e. Public Health Agency of Canada, Q2 Solutions Vaccine Testing Laboratory or CDC).[133-135,144] Other studies used a bead-based multiplex assay,[136-140,144] or western blot,[141] or an oral fluid anti-EBOV GP IgG capture assay.[18,142,143] Seropositivity thresholds for these tests were determined in different ways. For the Alpha Diagnostics test, a seropositivity cut-off was provided by the test manufacturer (1.0 units/mL), however, several studies used a higher threshold (2.5 or 4.7 units/mL) to be conservative.[122-124] Studies using in-house ELISA tests or the oral fluid anti-EBOV GP IgG capture assay determined seropositivity by comparing test results with the negative controls.[18,132,134,142] Some studies determined seropositivity based on simultaneous reactivity to different EBOV antigens,[136,137,144] other studies also measured neutralising antibodies.[124,127,128] Additional details on the tests and cut-offs used in the different studies can be found in Appendix 2.

Despite these limitations, some overall observations can be made:

- There is convincing evidence that EBOV can cause asymptomatic infections or infections that manifest with mild symptoms.[18,126,127,138,142,144,145] A lower infectious dose could explain a better ability of the human body to control the infection leading to fewer symptoms and this is supported by two studies that found that asymptomatic or minimally symptomatic EBOV infections were associated with a lower level of exposure to alive or deceased EVD cases (i.e. not sharing the same household or funeral attendance without direct involvement).[18,145] Subjects with minimally symptomatic or asymptomatic EBOV infections were not found to transmit EBOV to others.[18,145] However, this observation was based on a few cases and would need to be confirmed in more

detailed investigations of EBOV transmission chains with the inclusion of minimally symptomatic infections and unrecognised EVD cases.[18,145]

- Many studies have identified a level of seropositivity in the general population, including countries that have never reported an EVD outbreak or were not affected by EVD at the time of the investigation.[119,125,137,139,140] Whether this reflects waning antibodies from historical EBOV infections, which may or may not have been asymptomatic, or is a result of cross-reactivity with other infections (i.e. other ebolaviruses) is still not completely understood. Antibody seroprevalence in the general population seems to vary geographically and to increase with age in several studies.[128,132,135,137,146-149]
- Regarding risk factors for EVD transmission, several studies found that contact with EVD cases was associated with higher EBOV antibody seropositivity or concentration. In one study, seropositivity among asymptomatic contacts of EVD cases was weakly correlated with exposure level to an EVD case (i.e. direct contact with the body or body fluids).[142] In another study, seropositivity increased with participation in burial rituals and exposure to blood or vomit.[138]
- Several studies focused on HCWs and their exposure to EVD cases. In one study among 565 HCWs from Boende in DRC, the site of an EBOV outbreak in 2014, approximately 41% of HCWs were found to be reactive to at least one EBOV protein and 28% had IgG antibodies against the EBOV GP, despite never having developed EVD symptoms.[124] Interestingly, these high percentages of seroreactivity among HCWs were not confirmed in a more recent study conducted in the same area of DRC,[144] a discrepancy probably due to the different assay and seropositivity cut-off employed in this study. Using any form of PPE when interacting with a confirmed, probable, or suspect EVD case was found to be negatively associated with seroreactivity among DRC HCWs,[123] and lack of PPE use was also considered one of the reasons why community health volunteers were found more likely to be seroreactive against EBOV antigens than nurses in another study from DRC.[129]
- Several studies investigated environmental risk factors. EBOV seropositivity was found associated with visits to the forest or hunting, exposure to rodents or duikers, contact with bats and consumption of NHP meat in two studies in the

general population in DRC.[122,132] One study in Western Uganda found that miners or people living in an area close to a mine were more likely to be filovirus seropositive (IgG against SUDV, BDBV and MARV) compared to a control group living in central Uganda, away from any mining activity.[135]

2.3 The EBOVAC1 seroprevalence study

In the EBOVAC1 project, a cross-sectional study was conducted from 16 March 2016 to 29 June 2018 to assess the prevalence of IgG antibodies against EBOV GP in adults and children attending the screening visit of the EBOVAC-Salone trial. Participants were enrolled from three sites in Kambia District in Sierra Leone. Two sites were located in Kambia town and one site was located in the neighbouring community of Rokupr, a rural village on the Great Scarcies River about 15 km from Kambia town (Figure 4).

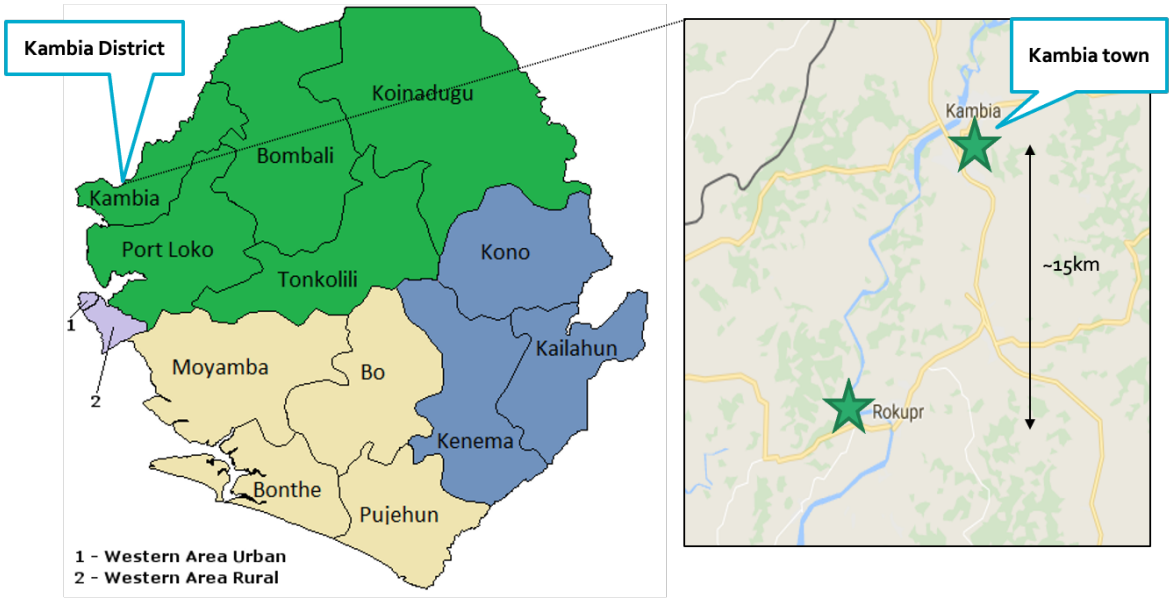


Figure 4 - EBOVAC-Salone trial and seroprevalence study sites in Sierra Leone

Source: Andyrom75

https://commons.wikimedia.org/wiki/File:Sierra_Leone_Colored_Provinces_with_Districts.png

Legend: Sierra Leone's territory divided per regions (color-coded) and districts with their name. Northern Region (green), Eastern region (blue), Southern region (beige), Western region (mauve).

The recruitment for both the trial and the seroprevalence study was age-staggered. Adults were recruited first followed by children in three age cohorts from the oldest

to the youngest age group (12-17, 4-11 and 1-3 years old). Potential participants were counselled on the importance of providing accurate medical information on previous EVD symptoms or prior vaccination with a candidate Ebola vaccine. Those who reported that they had been diagnosed with EVD in the past or had been previously vaccinated with an Ebola vaccine were considered ineligible for both the seroprevalence study and the vaccine trial. Study participants were interviewed to collect information on potential risk factors for EBOV transmission, including residence in areas where there were EVD cases during the 2014-16 outbreak, healthcare work during the outbreak, contact with EVD cases, funeral attendance, consumption of bushmeat and contact with wild animals. We also asked questions about contact with domestic animals, such as dogs and pigs, because there is evidence that EBOV can infect these species.[150-155]

IgG antibodies against EBOV GP were measured using the Filovirus Animal Non-Clinical Group (FANG) ELISA at Q2 Solutions Vaccine Testing Laboratory, USA. This test was developed and validated at the Battelle Memorial Institute in the USA.[156] Test validation was also conducted at the Q2 Solutions Laboratory and was later endorsed by the USA FDA in February 2017 (Q2 Solutions, private communication, December 2017). The assay had a lower limit of quantification (LLOQ) of 36.11 ELISA units (Eu)/ml and no established cut-off to distinguish seropositive from seronegative individuals (Q2 Solutions, private communication, September 2019). To determine seropositivity, we adopted a cut-off of >607 Eu/ml which had been calculated in a previous study using sera collected between 2004 and 2011 from 100 EBOV-naïve Malian individuals and was defined as the antibody concentration of three standard deviations (SDs) above the mean (log_{10} transformed).[157] This cut-off was considered appropriate to provide an estimate of the prevalence of IgG antibodies to EBOV GP in a West African setting like Sierra Leone. We also conducted a post-hoc analysis with an alternative cut-off calculated using sera from 388 EBOV-naïve subjects from the UK. Additional information on the study procedures is presented in the published paper and supplementary material (see portfolio Paper 1 in SECTION 2).[134]

A total of 1524 potential participants were screened for the EBOVAC-Salone trial, of whom 1315 (86.3%) consented to the seroprevalence study. Blood samples were available for 1282 (97.5%) of these participants, 687 (53.6%) of whom were children and 827 (64.5%) were male. As the FANG ELISA results were indeterminate in 10 out of the 1282 samples, the IgG seroprevalence and geometric mean concentration (GMC) estimation was based on results from 1272 participants. Among these, 684 (53.8%) had a result above the FANG ELISA LLOQ and 107 participants (8.4%, 95% CI 7.0-10.0) had a result above the prespecified seropositivity cut-off.[134]

The EBOV GP antibody concentration increased with age and was greater in participants aged five years or older than in younger children.[134] In the risk factor analysis, the only characteristic associated with seropositivity and concentration, after adjusting for age and sex, was living in a compound that had one or more pigs during the outbreak (adjusted odds ratio [OR] 4.5, 95% CI 1.6-13.0; p=0.01 for seropositivity and adjusted GMC ratio [GMR] 3.0, 95% CI 1.5-5.9; p<0.01 for antibody concentration). The post hoc analysis with the alternative cut-off calculated using sera from the UK showed similar results.[134]

2.3.1 Study strengths and limitations

The EBOVAC1 seroprevalence study had several strengths. The study was conducted in an area with prolonged EBOV transmission during the 2014-16 EVD epidemic and the sample size allowed the estimation of seroprevalence with a good level of precision. The study also explored a wide range of potential risk factors for EBOV transmission, including contact with wild and domestic animals.[134]

The study had several limitations. In normal circumstances, a serosurvey would have preceded the vaccine trial and used a random sampling technique to select participants from the study area. However, in 2015, during the EVD epidemic in West Africa, there was an urgency to find an effective vaccine against EVD and the EBOVAC1 project focused on implementing the vaccine trial as a priority. For this reason, a decision was made to embed the seroprevalence study in the screening phase of this trial. This meant that the seroprevalence study participants were not

selected as a random sample of the population in the area and this could have affected the generalisability of the results to the general population as the vaccine trial had strictly defined inclusion and exclusion criteria. Participants were also not recruited in the same period. The RCT procedures required age-staggered recruitment, meaning that the youngest age cohort (1-3 years old) was recruited last, more than two years after the end of the EVD outbreak. However, a sensitivity analysis suggested that the year of recruitment had only a negligible confounding effect on the lower EBOV GP antibody concentrations observed in the youngest children (see the Appendix of Paper 1 in SECTION 2).[134]

In our study, healthcare work, contact with EVD cases and funeral attendance, which were found to be associated with EBOV transmission or seropositivity in other studies, were not associated with EBOV antibody seropositivity or concentration.[123,124,133,138,142,158] However, because so few participants reported having these risk factors, our study may not have had the statistical power to find such a link. We do not know why these risk factors were uncommonly reported. One possible explanation is that our study was conducted at the end of the 2014-16 EVD epidemic in Sierra Leone when public health measures to contain the outbreak had been in place for several months and the population had received many messages on how to prevent EVD transmission. This could have caused an under-reporting of behaviours considered at risk. For example, hunting and consumption of bushmeat were rarely reported by our participants in contrast with some sources describing these as common practices in West Africa.[159,160] We suspect that, because the Ebola prevention campaign included specific messages about avoiding contact with wild animals and avoiding bushmeat consumption, participants may have been reluctant to admit to these practices. Another possible reason is that the study sites were situated near or on a large river, which may have resulted in the population consuming more fish and relying less on bush meat.

The association of both antibody seropositivity and concentration with pig ownership could have occurred by chance because it was based on only 18 participants who reported keeping one or more pigs in their household compound at the time of the

outbreak. The observed association could also be confounded by unrecorded risk factors or clustering of EVD-exposed participants in a household who kept pigs. In our seroprevalence study, participants' data were anonymised to avoid participants' fear of being stigmatised if they were known to be positive for EBOV antibodies. For this reason, we were not able to approach the seropositive participants who declared pig ownership to ask additional questions or to conduct further tests. However, among the 18 participants who declared having one or more pigs in their compound, the five seropositive participants did not come from the same household. In addition, none of them declared that they had been in contact with an EVD case. It is therefore unlikely that our results were due to EBOV transmission clustering in households that also had pigs.

The use of the FANG ELISA for the assessment of EBOV seroprevalence in our study also deserves some discussion. This test was considered the best option available at the time when the seroprevalence study was implemented since it had been validated and was the same assay used for the EBOVAC-Salone trial.[30,68,156] This test was also considered to be more precise and accurate than the commercially available Alpha Diagnostics test,[157] and had been previously used in Ebola vaccine trials of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen and the rVSV–ZEBOV vaccine.[50,62,63] However, positivity with this assay has been observed in samples from countries that are not known to have environmental risk factors for EBOV spillover, such as the UK or USA (Janssen private communication 2020). This suggests that the assay might not have a high specificity. In our study, we observed a background seroreactivity in approximately 50% of our participants who had a result above the LLOQ of the test and it is not clear how much the assay specificity contributed to this result.

Finally, an important limitation of the study is that we are not able to determine what the presence of EBOV GP antibodies means in this setting and whether this reflects true asymptomatic EBOV infection since we cannot exclude under-reporting of previous EVD symptoms and we have not yet investigated cross-reactivity with other infections. In addition, it is not clear whether EBOV seropositivity is a sign of

previously acquired immune memory to the EBOV GP, which might provide some protection against future EBOV infections.

2.3.2 Study contribution to knowledge

The study found an 8.4% prevalence of IgG antibodies against EBOV GP over a pre-defined threshold in healthy adults and children who reported no prior symptoms of EVD in northern Sierra Leone, near and after the end of the 2014-16 EVD epidemic. This estimate is within the range of estimates reported in other studies (1% to 17%),[119] and is similar to the baseline seroprevalence (4.0%) found in the PREVAIL Ebola vaccine trial which was conducted in Liberia during the 2014-16 EVD epidemic and used the same assay and same cut-off as our seroprevalence study.[51] The increase in EBOV GP antibody concentration with age is consistent with other studies and could be explained by older age groups being exposed to EBOV or other infections that could cause cross-reactive antibodies to the EBOV GP.[132,146-149]

Our study found a strong independent association of both EBOV GP antibody seropositivity and concentration with residence in a household compound that owned one or more pigs at the time of the outbreak. This type of association has not been found in other studies. However, the role of pigs as potential, occasional reservoirs of EBOV or other ebolaviruses, has been suggested. Pigs can be experimentally infected with EBOV and can transmit the virus to NHPs.[155] Ebolavirus-specific IgG antibodies have been detected in a few pigs in Sierra Leone and Guinea, suggesting the possibility that pigs can be naturally infected by ebolaviruses.[153,154] Pigs in the Philippines have been found to be naturally infected with RESTV, an ebolavirus that is not known to cause disease in humans. RESTV-specific antibodies were detected in healthy farmers in contact with the infected pigs suggesting potential transmission from pigs to humans.[161]

It is not clear if RESTV infection could explain our results. RESTV was believed to circulate only in Asia. However, RESTV-specific IgG antibodies were found in fruit bats and NHPs in Zambia, suggesting that this virus might circulate also in Africa.[162] Cross-reactivity of IgG antibodies between RESTV and EBOV antigens has been reported.[163]

In conclusion, the findings of the EBOVAC1 seroprevalence study suggest that the extent of EBOV infection during the 2014-16 EVD epidemic was probably higher than previously reported. It is not clear if these infections were all asymptomatic or paucisymptomatic since we cannot exclude an under-reporting of EVD symptoms. This result could also be partially due to the lack of specificity of the assay and to exposure to other infections including other ebolaviruses that generate cross-reactive antibodies. These aspects need further investigation to understand the extent, spread and dynamics of future Ebola disease outbreaks.

Chapter 3: Safety and immunogenicity of the Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen in Sierra Leone

One of the objectives of the EBOVAC1 project was to assess the safety, immunogenicity and, if feasible, efficacy of the two-dose heterologous Ad26.ZEBOV, MVA-BN-Filo vaccine regimen in Sierra Leone, a country heavily affected by the 2014-16 EVD epidemic in West Africa.

The Ad26.ZEBOV, MVA-BN-Filo vaccine regimen was developed by Janssen Vaccines & Prevention B.V. (Johnson & Johnson) and Bavarian Nordic and was based on previous technology and experience in designing vaccines for other infections. Heterologous two-dose regimens using adenovirus- and MVA-vectored vaccines had been used in clinical trials against malaria, human immunodeficiency virus (HIV), and hepatitis C, showing good tolerability and ability to induce robust humoral and cellular immune responses.[164-168]

This Ebola vaccine regimen had been shown to protect NHPs against an EBOV challenge,[104] and the EBOVAC1 project hoped to rapidly prove its safety and efficacy in humans so that it could be deployed in Sierra Leone to help control the ongoing EVD outbreak.

The EBOVAC1 project rapidly started phase 1 studies in the UK and Africa, which demonstrated the good tolerability of the Ad26.ZEBOV and MVA-BN-Filo vaccines and provided immune response data that helped to decide the best schedule and interval between doses.[59-63] A regimen with Ad26.ZEBOV as the first dose and MVA-BN-Filo as the second dose with a 56-day interval between doses induced the most rapid and robust immune response among different options considered and was chosen as the vaccine regimen for the trial in Sierra Leone (Figure 5).[59-63]

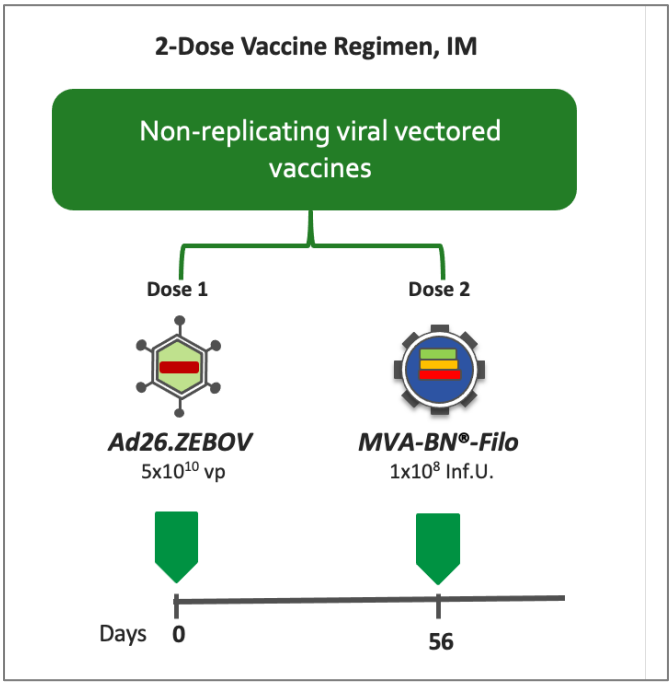


Figure 5 - Ad26.ZEBOV, MVA-BN-Filo vaccine regimen

Source: Johnson & Johnson

https://www.jnj.com/johnson-johnson-joins-world-health-organization-in-efforts-to-prevent-spread-of-ebola-in-west-africa

Legend: Ad26.ZEBOV=adenovirus type 26 vector-based vaccine encoding the Ebola virus glycoprotein. MVA-BN-Filo=modified vaccinia Ankara vector-based vaccine, encoding glycoproteins from the Ebola virus, Sudan virus, and Marburg virus, and the nucleoprotein from the Taï forest virus. IM=intramuscular, vp=viral particles, Inf.U.= infectious units.

The district of Kambia in the north of the country was selected for the study because there were no other Ebola vaccine trials ongoing in the area, there was stakeholder and community support and there was ongoing EVD transmission, which meant that the trial had a chance to assess VE. The number of EVD cases in Sierra Leone was declining at the beginning of 2015 and low numbers were expected in many districts after February 2015, except in Kambia.[169]

However, after March 2015 there was a substantial decline in EVD cases in the entire country, including Kambia district, which made it unfeasible to assess VE and this objective was, consequently, removed from the trial protocol. The study focus changed to vaccine safety and immunogenicity and the sample size in children was expanded because data in this group was limited. A subsequent protocol amendment included the provision of an Ad26.ZEBOV booster dose to a subset of previously vaccinated adults, while the administration of a booster dose to previously vaccinated children was evaluated in a subsequent study a few years later.

3.1 The EBOVAC-Salone trial (VAC52150EBL3001)

VAC52150EBL3001 or EBOVAC-Salone ('Salone' meaning Sierra Leone in Krio language), was a randomised, double-blind, controlled trial to evaluate the safety and immunogenicity of a two-dose heterologous vaccine regimen in which Ad26.ZEBOV at $5x10^{10}$ viral particles (vp) was administered as the first dose and MVA-BN-Filo at $1x10^{8}$ infectious units (Inf.U.) as the second dose 56 days later (Figure 5).

The trial comprised two stages: an open-label, non-randomised stage 1, and a randomised, double-blind, controlled stage 2 (Figure 6).

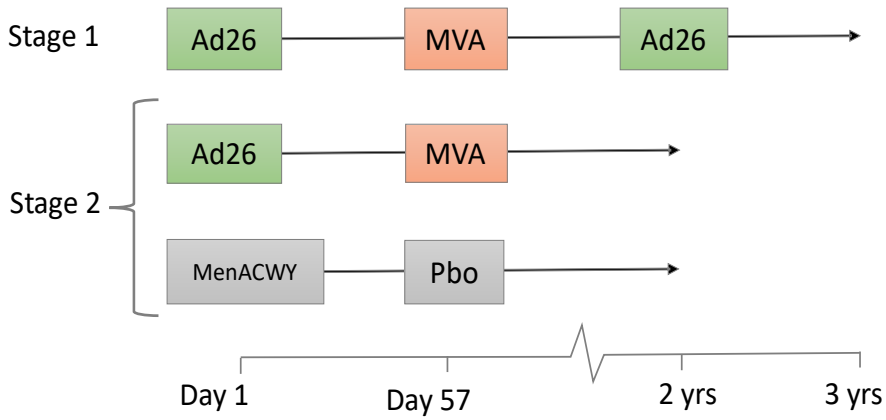


Figure 6 - EBOVAC-Salone study design

Source: Ishola et al. Lancet Infect Dis 2022; 22: 97-109.

Legend: Vaccine doses were 5×10^{10} viral particles for Ad26.ZEBOV (Ad26), 1×10^{8} infectious units for MVA-BN-Filo (MVA), 0.5 mL reconstituted vaccine solution for MenACWY, and 0.5 mL of 0.9% sodium chloride solution for the placebo (Pbo).

Stage 1 was included in the study design to obtain preliminary safety data, since the experimental vaccine regimen had never been used in Sierra Leone before and the national health authority requested the inclusion of this initial stage. Forty-three adults were enrolled in stage 1; they all received the Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen and were followed up for two years. After two years they were offered an Ad26.ZEBOV booster vaccination and those who received the booster (n=29) were followed up for an additional year. In stage 2, 400 adults and 576 children (aged 1-17 years) in three age cohorts (12-17, 4-11, 1-3 years) were enrolled and randomised (3:1) to the same Ebola vaccine regimen as in stage 1 or an active control, a meningococcal quadrivalent (serogroups A, C, W135, and Y) conjugate vaccine (MenACWY), as the first dose, followed by placebo as the second dose on day 57. Stage 2 was double-blind, meaning that the study team personnel (except those responsible for vaccine preparation) and the participants were not aware of the study vaccine allocation. Stage 2 follow-up was two years in adults and one year in children.

The primary objective of the EBOVAC-Salone trial was the evaluation of the safety and tolerability of the Ebola vaccine regimen by collecting solicited local and systemic AEs in the first seven days after each vaccination, unsolicited AEs in the first 28 days after each vaccination and SAEs until each participant's last study visit. The secondary outcomes were the evaluation of binding antibody responses 21 days after dose 2 in a per-protocol set of participants using the FANG ELISA (Q2 Solutions Vaccine Testing Laboratory, USA) and to assess the Ad26.ZEBOV booster safety and tolerability in stage 1 participants. The study also had exploratory outcomes, including the evaluation of humoral immune responses at other relevant time points and following the booster vaccination in stage 1, the assessment of neutralising antibodies (nAbs) against the EBOV GP using a pseudovirion neutralisation assay (psVNA) (Monogram Biosciences, USA) and nAbs against the Ad26 and MVA vaccine vectors using, respectively, an Ad26-specific virus neutralisation assay (Ad26 VNA, Janssen) and a plaque reduction neutralisation test (PRNT, Bavarian Nordic). Additional information on the trial methodology and procedures is available on the trial registration page in ClinicalTrials.gov (NCT02509494) and was presented in two published papers.[30,68] One of these papers is included in my PhD portfolio (see Paper 2 in SECTION 2).[30]

The Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen was well tolerated in both adults and children.[30,68] The most commonly reported solicited local AE in both groups was mild to moderate injection site pain, which occurred in up to 28%* of adults and 21%* of children after Ad26.ZEBOV vaccination and in up to 23%* of adults and 15%* of children after MVA-BN-Filo vaccination.[30,68] Solicited systemic AEs occurred in up to 54%* of adults and 36%* of children after Ad26.ZEBOV vaccination and up to 43%* of adults and 19%* of children after MVA-BN-Filo vaccination.[30,68] Headache, myalgia, fatigue, and arthralgia were the most frequently reported solicited systemic AEs in adults and were mostly mild to moderate.[30] Headache, fatigue, and chills were also frequently reported in the 12-17-year-old adolescents and in the 4-11-year-old children while fever, decreased appetite and decreased activity were the most frequently observed solicited systemic AEs in the 1-3-year-old toddlers.[68] Approximately 5% of adults and up to 10% of children reported at least one SAE during the study. None of the SAEs was considered related to the study vaccine. The percentage of participants reporting local AEs (i.e. injection site pain) was higher in the Ebola vaccine group compared to the control group but the percentages of participants reporting systemic AEs were similar between the two groups.[30,68] Among the stage 1 participants that received the Ad26.ZEBOV booster, the post-booster vaccination safety profile was not notably different to that observed after dose 1.[30]

The Ad26.ZEBOV, MVA-BN-Filo vaccine regimen was immunogenic in both adults and children (Figures 7 and 8).[30,68] EBOV GP-specific binding antibodies were observed after dose 1 vaccination and increased substantially after the second dose. At 21 days post dose 2, binding antibody responses (defined as 2.5 times the pre-dose 1 baseline value if this was positive, or 2.5 times the LLOQ if the baseline value was negative) were observed in 98% of adults and in more than 98% of children who received the Ebola vaccine regimen, across all age groups.[30,68]

* In the EBOVAC-Salone trial, percentages of participants experiencing AEs were calculated separately for Stage 1 and Stage 2 adults and for each of the paediatric age groups in Stage 2. The percentages indicated in this summary are the highest observed either between the two stages in adults or across the different age groups in children.

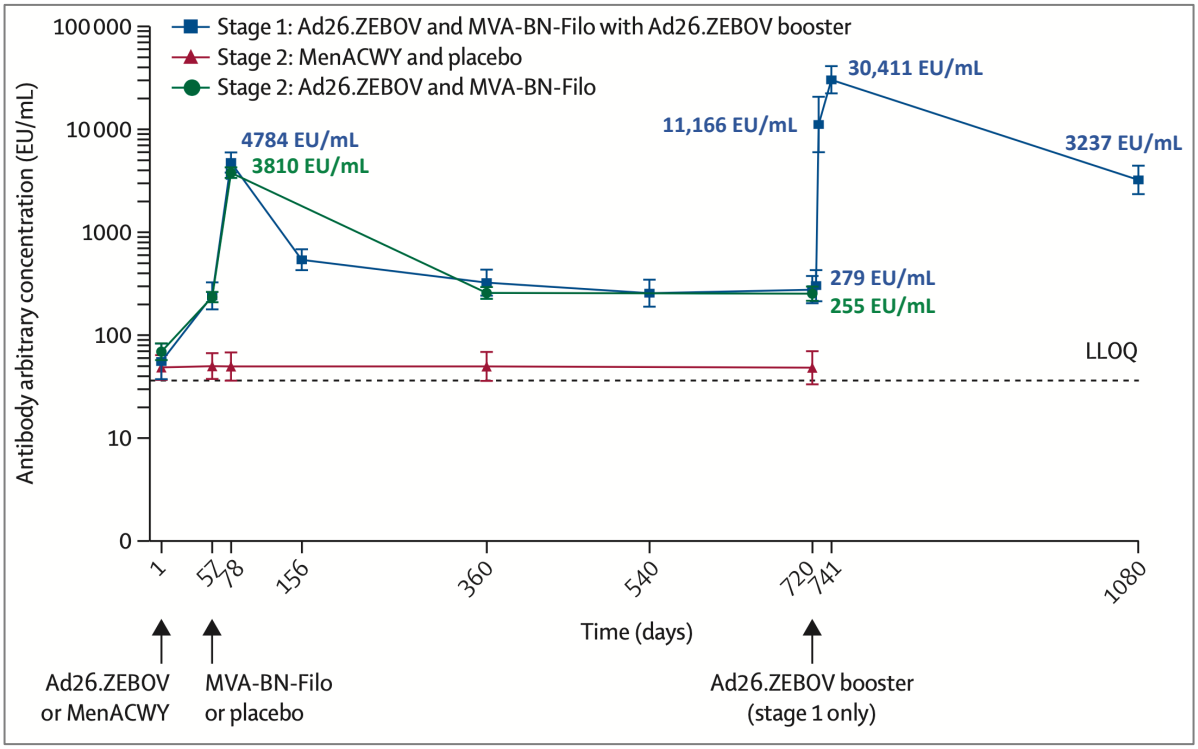


Figure 7 - EBOVAC-Salone study: anti-EBOV GP binding antibody response in adults

Source: Ishola et al. Lancet Infect Dis 2022; 22: 97-109.

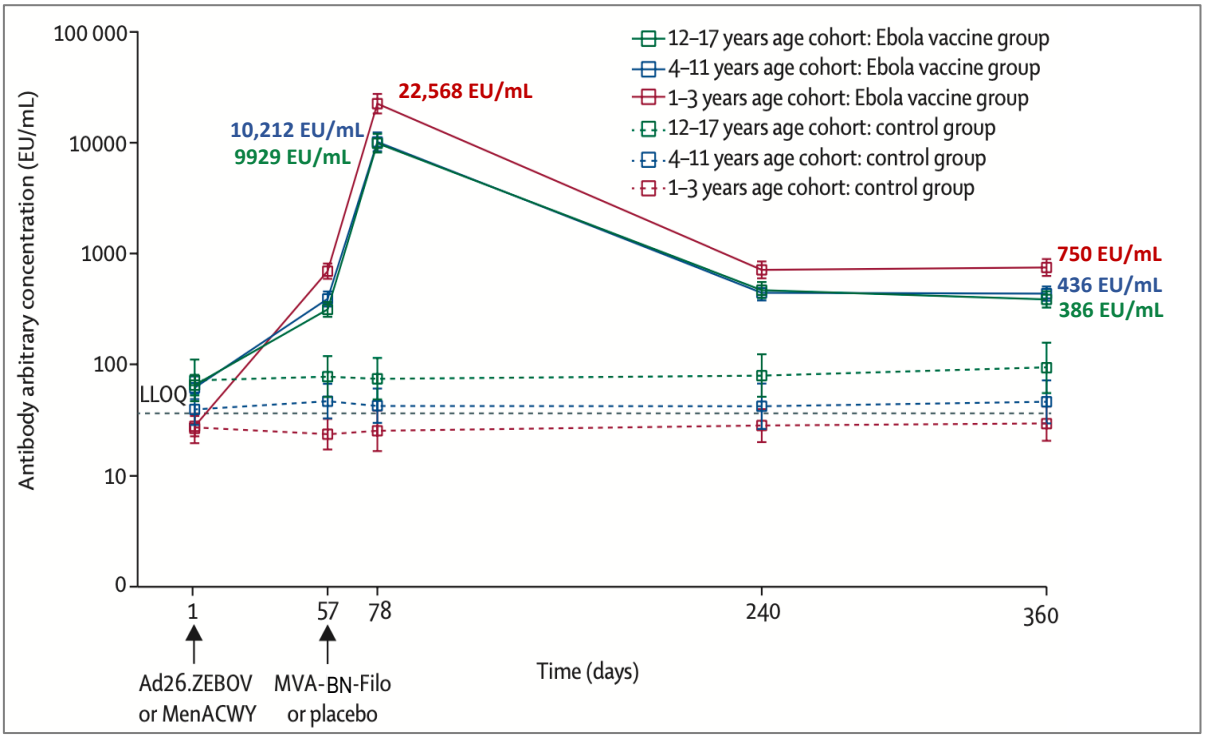


Figure 8 - EBOVAC-Salone study: anti-EBOV GP binding antibody response in children

Source: Afolabi et al. Lancet Infect Dis 2022; 22: 110–22.

Antibody GMCs decreased over time but at one year post dose 1, responses were still observed in 77% of stage 1 adults, 49% stage 2 adults, and in 70%, 71% and 96% of the children in the different age groups, 12-17 years, 4-11 years and 1-3 years old, respectively. Two years post dose 1, 68% of stage 1 adults and 50% of stage 2 adults who received the Ebola vaccine regimen were still responders. Booster vaccination with Ad26.ZEBOV in stage 1 adults induced a 40-times increase in binding antibody GMC at seven days and a 110-times increase at 21 days compared to the pre-booster GMC (Figure 7).[30] Antibody responses were detected in 96% and 100% of participants at seven and 21 days post booster, respectively.[30] Binding antibody GMC decreased at one year post booster, however, persistent responses were observed in all 26 participants still on follow-up, at a level approximately 10-fold higher than that observed at one and two years post dose 1 (Figure 7).[30]

The Ad26.ZEBOV, MVA-BN-Filo vaccine regimen also elicited nAbs in both adults and children (Figures 9 and 10).

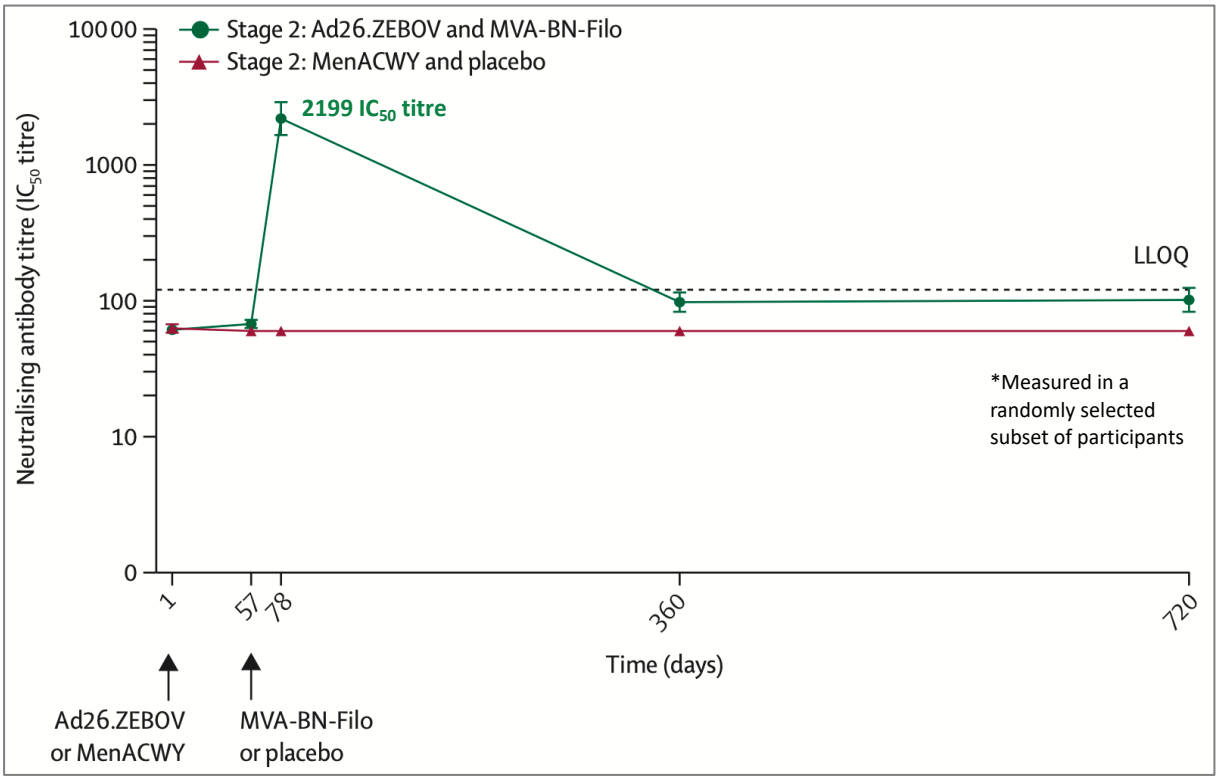


Figure 9 - EBOVAC-Salone study: anti-EBOV GP neutralising antibody response in adults

Source: Ishola et al. Lancet Infect Dis 2022; 22: 97-109.

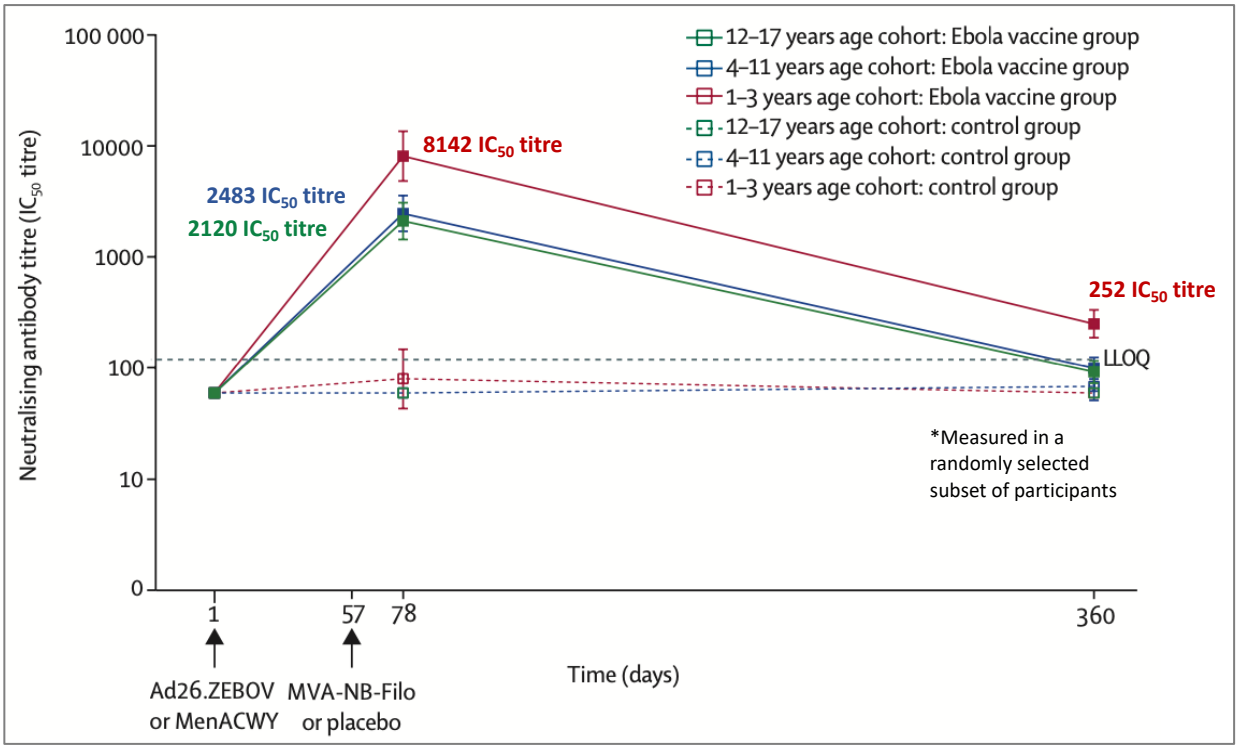


Figure 10 - EBOVAC-Salone study: anti-EBOV GP neutralising antibody response in children

Source: Afolabi et al. Lancet Infect Dis 2022; 22: 110–22.

At 21 days post dose 2, an EBOV GP-specific nAb response (defined as two times the pre-dose 1 baseline value if this was positive, or two times the LLOQ if the baseline value was negative) was detected in 98% of adults and in more than 94% of children across different age groups.[30,68] Geometric Mean Titres (GMTs) decreased over time and at one year post dose 1, nAb responses were only detected in 6% of adults, 8% of 12-17 years old, 15% of 4-11 years old and 49% of 1-3 years old. At about two years post dose 1, nAb responses were observed in 12% of adults receiving the Ebola vaccine regimen.[30,68] In general, the younger children (1-3 years old) had higher levels of binding and neutralising antibodies compared to the older age groups.[30,68]

Pre-existing Ad26-specific nAbs were detected in 93% of adults and up to 78% of children receiving Ebola vaccination, while pre-existing MVA-specific nAbs were detected in only 5% of adults and in none of the children who received Ebola vaccination.[30,68] There was a weak negative correlation between the baseline Ad26-specific nAb titres and the EBOV GP-specific binding antibody concentrations at 21 days post dose 2 in both adults (Spearman correlation coefficient [CC]=-0.145) and children (CC=-0.204).[30,68] However, pre-existing immunity against the Ad26 vector did

not seem to substantially impair the vaccine regimen immunogenicity at 21 days post dose 2 because the majority of participants were responders (>98%) at this time point.[30,68]

Some participants had antibodies against the EBOV GP at baseline on day 1, before dose 1 administration. A post hoc analysis of the EBOVAC-Salone trial data in adults found a weak positive correlation between the baseline EBOV GP-specific binding antibody concentration and the concentration of the same antibodies at 21 days post dose 2 (CC=0.104).[30] In children, a very weak negative correlation between the two measurements was found (CC=-0.084).[68] However, the baseline EBOV GP-specific binding antibodies did not seem to substantially affect the immune response to the vaccine regimen at 21 days post dose 2.[30,68]

3.2 The EBOVAC booster study in children (VAC52150EBL2011)

VAC52150EBL2011 or EBOVAC booster study in children was an open-label, non-randomised, phase 2 trial to assess the safety and immunogenicity of an Ad26.ZEBOV booster dose in children previously vaccinated with the Ad26.ZEBOV, MVA-BN-Filo regimen in the EBOVAC-Salone trial.[69]

Children were eligible for the booster study if they were considered healthy, had received the Ad26.ZEBOV, MVA-BN-Filo regimen at least two years earlier in the EBOVAC-Salone trial, and were between one and 11 years old at the time of dose 1 vaccination in the initial trial. We planned to enrol approximately 50 participants equally split between the 1-3 years and 4-11 years age cohorts of the EBOVAC-Salone trial.[69]

Safety and immunogenicity were the primary outcomes of the booster study. Safety was assessed in all participants who had received the booster vaccination by collecting solicited local and systemic AEs in the first seven days after booster vaccination and unsolicited AEs, including SAEs, for 28 days after the booster vaccination.

Binding antibody responses against the EBOV GP were assessed in all boosted participants who had at least one evaluable post-booster sample and no major protocol deviations that could have influenced the immune response at seven and 21 days after booster vaccination using the FANG ELISA. A planned exploratory outcome was the nAb response against the Ad26 vector before booster vaccination as measured by a virus neutralisation assay. Additional information on the EBOVAC booster study methodology and procedures is available on the trial registration page in ClinicalTrials.gov (NCT04711356) and was presented in one of the published papers in my PhD portfolio (see Paper 3 in SECTION 2).[69]

A total of 50 children were enrolled and received the Ad26.ZEBOV booster vaccination more than three years after their first vaccine dose in the EBOVAC-Salone trial. Of those, 27 (54%) were in the 1-3 years age cohort (aged 4-7 years at the time of screening for the booster study), and 23 (46%) were in the 4-11 years age cohort (aged 9-15 years at the time of the booster study screening).[69]

The booster was well tolerated. Injection site pain was the most common solicited local AE, reported by 18 (36%) of participants, while headache was the most common solicited systemic AE, reported by 11 (22%) of participants. No SAEs were observed during the study period.[69]

Before the booster vaccination, 40 (87%) of 46 participants with available data, were still vaccine responders at a median of 3.2 years from the time of dose 1 vaccination in the EBOVAC-Salone trial. This percentage was 96% in the 1-3 years age cohort at a median of 3.1 years from dose 1 vaccination, while in the age 4-11 years cohort the percentage of responders was 77% at a median of 3.8 years after dose 1 vaccination.[69]

The booster vaccination induced a rapid and robust increase in EBOV GP-specific binding antibodies in all participants, with GMCs that were 44 times higher at seven days post booster and 101 times higher at 21 days post booster compared to the pre-booster baseline GMC (Figure 11).[69]

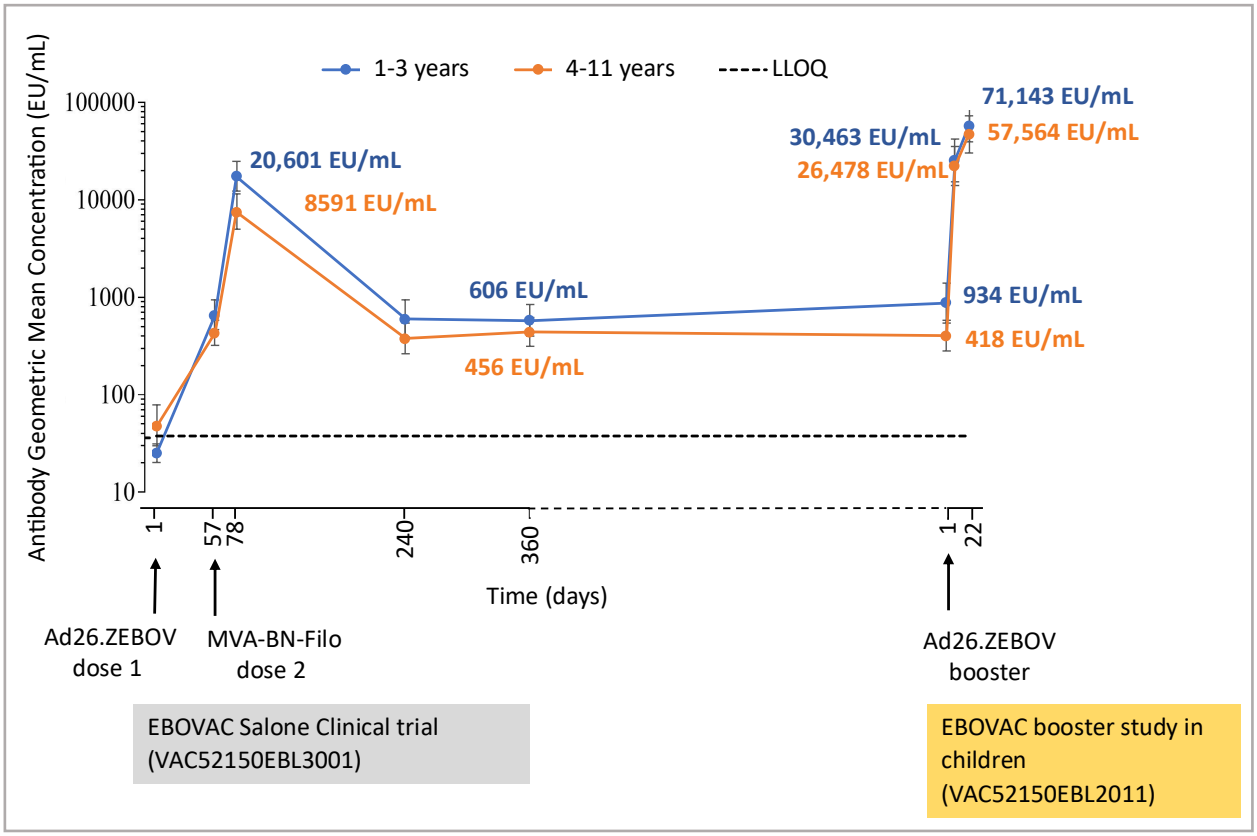


Figure 11 - EBOVAC booster study in children: anti-EBOV GP binding antibody response

Source: Manno et al. Lancet Infect Dis 2023; 23(3): 352-360.

3.3 Strengths and limitations of the clinical trials

Both the EBOVAC-Salone trial and the booster study had several strengths. They provided data on the safety and immunogenicity of a novel Ebola vaccine regimen in adults and children in an area that was affected by the 2014-16 EVD epidemic.[30,68,69] They also provided data on the durability of the immune response for up to two years in adults and up to almost four years in children and demonstrated the safety and immunogenicity of a booster dose given to previously vaccinated adults and children.[30,69]

Both studies also had some limitations. The EBOVAC-Salone trial, as previously explained, was initially designed to evaluate vaccine efficacy but due to the decline of the EVD epidemic, the study design and outcomes were changed to focus only on safety and immunogenicity.[30] The study also had the limitation that pregnant and breastfeeding women were excluded.[30] This meant that the safety of the vaccine

could not be assessed in this population, which is among the most vulnerable during EVD outbreaks.[170] Women were also difficult to recruit due to the contraception requirements of the trial and local socioeconomic and cultural factors. This caused a gender imbalance among the adult participants.[30]

The main limitation of the EBOVAC booster study was the short post-booster follow-up due to the end of the EBOVAC1 grant.[69] This did not affect the assessment of short-term safety up to 28 days post booster, which is the standard period for the evaluation of this outcome after vaccination, but limited the evaluation of long-term safety. However, in clinical trials in which an Ad26.ZEBOV booster dose was given to adults who were followed up for longer periods, such as EBOVAC-Salone and the VAC52150EBL2002 study in Kenya, Burkina Faso, Côte d'Ivoire and Uganda, none of the SAEs was considered related to the Ad26.ZEBOV booster dose.[30,65] On the other hand, all the SAEs that were considered related to the Ad26.ZEBOV vaccination in children in the UMURINZI campaign in Rwanda, such as febrile convulsions, diarrhoea and/or vomiting, occurred within 24 hours of vaccine administration.[84] We did not observe any of these SAEs in our studies, however, the number of 2-8-year-old children in UMURINZI was more than 20,000 and the incidence of these SAEs was small (e.g. 0.031% for febrile seizure),[84] which means that it was very unlikely that any of these SAEs would be seen in our studies which had a sample size of a few hundred children.[68,69]

The short follow-up period also meant that the study could not assess the long-term immunogenicity of the booster dose in children. In the previous trials mentioned above, adults followed for one year after receiving the Ad26.ZEBOV booster had binding antibody GMCs at this time point that were greater than one year after the initial Ad26.ZEBOV, MVA-BN-Filo vaccine regimen administration.[30,65] Since the children in our study had binding antibody responses similar to that previously observed in adults at seven and 21 days post booster, their binding antibody kinetics likely resembled those of adults also at later time points and their antibody GMCs were maintained at higher levels than after the initial Ad26.ZEBOV, MVA-BN-Filo vaccine regimen for at least one year post-booster vaccination.[30]

A limitation of both the EBOVAC-Salone trial and the booster study in children was that the assessment of EBOV GP nAbs was not done after booster vaccination.[30,69] This happened because the laboratory performing the nAb analysis (Monogram, USA) required that samples were negative for EBOV polymerase chain reaction (PCR) before sample shipment and this test was not performed at the beginning of the EBOVAC-Salone trial in stage 1 participants.[30] The end of the EBOVAC1 grant also meant that nAbs could not be assessed in the booster study in children.[69] However, in previous clinical trials, and NHP challenge studies, titres of nAbs strongly correlated with the concentration of binding antibodies after the initial Ad26.ZEBOV, MVA-BN-Filo vaccine regimen administration. Therefore it is plausible that nAbs mirrored the increase of binding antibodies also after booster vaccination.[30,68,104,171] Binding antibodies were also correlated with NHP survival in challenge studies and were selected as the immune parameter to support the Ad26.ZEBOV and MVA-BN-Filo vaccines licensure in the EU.[104,171]

The evaluation of cellular immune responses was not performed in either study because we were unable to establish the processing of peripheral blood mononuclear cells (PBMC) in the laboratory in Sierra Leone within the EBOVAC1 project timeframe.[69] Cellular immune responses after vaccination with the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen have been described in previous studies in adults and children, but data in children are limited.[59,62,63,65,66] An ongoing study (VAC52150EBL2012, NCT05284097) aims to collect further data on cellular immune responses in children in Sierra Leone.

3.4 Contribution to knowledge of the EBOVAC-Salone trial and the EBOVAC booster study in children

The EBOVAC-Salone trial demonstrated that the Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen was well tolerated and immunogenic in both adults and children.[30,68] These results supported the decision of the European Commission to grant marketing authorisation, under exceptional circumstances, for this vaccine regimen in the EU for EBOV infection prophylaxis in adults and children aged one year or older.[106] In the absence of data on VE, the licensure of the Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine

regimen was based on the results of a statistical approach called 'immunobridging', in which the likelihood of protection induced by vaccination in people was inferred by correlating the magnitude of immune responses in vaccinated individuals in the EBOVAC-Salone and other similar trials with those observed in vaccinated NHPs in EBOV challenge studies.[171,172]

The EBOVAC-Salone trial and the booster study also demonstrated that a booster vaccination with Ad26.ZEBOV, given two or three years after the initial vaccination, induced a robust anamnestic response within seven days in both adults and children.[30,69] This finding is important for the prophylactic use of this vaccine regimen and supports the strategy of giving an Ad26.ZEBOV booster to previously immunised individuals at risk of EVD transmission (i.e. HCWs involved in the response to an EVD outbreak). This possibility was included in the marketing authorisation of the vaccine regimen on the basis of the results of the EBOVAC-Salone trial.[106]

Chapter 4: The effect of malaria on the immune response to the two-dose Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen

All EVD outbreaks to date have occurred in Sub-Saharan Africa (Table 1) and vaccines against EVD will likely be used mainly in this region. Sub-Saharan Africa also carries the highest share of the global burden of malaria. The WHO estimated that 95% of all malaria cases and 96% of all malaria deaths in 2021 occurred in the African region.[173]

Malaria infection impairs the immune response to some vaccines, such as tetanus toxoid and typhoid,[174] *Hemophilus influenzae* type b conjugate vaccine,[175] and meningococcal polysaccharide vaccine.[176,177] However, there was no evidence that malaria infection affected the immunogenicity of some other vaccines, such as the HPV-16/18 virus-like particle AS04-adjuvanted vaccine.[178,179]

At the time of the implementation of the EBOVAC-Salone trial in 2015, it was deemed important to study the effect of malaria on the immunogenicity of the Ebola vaccine

regimen because Kambia district, where the trial was taking place, is among the areas with the highest prevalence of malaria in children under the age of five in Sierra Leone.[180,181] When the results of the trial became available, the antibody concentrations achieved 21 days after dose 2 of the Ebola vaccine regimen, appeared to be lower in Sierra Leone compared to other countries (Appendix 3). In trials that enrolled both adults and children, antibody concentrations 21 days after the second dose were higher in young children compared to older children and adults (Appendix 3). Exposure to malaria infection was proposed as one of the possible reasons for this geographical variation and difference in antibody response by age.[180,181]

4.1 The EBOVAC malaria study

A cohort study was nested within the EBOVAC-Salone trial to investigate the effect of malaria infection on the immune response after vaccination with the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen. All participants in Stage 2 of the EBOVAC-Salone trial,[30,68] were invited to take part in the malaria study on the day of their dose 1 vaccination. However, after the data unblinding at the end of the trial, only participants who received the Ebola vaccine regimen and consented to participate in the malaria study were included in the data analysis.

Exposure to malaria infection was considered in three ways: 1) exposure to malaria before vaccination, 2) exposure to malaria at the time of vaccination and 3) exposure to malaria after vaccination but before assessment of vaccine immunogenicity (Figure 12).[182]

These three different components were assessed as follows:

1) Exposure to malaria before vaccination was based on participants' antibody responses to six *Plasmodium falciparum* (*P. falciparum*) recombinant antigens in samples collected at the trial screening visit using a Luminex MAGPIX quantitative suspension array technology (qSAT).[183,184] The following antigens were considered the most suitable to ascertain previous malaria infection according to previous research:[183] apical membrane antigen 1 (AMA-1), merozoite surface protein 1.19 (MSP-1.19), and glutamate-rich protein R2 region (GLURP.R2), indicative of long-term

exposure to malaria; reticulocyte-binding protein homologue (Rh2.2030), gametocyte exported protein (GEXP18), and early transcribed membrane protein (Etramp5.Ag1), indicative of recent exposure to malaria (i.e. infection in the past nine months). The antigens indicative of long-term exposure to malaria (AMA-1, MSP-1.19 and GLURP.R2) were selected because they are important targets of immunity against malaria. Both AMA-1 and MSP-1.19 are proteins expressed by merozoites, the asexual blood-stage form of malaria parasites, and play a critical role in their ability to invade erythrocytes and grow inside the infected cells.[185,186] Antibodies against these antigens are known to interfere with malaria blood-stage replication and are found in people with long-term exposure to malaria.[186-188] The glutamate-rich protein (GLURP) is an antigen expressed by mature schizont-infected erythrocytes and elicits antibodies able to suppress malaria parasite growth in vitro in the presence of monocytes.[189] Antigens indicative of recent malaria exposure (Rh2.2030, GEXP18, Etramp5.Ag1) were originally identified by studying the antibody response of children after *P. falciparum* infection in Papua New Guinea and Uganda.[190,191] In our malaria study, antibody responses to each of these six antigens were used to calculate an age-adjusted cumulative quartile score, which was then used to categorise participants into high-exposure, intermediate-exposure and low-exposure to malaria infection before dose 1 vaccination.[182]

2) Malaria infection at vaccination was measured using light microscopy on samples collected on the day of dose 1 vaccination. However, since the presence of fever was a contraindication to vaccination in the EBOVAC-Salone trial, the study could only evaluate asymptomatic infection.[192] Participants with clinical malaria were not vaccinated until malaria was treated.[192]

3) To assess malaria after vaccination, we considered episodes of symptomatic malaria, AEs or SAEs, recorded in the EBOVAC-Salone trial after each vaccination. In this trial, malaria was the most frequent unsolicited AE in all age groups and the most frequent SAE in the 1-3-year-old children.[30,68] Diagnosis of malaria was based on clinical symptoms and positivity to a rapid diagnostic test (RDT; i.e. First Response Malaria Ag. pLDH HRP2 Combo Rapid Diagnostic Test, Premier Medical Corporation Private Limited, Mumbai). All participants with clinical malaria were treated with a

course of age-appropriate antimalarial drugs following national malaria treatment guidelines in Sierra Leone.[182,193] Additional information on the malaria cohort study methodology and procedures have been presented in two published papers.[182,192] One of them is included in my PhD portfolio (see Paper 4 in SECTION 2).

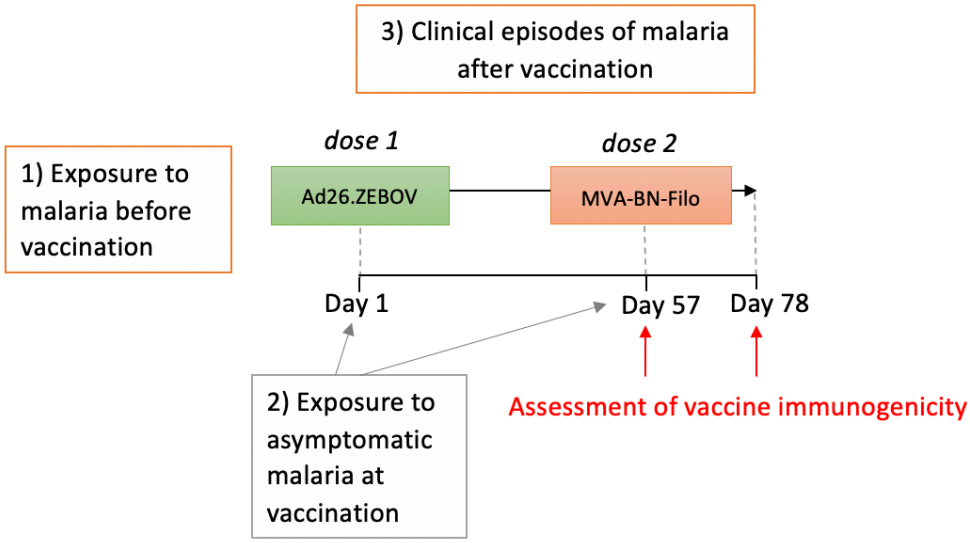


Figure 12 - EBOVAC malaria study conceptual framework

Source: Manno et al. Vaccines 2023, 11, 1317. https://doi.org/10.3390/vaccines11081317

A total of 587 participants were included in the malaria study, of whom 188 (32.0%) were adults aged ≥18 years, and 399 (68.0%) were children (125 aged 1-3 years, 133 aged 4-11 years, and 141 aged 12-17 years).[182,192] The effect of previous exposure to malaria at screening could only be evaluated in 474 (80.7%) participants who had an available serum sample for the Luminex analysis.

The study found no evidence that the EBOV GP binding antibody GMC post dose 1 (day 57) and post dose 2 (day 78) differed between different categories of previous exposure to malaria.[182] A similar result was obtained in a secondary analysis in which participants were ranked separately for their responses to the long-term exposure antigens (AMA-1, MSP-1.19, GLURP.R2) and antigens indicative of recent malaria infection (Rh2.2030, GEXP18, Etramp5.Ag1).[182]

Post-dose 1 GMC was lower in 1-3-year-olds with asymptomatic malaria compared with malaria-negative children (age-group-specific GMR=0.56, 95%CI=0.39-0.81) but this was not seen in older age groups.[192] Asymptomatic malaria had no consistent effect across age groups post dose 2.[192]

After adjusting for age, there was evidence that participants with at least one clinical malaria episode during the 56 days post dose 1 had lower post-dose 1 GMC (GMR=0.82, 95%CI=0.69-0.98) compared to participants without clinical malaria in the same period.[182] However, post-dose 2 GMC measured at 21 days post dose 2 was not reduced in participants who experienced clinical malaria between dose 1 and this time point.[182]

4.1.2 Study strengths and limitations

The EBOVAC malaria cohort study had several strengths, such as the inclusion of participants of different ages, including young children, since malaria is known to affect children more than older age groups. The use of a conceptual framework (Figure 12) was a useful tool to guide the analysis of data and to break down the different pathways in which malaria could affect vaccine immunogenicity.[182]

The study also has some limitations. Serum samples were no longer available from the EBOVAC-Salone trial for the Luminex analysis and, therefore, we had to use serum samples obtained for the EBOVAC1 Ebola seroprevalence study (see Chapter 2).[134] Since this study was not linked directly to the malaria study, we utilised a matching algorithm based on participants' characteristics, such as date of birth, age, sex and clinic number, to identify available serum samples, which could only be retrieved in 81% of the study participants.[182]

The assessment of clinical episodes of malaria also had some limitations. Since in the EBOVAC-Salone trial AEs were collected up to 28 days after each vaccine dose, while only SAEs were collected throughout the study, episodes of non-serious clinical malaria were not recorded from day 30 to day 56 after dose 1, which could have caused a misclassification of participants who had a non-serious malaria infection in that time interval. This misclassification, if present, is likely to have occurred

randomly and independently from the assessment of vaccine immunogenicity and this could have resulted in a dilution of the effect of clinical malaria episodes on vaccine immunogenicity.[194]

Another drawback to the study is that the diagnosis of clinical episodes of malaria was based on RDT positivity, which in a population with a high background of malaria infection, as the one in our study, may last for several weeks after recovery from malaria. This means that our participants could have tested positive even if malaria was not the immediate cause of their illness and this could have caused an overestimation of clinical malaria episodes. However, the RDT positivity still indicated recent malaria infection and therefore recent exposure to malaria.

The association between asymptomatic malaria infection at dose 1 vaccination and the lower concentration of EBOV GP binding antibodies noted in the younger children could also be due to unrecorded confounding factors such as other conditions that affect vaccine immune response, e.g. malnutrition.

Finally, as previously mentioned, since the presence of fever was a contraindication to vaccination in the EBOVAC-Salone trial, we were unable to assess whether the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen was equally immunogenic in people with symptomatic malaria infection at vaccination, a situation that could happen outside a clinical trial, particularly during mass vaccination in response to an ongoing EVD outbreak.

4.1.3 Study contribution to knowledge

The EBOVAC malaria cohort study was the first study to assess the effect of malaria on the immune response to the Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen. Its results showed that exposure to malaria infection did not substantially affect the immunogenicity of the vaccine regimen.

These results are in agreement with those of a different study carried out in adults in Sierra Leone, which demonstrated that asymptomatic malaria infection at vaccination did not affect the immune responses to rVSV-ZEBOV.[195] This vaccine was

also shown to be effective in preventing EVD in Guinea and DRC, which are malaria-endemic countries.[41,42,88]

Our results and the other study results are reassuring because they confirm that both vaccines are suitable for EVD prophylaxis in areas where malaria is highly endemic and where the vaccines may be most used in the future.[182,192,195]

Chapter 5: Discussion

Ebola disease is a severe infection by one of four ebolaviruses (EBOV, SUDV, BDBV and TAFV). These viruses are part of the Filoviridae family, which also includes MARV, a virus that shares with them many similarities in terms of epidemiology, transmission and clinical features. Since its discovery in 1976, Ebola disease had been mostly neglected until a large epidemic in West Africa in 2014 captured the world's attention.

Ebola disease is rare with fewer than 40,000 reported cases in 32 outbreaks.[12] However, the large outbreak in West Africa and the following one in North Kivu and Ituri provinces in DRC between 2018 and 2020 showed that, in particular settings with weak surveillance systems and poor health infrastructure, the viruses can transmit uncontrolled for months or years claiming thousands of lives. The response to the outbreak in DRC was also complicated by the fact that the area where the epidemic occurred was affected by armed conflicts and there was a high community mistrust, which resulted in Ebola treatment centres being attacked and healthcare workers being injured or killed.[12,196] Unfortunately, these challenging situations occur in other African regions at potential risk of ebolavirus outbreaks.[197]

It has been estimated that the area at risk of future transmission from the animal reservoirs extends over 22 African countries and is inhabited by 22 million people.[197] However, the number of countries and people at potential risk of future outbreaks is probably much larger because human-to-human transmission after the start of an outbreak spreads the virus to other areas that are distant from the point of origin, as

happened in the 2014-16 West African epidemic when the disease quickly reached the capital cities of Guinea, Sierra Leone and Liberia, and was exported to other neighbouring countries including Nigeria, Mali and Senegal.[198]

There is the possibility that Ebola disease outbreaks will be less rare in the future since modelling work predicts a 1.75 to 3.2-fold increase in the endemic rate of animal-human virus spillover in Africa by 2070.[14] Moreover, as large outbreaks leave a large pool of survivors, there is the possibility that future outbreaks may also be caused by the reintroduction of the virus from persistently infected survivors,[15] as was demonstrated for two recent outbreaks, which were genetically linked to survivors infected during previous outbreaks.[7,16]

Large outbreaks can have a devastating effect on the affected communities, not only due to the number of lives lost to the disease, a great proportion of these being among the healthcare workforce, but also due to other diseases or health conditions that are neglected during outbreaks, like vaccine-preventable diseases in childhood or emergency obstetric and neonatal care.[26] A study that considered all these aspects when calculating the economic and social burden cost of the 2014-16 EVD epidemic estimated a cumulative cost of over 50 billion USD.[26]

In light of these considerations, it is an absolute priority for countries previously affected by Ebola disease or at risk of future outbreaks, to adequately prepare for the next outbreak.

Vaccines play an important role in preparedness plans because they can be used in anticipation of outbreaks. For example, vaccination of HCWs and other high-risk groups (traditional healers, pharmacists, taxi drivers, etc.) before the occurrence of an outbreak has been proposed as an effective strategy to contain outbreaks.[12] HCWs or traditional healers are at high risk of infection before the outbreak is detected and EVD outbreaks can initially be amplified by nosocomial transmission.[199] Once an outbreak occurs, vaccines are also an integral part of the response effort to protect healthcare and frontline workers involved in the control activities or for ring vaccination of contacts of cases and contacts of contacts to prevent disease spread.

In response to the largest outbreak of Ebola disease in West Africa in 2014-16 caused by EBOV, the EBOVAC1 project conducted several clinical trials of a two-dose heterologous vaccine regimen combining the Ad26.ZEBOV and MVA-BN-Filo Ebola vaccines and provided important data that supported the marketing authorisation of these vaccines in the EU in 2020.[106] The EBOVAC-Salone trial (VAC52150EBL3001) was particularly relevant because it was conducted in a country affected by the EVD epidemic, recruited a relatively large number of adults and children and also evaluated the safety and immunogenicity of a booster dose in adults.[30,68] The data from this trial helped to guide the European Medicines Agency (EMA) indication for the use of this vaccine regimen, which is the prevention of EVD in adults and children ≥1 year of age with the possibility of an Ad26.ZEBOV booster in people who are at imminent risk of EBOV infection and have received the Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen more than four months earlier.[200] The results of the EBOVAC booster study in children (VAC52150EBL2011) supported the strategy of providing the additional Ad26.ZEBOV booster to previously immunised children.[69]

Requiring two doses, the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen is not an ideal regimen for ring vaccination. The rVSV-ZEBOV, which is a single-dose vaccine and proven to be efficacious during ring vaccination, is the vaccine of choice for this approach.[42] However, the Ad26.ZEBOV, MVA-BN-Filo regimen could be used for prophylactic immunisation of HCWs and other high-risk groups in endemic areas, as this is an effective strategy to prevent amplification of EVD outbreaks, as explained before. A vaccination campaign with the Ad26.ZEBOV, MVA-BN-Filo regimen targeting HCWs and other high-risk occupational groups was implemented in 2021 in areas of Sierra Leone neighbouring Guinea, where there was an ongoing EVD outbreak.[201] Vaccination could be offered regularly to HCWs likely to encounter cases of EVD to account for the frequent staff turnover and considered an occupational requirement such as other vaccinations like hepatitis B.[12]

The administration of a booster dose after the initial vaccine regimen is also important. NHP studies have shown that animals vaccinated 1.5 years before with the Ad26.ZEBOV, MVA-BN-Filo regimen were not protected against EBOV inoculation

despite having some level of binding antibodies, while animals who received an Ad26.ZEBOV booster dose before the EBOV challenge were protected.[202] In humans, binding antibodies have been shown to wane over six months after the second dose and then to reach a plateau for at least 3.8 years.[202] It is possible that in humans the persisting binding antibodies could confer some protection, considering that the EVD incubation time is longer and the progression of the disease is slower than in EBOV-challenged NHPs,[202] but this is not proven and, based on current knowledge, it would be safer to provide at least one booster after the initial vaccine regimen.

The Ad26.ZEBOV, MVA-BN-Filo regimen could also be used for mass vaccination of the general population living in proximity to an outbreak area and thus at potential risk of EVD transmission.[12] During the 2018-20 EVD outbreak in North Kivu and Ituri provinces in DRC, WHO SAGE recommended this regimen for vaccination of lower-risk populations,[108] and a clinical trial (DRC-EB-001, NCT04152486) was implemented following these recommendations in DRC.[203] During the same outbreak, the UMURINZI campaign implemented in Rwanda in areas bordering DRC, showed that 94% of the adults and children who received their first dose returned for their second dose, confirming the feasibility of a two-dose regimen administration during mass vaccination campaigns.[84] The UMURINZI project did not include pregnant women, but this group was included in the DRC-EB-001 trial and must be included in future campaigns considering their vulnerability during EVD outbreaks.[170,203] Data on the safety of the Ad26.ZEBOV, MVA-BN-Filo regimen in pregnant women and their newborns will be available from the DRC-EB-001 study and a recently completed RCT in Rwanda (NCT04556526).[204]

After an outbreak is over, the use of the Ad26.ZEBOV, MVA-BN-Filo regimen for vaccination of sex partners of male survivors could also be considered to prevent sexual transmission.[12]

Beyond these uses, it is unlikely that the vaccine regimen will be included in routine vaccinations of the general population outside outbreak conditions.[12] Countries at risk of EVD outbreaks have some of the most under-resourced healthcare systems in the world and resources would probably be spent better on tackling more common

health issues, such as malaria or other vaccine-preventable diseases with a higher burden of morbidity and mortality. More research is needed to better understand the ecology of ebolaviruses, which would allow spatio-temporal predictions of EVD risk in order to consider a more targeted use of vaccines for the general population.[12]

An attractive solution for Ebola disease prophylaxis outside outbreak conditions would be vaccination with a multivalent vaccine covering different ebolaviruses, which in case of an outbreak, could be boosted with a monovalent vaccine against the virus causing the outbreak. An Ad26.Filo vaccine encoding the GPs of EBOV, SUDV, and MARV, in a two-dose regimen with MVA-BN-Filo, has shown a robust immune response against EBOV, but suboptimal responses against SUDV and MARV GPs,[71] and, unfortunately, will not be developed further (Janssen private communication 2023). The development of a multivalent ebolavirus or filovirus vaccine deserves further research.

It is also important to consider that vaccines do not work in isolation but are only one of multiple interventions needed to contain outbreaks. Measures such as early identification of cases, contact tracing, safe burials, communities' awareness of transmission risks and their cooperation in the outbreak control effort are paramount. As the 2018-20 EVD outbreak in North Kivu and Ituri provinces of DRC has demonstrated, if these measures are not implemented properly, the availability of a vaccine is not enough to control the spread of the disease. It is therefore important that countries at risk of future Ebola disease outbreaks have emergency response plans and resources that include all the pillars of outbreak control response.[205]

In conclusion, the EBOVAC1 project has advanced knowledge about vaccination against EVD and has supported the licensure of a vaccine regimen against the disease. I believe the work I have done in collaboration with colleagues has contributed to some of these advances, providing information about the safety and immunogenicity of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen and of an Ad26.ZEBOV booster in previously vaccinated adults and children. My and my team's work has also provided insight into the seroprevalence of EBOV antibodies in Kambia district and the effect

of malaria on the immunogenicity of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen. I hope that the knowledge acquired will inform the use of this vaccine regimen alongside outbreak preparedness plans to better respond to future outbreaks.

SECTION 2: RESEARCH PUBLICATIONS

Paper 1: Ebola Virus Glycoprotein IgG Seroprevalence in Community Previously Affected by Ebola, Sierra Leone

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	150330	**Title**	Dr
First Name(s)	Daniela		
Surname/Family Name	Manno		
Thesis Title	Testing a prophylactic vaccine regimen against Ebola virus disease in Sierra Leone: vaccine safety, immunogenicity and factors affecting immunogenicity		
Primary Supervisor	Professor Deborah Watson Jones		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	Emerging Infectious Diseases		
When was the work published?	March 2022		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	PhD by Prior Publication		
Have you retained the copyright for the work?*	**Yes**	Was the work subject to academic peer review?	**Yes**

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	

Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I finalised the protocol and supervised the study conduct. I drafted the statistical analysis plan and conducted the statistical analysis in collaboration with the study statistician, Philip Ayieko. I am the first and corresponding author of the paper. I drafted the manuscript and I was responsible for communication with the journal during the manuscript submission, peer-review and publication process.

SECTION E

Student Signature	
Date	

Supervisor Signature	
Date	

RETENTION OF COPYRIGHT EVIDENCE

Paper 1: Ebola Virus Glycoprotein IgG Seroprevalence in Community Previously Affected by Ebola, Sierra Leone

Emerging Infectious Disease Journal: copyright policy

https://wwwnc.cdc.gov/eid/about/general

Open Access and Usage/Copyright
Emerging Infectious Diseases is an open access journal published by the Centers for Disease Control and Prevention, a U.S. Government agency. The journal is a member in good standing of the Directory of Open Access Journals [External Link].
All materials published in *Emerging Infectious Diseases*, including text, figures, tables, and photographs, are in the public domain and can be reprinted or used without permission with proper citation. All content is freely available without charge to the user or their institution. In accordance with the Budapest Open Access Initiative [External Link] definition of open access, users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author. Because the journal is in the public domain, its usage policy also conforms to conditions set forth by Creative Commons [External Link]. *Emerging Infectious Diseases* does request a proper citation be included for its content and that any changes made to any content be clearly indicated. Suggested citation wording is included with each article. ***Emerging Infectious Diseases*** **grants authors permission to self-archive their articles without fees or permission and grants institutions permission to preserve a second copy of articles published by their researchers in the institutional repository.**

Ebola Virus Glycoprotein IgG Seroprevalence in Community Previously Affected by Ebola, Sierra Leone

Daniela Manno, Philip Ayieko, David Ishola, Muhammed O. Afolabi, Baimba Rogers, Frank Baiden, Alimamy Serry-Bangura, Osman M. Bah, Brian Köhn, Ibrahim Swaray, Kwabena Owusu-Kyei, Godfrey T. Otieno, Dickens Kowuor, Daniel Tindanbil, Elizabeth Smout, Cynthia Robinson, Babajide Keshinro, Julie Foster, Katherine Gallagher, Brett Lowe, Macaya Douoguih, Bailah Leigh, Brian Greenwood, Deborah Watson-Jones

We explored the association of Ebola virus antibody seropositivity and concentration with potential risk factors for infection. Among 1,282 adults and children from a community affected by the 2014–2016 Ebola outbreak in Sierra Leone, 8% were seropositive for virus antibodies but never experienced disease symptoms. Antibody concentration increased with age.

Ebola virus (EBOV) antibodies have been found in populations that have never experienced documented Ebola outbreaks and in persons who reported no history of Ebola virus disease (EVD) (*1*). The clinical significance of these findings is unknown. We conducted a cross-sectional study in healthy adults and children from a population affected by the 2014–2016 EVD outbreak in Sierra Leone and explored the association of antibody seropositivity and concentration with potential risk factors for EBOV infection.

Author affiliations: London School of Hygiene and Tropical Medicine, London, UK (D. Manno, P. Ayieko, D. Ishola, M.O. Afolabi, F. Baiden, B. Köhn, K. Owusu-Kyei, G.T. Otieno, D. Kowuor, D. Tindanbil, E. Smout, J. Foster, K. Gallagher, B. Lowe, B. Greenwood, D. Watson-Jones); Mwanza Intervention Trials Unit, National Institute for Medical Research, Mwanza, Tanzania (P. Ayieko, D. Watson-Jones); University of Sierra Leone College of Medicine and Allied Health Sciences, Freetown, Sierra Leone (B. Rogers, A. Serry-Bangura, O.M. Bah, I. Swaray, B. Leigh); Janssen Vaccines and Prevention, Leiden, the Netherlands (C. Robinson, B. Keshinro, M. Douoguih)

DOI: https://doi.org/10.3201/eid2803.211496

The Study

We conducted a seroprevalence study in Kambia District, Sierra Leone, during March 2016–June 2018. We nested the study within the screening visit of the EBOVAC-Salone (https://www.ebovac.org) randomized controlled trial (RCT), which evaluated the safety and immunogenicity of the 2-dose Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen (ClinicalTrials.gov, no. NCT02509494) (*2*,*3*). Persons who reported having a previous EVD diagnosis and persons who previously received a candidate Ebola vaccine were ineligible for the RCT, and we excluded them from the seroprevalence study. We recruited adults first, then recruited children in 3 age cohorts: 12–17, 4–11, and 1–3 years of age.

We measured IgG to EBOV glycoprotein (GP) by using the Filovirus Animal Non-Clinical Group (FANG) ELISA (Q2 Solutions Vaccine Testing Laboratory, https://www.q2labsolutions.com). We determined seropositivity by using a cutoff of >607 ELISA units (EU)/mL, which was calculated previously in an EBOV-naive population in West Africa (*4*) (Appendix, https://wwwnc.cdc.gov/EID/article/28/3/21-1496-App1.pdf).

Among 1,282 study participants (Figure), 687 (53.6%) were <18 years of age (median 16 years, IQR 7–25 years), and 827 (64.5%) were male. Among 1,272 participants with antibody results, we considered 107 (8.4%, 95% CI 7.0%–10.0%) seropositive for EBOV GP IgG by using the prespecified cutoff.

Risk factor analysis showed that, after adjusting for age and sex, the only characteristic associated with seropositivity was living in a household compound with ≥1 pigs during the outbreak (adjusted odds ratio [OR] 4.5, 95% CI 1.6–13.0; p = 0.01) (Tables 1, 2; Appendix

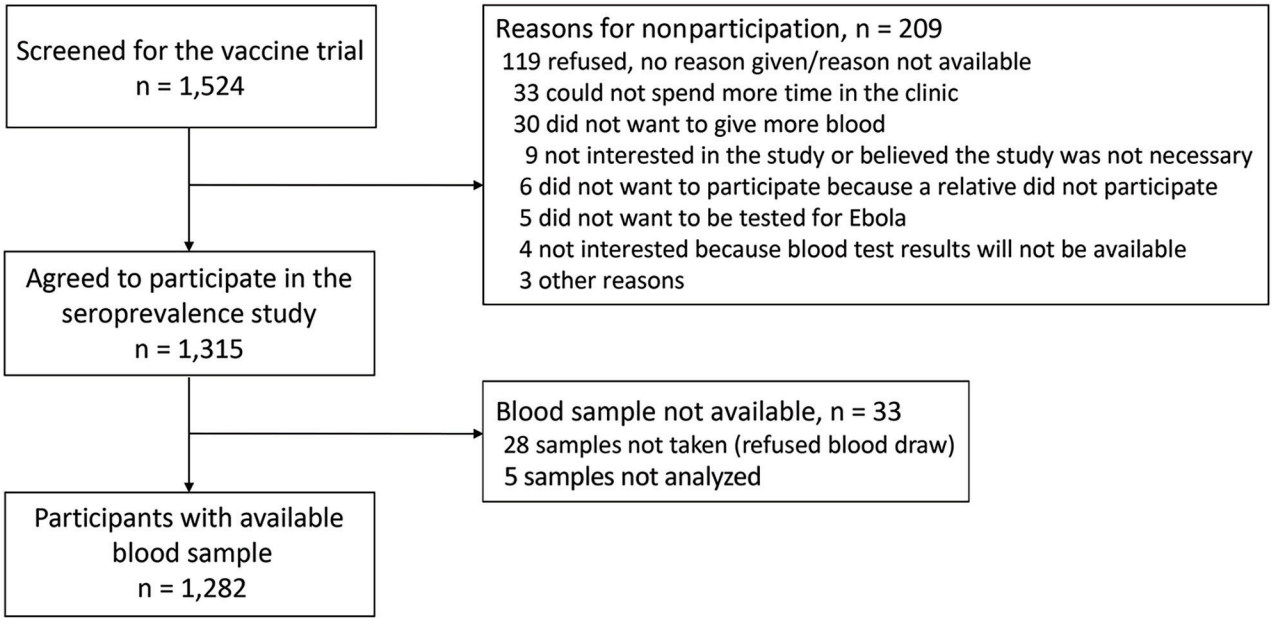


Figure. Flow chart of participants screened for the Ebola virus vaccine trial and subsequent seropositivity study of community members affected by the 2014–2016 Ebola outbreak, Sierra Leone.

Table 1). The EBOV antibody geometric mean concetration (GMC) was higher in participants ≥5 years of age than in young er children (Appendix Table 1). After adjusting for age and sex, only pig ownership remained associated with antibody concentration (adjusted GMC ratio 3.0, 95% CI 1.5–5.9; p<0.01) (Table 2).

The 8.4% seroprevalence in our study is within the range of estimates (0%–24%) from prior studies; however, this range is large because of the use of different assays, different seroprevalence thresholds, different levels of exposure to EVD cases, and studies undertaken in different geographic areas and at different timepoints relative to reported outbreaks (*1*). Our estimate is similar to the baseline EBOV antibody seroprevalence (4.0%) measured in another Ebola vaccine trial conducted in Liberia during the 2014–2016 EVD outbreak that used the same assay and cutoff (*5*).

Similar to results from previous studies, our findings showed a statistically significant increase in EBOV antibody concentration with participants' age in our study, possibly because of increased exposure of older age groups to EBOV or to other infections that could induce cross-reactive antibodies to the EBOV GP (*6*,*7*). Potential exposures to EVD, such as healthcare work, contact with EVD cases, and funeral attendance, which were associated with EBOV transmission in other studies (*8*), were not associated with EBOV antibody seropositivity or concentration in our study. However, few participants reporting those risk factors, and our study might have lacked the power to detect such associations.

We found an independent association of both EBOV antibody seropositivity and concentration with residence in a household compound that owned ≥1 pigs during the Ebola outbreak. Pigs can be experimentally infected with EBOV and can transmit the virus to nonhuman primates (*9*). EBOV-specific antibodies have been found in pigs in Sierra Leone and Guinea, suggesting that pigs can be naturally infected by EBOV (*10*,*11*). Pigs in the Philippines have been found to be naturally infected with Reston virus, an EBOV strain that is not known to cause disease in humans. Reston virus–specific antibodies were found in healthy farmers in contact with the infected pigs, suggesting potential transmission from pigs to humans (*12*). However, we found no association of EBOV antibody with having other domestic animals, in particular dogs, which also could be infected with EBOV (*13*,*14*).

One strength of our study is that we conducted our study in an area with prolonged EBOV transmission during the 2014–2016 EVD outbreak. Further, we explored a wide range of potential risk factors for EBOV acquisition, and we used the FANG ELISA, which has been proven to be more precise and accurate than a commercial alternative (*4*).

The first limitation of our study is that the parent RCT did not require random sampling of potential participants' households, which could have affected the generalizability of our results to the

general population. The RCT recruitment was age-staggered, and the youngest age cohort (1–3 years of age) was recruited >2 years after the EVD outbreak ended. However, a sensitivity analysis suggested that year of recruitment had a negligible confounding effect on the lower EBOV antibody concentrations observed in the youngest children (Appendix Table 2). Our study was conducted at the end of the 2014–2016 EVD outbreak in Sierra Leone, when public health measures to contain EBOV transmission had been in place for several months and the population had received messages about EVD prevention. This factor could have caused an underreporting of behaviors considered to put persons at risk for EVD. For example, hunting and consumption of bushmeat was rarely reported by our participants, in contrast with some reports that describe frequent hunting and bushmeat consumption in West Africa (*15*). The association of both antibody seropositivity and concentration with pig ownership is based on only 18 participants who reported keeping ≥1 pigs in their household compound at the time of the

Table 1. Potential EVD exposure in community or work during the 2014–2016 EVD outbreak and antibody seropositivity and GMC among participants in a study of EBOV GP–specific binding antibody seropositivity, Sierra Leone*

Risk factors	No. (%), n = 1,282	No. seropositive/ no. tested (%)	OR (95% CI)	Adjusted OR (95% CI)†	GMC, EU/mL (95% CI)	GMC ratio (95% CI)	Adjusted GMC ratio (95% CI)†
Living in a village or town with Ebola cases, n = 1,281							
N	199 (15.5)	10/198 (5.1)	Referent, p = 0.049	Referent, p = 0.125	49 (40–58)	Referent, p = 0.010	Referent, p = 0.882
Y	1,082 (84.5)	97/1,073 (9.0)	1.9 (1.0–3.6)	1.7 (0.8–3.3)	65 (60–71)	1.3 (1.1–1.6)	1.0 (0.8–1.3)
Knowing someone who had Ebola							
No, don't know	1,044 (81.4)	82/1,036 (7.9)	Referent, p = 0.193		61, 56–67)	Referent, p = 0.204	
Y	238 (18.6)	25/236 (10.6)	1.4 (0.9–2.2)		70 (57–85)	1.1 (0.92–1.4)	
No. EVD cases known by participant							
0	1,044 (81.4)	82/1,036 (7.9)	Referent, p = 0.55		61 (56–67)	Referent, p = 0.382	
1	125 (9.8)	13/125 (10.4)	1.4 (0.7–2.5)		64 (49–85)	1.1 (0.8–1.4)	
2–3	66 (5.2)	8/65 (12.3)	1.6 (0.8–3.5)		84 (57–124)	1.4 (0.9–2.0)	
>3	47 (3.7)	4/46 (8.7)	1.1 (0.4–3.2)		66 (44–99)	1.1 (0.7–1.6)	
Closest relationship with an EVD case, n = 1,280							
No relationship‡	1,044 (81.5)	82/1,036 (7.9)	Referent, p = 0.197		61, 56–67)	Referent, p = 0.259	
Close family§	27 (2.1)	1/27 (3.7)	0.5 (0.1–3.3)		52 (33–81)	0.9 (0.5–1.3)	
Other relative	52 (4.1)	6/51 (11.8)	1.6 (0.6–3.7)		64 (42–96)	1.0 (0.7–1.6)	
Friend	59 (4.6)	4/59 (6.8)	0.8 (0.3–2.4)		64 (45–91)	1.1 (0.7–1.5)	
Community member	98 (7.7)	14/97 (14.4)	2.0 (1.1–3.7)		86 (62–120)	1.4 (1.0–2.0)	
Living in the same household with an EVD case, n = 1,280							
N	1,269 (99.1)	107/1,260 (8.5)	–		63 (58–68)	Referent, p = 0.814	
Y	11 (0.9)	0/10 (0.0)	–		56 (31–102)	0.9 (0.5–1.6)	
Caring for an EVD case, n = 1,281							
N	1,272 (99.3)	107/1,262 (8.5)	–		63 (58–68)	Referent, p = 0.600	
Y	9 (0.7)	0/9 (0.0)	–		48 (24–98)	0.8 (0.4–1.6)	
Direct body contact with an EVD case, n = 1,281							
N	1,275 (99.5)	107/1,265 (8.5)	–		62 (57–67)	Referent, p = 0.640	
Y	6 (0.5)	0/6 (0.0)	–		83 (28–242)	1.3 (0.5–3.9)	
Attending a funeral of an EVD case							
N	1,263 (98.5)	105/1,254 (8.4)	Referent, p = 0.691		62 (57–67)	Referent, p = 0.346	
Y	19 (1.5)	2/18 (11.1)	1.4 (0.3–6.0)		87 (37–204)	1.4 (0.6–3.3)	
Healthcare frontline worker during EVD outbreak							
No, NA¶	1,254 (97.8)	105/1,244 (8.4)	Referent, p = 0.802		63 (58–69)	Referent, p = 0.798	
Y	28 (2.2)	2/28 (7.1)	0.8 (0.2–3.6)		58 (36–93)	0.9 (0.6–1.5)	

*Seropositivity defined as >607 EU/mL. EBOV GP–specific binding antibodies were indeterminate in 10 participants. p values calculated by using likelihood ratio test. EBOV GP, Ebola virus glycoprotein; EU, ELISA units; EVD, Ebola virus disease; GMC, geometric mean concentration; NA, not applicable; OR, odds ratio.
†Adjusted for age and sex.
‡Participant did not know anyone with Ebola.
§Participant was the parent or child or spouse or sibling of an EVD case.
¶Not applicable because participant was a child or did not have a job.

Table 2. Potential risk factors for transmission of Ebola virus from animals during the 2014–2016 EVD outbreak and antibody seropositivity and GMC among participants in a study of EBOV GP–specific binding antibody seropositivity, Sierra Leone*

Risk factors	No. (%), n = 1,282	No. seropositive/ no. tested (%)	OR (95% CI)	Adjusted OR (95% CI)†	GMC, EU/mL (95% CI)	GMC ratio (95% CI)	Adjusted GMC ratio (95% CI)†
Number of domestic animals in the participant's compound							
0	503 (39.2)	45/498 (9.0)	Referent, p = 0.558		59 (51–67)	Referent, p = 0.462	
1–5	374 (29.2)	33/371 (8.9)	1.0 (0.6–1.6)		65 (55–75)	1.1 (0.9–1.3)	
>5	405 (31.6)	29/403 (7.2)	0.8 (0.5–1.3)		66 (57–76)	1.1 (0.9–1.3)	
Having the following domestic animals in the compound‡							
Dog							
N	1,116 (87.1)	90/1,107 (8.1)	Referent, p = 0.349		66 (52–84)	Referent, p = 0.559	
Y	165 (12.9)	17/164 (10.4)	1.3 (0.8–2.3)		62 (57–67)	1.1 (0.8–1.4)	
Cat							
N	951 (74.2)	80/943 (8.5)	Referent, p = 0.887		61 (56–67)	Referent, p = 0.400	
Y	330 (25.8)	27/328 (8.2)	1.0 (0.6–1.5)		66 (56–78)	1.1 (0.9–1.3)	
Goat, sheep							
N	870 (67.9)	76/863 (8.8)	Referent, p = 0.465		62 (56–68)	Referent, p = 0.781	
Y	411 (32.1)	31/408 (7.6)	0.9 (0.6–1.3)		62 (57–67)	1.0 (0.9–1.2)	
Pig							
N	1,263 (98.6)	102/1,253 (8.1)	Referent, p = 0.015	Referent, p = 0.014	61 (57–67)	Referent, p<0.001	Referent, p = 0.001
Y	18 (1.4)	5/18 (27.8)	4.3 (1.5–12.4)	4.5 (1.6–13.0)	200 (93–431)	3.3 (1.5–7.1)	3.0 (1.5–5.9)
Other							
N	825 (64.4)	73/817 (8.9)	Referent, p = 0.370		61 (55–68)	Referent, p = 0.513	
Y	456 (35.6)	34/454 (7.5)	0.8 (0.5–1.3)		65 (57–74)	1.1 (0.9–1.3)	
Touching sick or dead domestic animals							
N	1,253 (97.7)	106/1,243(8.5)	Referent, p = 0.275		63 (58–68)	Referent, p = 0.824	
Y	29 (2.3)	1/29 (3.5)	0.4 (0.1–2.8)		59 (36–97)	0.9 (0.6–1.6)	
Hunting for wild animals§							
N	1,261 (99.3)	105/1,251(8.4)	Referent, p = 0.779		63 (58–68)	Referent, p = 0.859	
Y	9 (0.7)	1/9 (11.1)	1.4 (0.2–11.0)		57 (17–191)	0.9 (0.3–3.1)	
Touching sick or dead wild animals							
N	1,277 (99.6)	106/1,267 (8.4)	Referent, p = 0.419		62 (58–68)	Referent, p = 0.825	
Y	5 (0.4)	1/5 (20.0)	2.7 (0.3–24.7)		54 (8–369)	0.9 (0.1–5.9)	
Consuming bushmeat							
N	1,275 (99.4)	106/1,265 (8.4)	Referent, p = 0.606		62 (58–68)	Referent, p = 0.962	
Y	7 (0.6)	1/7(14.3)	1.8 (0.2–15.3)		61 (14–274)	1.0 (0.2–4.4)	

*Seropositivity defined as >607 EU/mL. EBOV GP–specific binding antibodies were indeterminate in 10 participants. p values calculated by using likelihood ratio test. EBOV, Ebola virus; EU, ELISA units; GMC, geometric mean concentration; GP, glycoprotein; OR, odds ratio.
†Adjusted for age and sex.
‡Participants could indicate >1 type of domestic animal.
§Types of wild animals hunted by participants who answered yes included monkeys, duiker antelopes, bats, and rodents.

outbreak. This association could have occurred by chance, although the evidence of an association is quite strong. The observed association also could be confounded by unrecorded risk factors among participants who also kept pigs, such as EBOV transmission clustering in participants from a household that also owned pigs. However, that possibility seems unlikely because none of the seropositive participants who owned pigs reported contact with an EVD case, and these participants all came from different households. Finally, we are not able to determine whether EBOV antibody seropositivity in this setting reflects true asymptomatic infection because we cannot exclude underreporting of earlier EVD symptoms and we have not yet investigated cross-reactivity with other viral infections. Whether EBOV seropositivity reflects acquired immunity that might provide some protection against future EBOV infections also is unclear.

Our findings suggest that the role of pigs as potential, occasional reservoirs of EBOV needs to be investigated further. The presence of antibodies binding the EBOV GP could also suggest circulation of other infectious agents, probably viruses, inducing cross-reactivity with the EBOV GP, but this possibility needs further investigation.

Conclusions

The incidence of EBOV infection during the 2014–2016 EVD outbreak in Sierra Leone could have been higher than previously reported; 8.4% of adults and children from a community affected by the outbreak who never experienced symptoms of EVD had serologic responses to EBOV above a cutoff threshold. Our study suggests that EBOV might cause asymptomatic infection, but whether underreporting of symptoms, FANG assay specificity, or exposure to other viral infections that could generate cross-reactive antibodies also contributed to the results is unclear. These questions would benefit from further investigation to help define the extent of future EVD outbreaks. Countries at high risk for EVD outbreaks should be aware of the risk of asymptomatic or paucisyntomatic infections.

Acknowledgments

We thank Christian Hansen for support in the sample size calculation for the study protocol and Kathy Baisley for advice on the statistical analysis. We thank Viki Bockstaland Kerstin Luhn for advice on the interpretation of FANG ELISA results. We thank the site study team, including study physicians and other clinicians; the data management team; the quality assurance, social science,and community engagement teams; the laboratory team, the College of Medicine and Allied Health Sciences (COMAHS) and the London School of Hygiene & Tropical Medicine (LSHTM) project management and administrative teams; and colleagues from World Vision, GOAL, and the Ebola Vaccine Deployment, Acceptance and Compliance (EBODAC) project. We thank all the study participants and their families. We also thank the other EBOVAC 1 Consortium partners, the University of Oxford, and Institut National de la Santé et de la Recherche Médicale (INSERM) in France for their support for this study.

This study was funded by the Innovative Medicines Initiative 2 Joint Undertaking (grant no. 115854, EBOVAC 1 project).

About the Author

Dr. Manno is a clinical epidemiologist and assistant professor at the London School of Hygiene and Tropical Medicine. Her primary research interests include epidemiological studies and clinical trials in infectious diseases.

References

1. Bower H, Glynn JR. A systematic review and meta-analysis of seroprevalence surveys of ebolavirus infection. Sci Data. 2017;4:160133. https://doi.org/10.1038/sdata.2016.133
2. Ishola D, Manno D, Afolabi MO, Keshinro B, Bockstal V, Rogers B, et al. Safety and long-term immunogenicity of the two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in adults in Sierra Leone: a combined open-label, non-randomised stage 1, and a randomised, double-blind, controlled stage 2 trial. Lancet Infect Dis. 2022;22:97–109. https://doi.org/10.1016/S1473-3099(21)00125-0
3. 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;22:110–22. https://doi.org/10.1016/S1473-3099(21)00128-6
4. Logue J, Tuznik K, Follmann D, Grandits G, Marchand J, Reilly C, et al. Use of the Filovirus Animal Non-Clinical Group (FANG) Ebola virus immuno-assay requires fewer study participants to power a study than the Alpha Diagnostic International assay. J Virol Methods. 2018;255:84–90. https://doi.org/10.1016/j.jviromet.2018.02.018
5. Kennedy SB, Bolay F, Kieh M, Grandits G, Badio M, Ballou R, et al.; PREVAIL I Study Group. Phase 2 placebo-controlled trial of two vaccines to prevent ebola in Liberia. N Engl J Med. 2017;377:1438–47. https://doi.org/10.1056/NEJMoa1614067
6. Mulangu S, Borchert M, Paweska J, Tshomba A, Afounde A, Kulidri A, et al. High prevalence of IgG antibodies to Ebola virus in the Efé pygmy population in the Watsa region, Democratic Republic of the Congo. BMC Infect Dis. 2016;16:263. https://doi.org/10.1186/s12879-016-1607-y
7. Bouree P, Bergmann JF. Ebola virus infection in man: a serological and epidemiological survey in the Cameroons. Am J Trop Med Hyg. 1983;32:1465–6. https://doi.org/10.4269/ajtmh.1983.32.1465
8. Brainard J, Hooper L, Pond K, Edmunds K, Hunter PR. Risk factors for transmission of Ebola or Marburg virus disease: a systematic review and meta-analysis. Int J Epidemiol. 2016;45:102–16. https://doi.org/10.1093/ije/dyv307
9. Weingartl HM, Embury-Hyatt C, Nfon C, Leung A, Smith G, Kobinger G. Transmission of Ebola virus from pigs to non-human primates. Sci Rep. 2012;2:811. https://doi.org/10.1038/srep00811
10. Fischer K, Jabaty J, Suluku R, Strecker T, Groseth A, Fehling SK, et al. Serological evidence for the circulation of ebolaviruses in pigs from Sierra Leone. J Infect Dis. 2018;218(suppl_5):S305–11. PubMed https://www.doi.org/10.1093/infdis/jiy330
11. Fischer K, Camara A, Troupin C, Fehling SK, Strecker T, Groschup MH, et al. Serological evidence of exposure to ebolaviruses in domestic pigs from Guinea. Transbound Emerg Dis. 2020;67:724–32. https://doi.org/10.1111/tbed.13391
12. Barrette RW, Metwally SA, Rowland JM, Xu L, Zaki SR, Nichol ST, et al. Discovery of swine as a host for the Reston ebolavirus. Science. 2009;325:204–6. https://doi.org/10.1126/science.1172705
13. Haun BK, Kamara V, Dweh AS, Garalde-Machida K, Forkay SSE, Takaaze M, et al. Serological evidence of Ebola virus exposure in dogs from affected communities in Liberia: A preliminary report. PLoS Negl Trop Dis. 2019;13(7):e0007614. PubMed https://doi.org/10.1371/journal.pntd.0007614
14. Fischer K, Suluku R, Fehling SK, Jabaty J, Koroma B, Strecker T, et al. Ebola virus neutralizing antibodies in dogs from Sierra Leone, 2017. Emerg Infect Dis. 2020;26:760–3. https://doi.org/10.3201/eid2604.190802
15. Luiselli L, Hema E, Segniagbeto G, Ouattara V, Eniang EA, Parfait G, et al. Bushmeat consumption in large urban centres in West Africa. Oryx. 2020;54:731–4.

Address for Correspondence: Daniela Manno, London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT, UK; email: daniela.manno@lshtm.ac.uk

Article DOI: https://doi.org/10.3201/eid2803.211496

Ebola Virus Glycoprotein IgG Seroprevalence in Community Previously Affected by Ebola, Sierra Leone

Appendix

Methods

Study Design

We conducted a cross-sectional seroprevalence study of immunoglobulin G (IgG) antibodies against Ebola virus (EBOV) glycoprotein (GP) during March 16, 2016–June 29, 2018. We nested the study within the screening visit of the EBOVAC-Salone (https://www.ebovac.org) randomized controlled trial (RCT), which was being conducted to evaluate the safety and immunogenicity of a 2-dose heterologous vaccination regimen with Ad26.ZEBOV and MVA-BN-Filo Ebola vaccines (protocol no. VAC52150EBL3001; ClinicalTrials.gov no. NCT02509494).

Study Participants

We enrolled participants from 3 sites in Kambia District, northern Sierra Leone; 2 sites in Kambia town and 1 site in the neighboring community of Rokupr, a rural village ≈15 km from Kambia town. Both areas were affected by widespread and prolonged EBOV transmission during the Ebola virus disease (EVD) epidemic in West Africa (*1*).

We recruited adults first, during March 16, 2016–December 29, 2016; then we enrolled children during March 21 2017–June 29, 2018 in 3 age cohorts: 12–17, 4–11, and 1–3 years of age. We counselled potential participants on the importance of providing accurate medical information, including any history of EVD, close contact with a person who had EVD, or prior vaccination with a candidate Ebola vaccine. Persons who reported having an EVD diagnosis in the past or who previously had been vaccinated with a candidate Ebola vaccine were considered ineligible for both the RCT and we did not include them in the seroprevalence study.

We obtained informed consent from adult participants and from parents or guardians for participants who were <18 years of age. We also asked children ≥7 years of age to give their assent for participation. Ethical approval for the study was received from the Sierra Leone Ethics Committee and the London School of Hygiene & Tropical Medicine (LSHTM) Ethics Committee (reference no. 10537).

Study Procedures

We interviewed study participants to collect information on potential risk factors for EBOV infection, including residence in areas where EVD cases occurred during the 2014–2016 outbreak, healthcare work during the outbreak, travel, contact with EVD cases, funeral attendance, and contact with or consumption of wild animals. Because EBOV also is known to infect domestic animals, including dogs and pigs, we also collected information on contacts with these animals during the outbreak (*2*–*7*).

Approximately 2 mL of blood was collected at enrollment. Samples were left to clot for 30 minutes, then centrifuged at 1,500 g (rpm) for 10 min at the study clinics. At the research laboratory, we aliquoted serum and froze it at −20°C. We stored serum samples at −20°C until shipped in controlled temperature containers to the laboratory in the United States for sample analysis. Q2 Solutions Vaccine Testing Laboratory (https://www.q2labsolutions.com) measured IgG against EBOV GP by using the EBOV GP Filovirus Animal Non-Clinical Group (FANG) ELISA. Validation of the FANG ELISA was endorsed by the US Food and Drug Administration (FDA) in February 2017 (Q2 Solutions, pers. comm., 2017. FANG ELISA has a lower limit of quantification (LLOQ), 36.11 ELISA units (EU)/mL, and has no established cutoff to distinguish seropositive persons after EBOV infection from seronegative persons (Q2 Solutions, pers. comm., 2019). To determine seropositivity, we used a cutoff of >607 EU/mL, which was calculated in a previous study using serum samples collected from 100 EBOV-naive persons from Mali during 2004–2011 and was defined as the antibody titer of 3 SD above the mean ($\log_{10}$ transformed) (*8*). This cutoff was considered appropriate to provide an estimate of the prevalence of IgG to EBOV GP in a setting in West Africa. We also conducted a post-hoc analysis with an alternative cutoff calculated by using serum samples from 388 EBOV-naive persons from the United Kingdom (See Alternative cutoff calculation).

Sample Size and Statistical Analysis

We did not conduct a formal sample size calculation for this study because the number of enrolled participants was determined by the number of participants screened for the RCT. We also had limited data on the estimated prevalence of IgG to EBOV GP in the general population afer an EVD outbreak. However, we estimated that a sample of 1,250 persons would enable us to estimate a prevalence of 1.0% with a precision of approximately ±0.55% (i.e., 95% CI 0.45%–1.55%).

We conducted our statistical analysis for all participants with an available FANG ELISA result. We calculated the seroprevalence of IgG to EBOV GP as a percentage of study participants who had an antibody concentration above the prespecified cutoff of >607 EU/mL. We obtained the antibody geometric mean concentration (GMC) and 95% CI by calculating the mean and 95% CI of the log-transformed values, and then transforming these results back into the original units by taking the antilogs. To calculate GMC, we imputed values below the LLOQ as LLOQ/2 (18.055 EU/mL). We calculated the odds ratio (OR) and 95% CI to measure the association between potential risk factors for acquisition of EBOV and seropositivity, using logistic regression. We calculated the GMC ratio and 95% CI to measure the association between the same risk factors and IgG antibody concentration, using linear regression. For the risk factor analysis, we selected a total of 26 variables out of 47 questions related to risk factors or potential confounders obtained from participants' interviews. Among those questions, we used 11 questions about household characteristics (ownership of goods, such as television, radio, etc.) to calculate the socioeconomic status of the household with principal component analysis. We adjusted the multivariable analyses for age and sex (a priori confounding factors). We conducted a post-hoc sensitivity analysis adjusting for year of enrollment to explore whether the age distribution of the EBOV GP antibody concentration could have been influenced by the age-staggered recruitment procedure. We used Stata 16 (StataCorp LLC, https://www.stata.com) for all the statistical analyses.

Alternative Cutoff Calculation

In a post-hoc analysis, we calculated an alternative seropositivity cutoff by using baseline Ebolavirus IgG levels from 388 healthy persons from the United Kingdom who were enrolled in an Ebola vaccine trial (protocol no. VAC52150EBL2001) during 2014–2015 (*9*). The investigators of this study conducted the sample analysis by using the FANG ELISA at Q2

Solutions Vaccine Testing Laboratory. Among the 388 participants, 26 had a baseline result above the LLOQ of 36.11 EU/mL. We imputed values below the LLOQ as LLOQ/2 (18.055 EU/mL). We defined the seropositivity cutoff as the antilog value of 3 SD above the mean of the $\log_{10}$ transformed values, as calculated in a previous study (*8*).

The EBOV GP antibody GMC in the 388 EBOV-naive persons from the UK was 20.44 EU/mL, with a geometric standard deviation (GSD) of 1.69 EU/mL. To calculate seropositivity cutoff we use the formula: GMC × $(GSD)^3$.

Seropositivity cutoff = $20.44 \times (1.69)^3 = 99.03$ EU/mL

Results

Detailed Description of Study Results

A total of 1,524 potential participants were screened for the VAC52150EBL3001 trial, of whom 1,315 (86.3%) agreed to participate in the seroprevalence study (Figure). Blood samples were available for 1,282 (97.5%) participants, 687 (53.6%) of whom were aged <18 years (median age 16 years, IQR = 7–25 years) and 827 (64.5%) of whom were male (Appendix Table 1).

Only 238 (18.6%) participants reported that they knew someone who had EVD during the outbreak (Table 1). Eleven (0.9%) participants reported that someone in their household had experienced EVD and 9 (0.7%) participants cared for someone with EVD. Six (0.5%) participants had direct body contact with an EVD patient. Only 28 participants (2.2%) undertook healthcare or frontline (i.e., burial team) work during the EVD outbreak. Only 9 (0.7%) reported hunting for wild animals and only 7 (0.6%) said that they had consumed bushmeat (Table 2).

Because the FANG ELISA results were indeterminate in 10 of the 1,282 samples, the estimation of IgG seroprevalence and GMC were based on results from 1,272 participants. Of those 1,272 samples, 684 (53.8%) had a result that was above the LLOQ of 36.11 EU/mL for the FANG ELISA. Overall, 107 participants (8.4%, 95% CI 7.0%–10.0%) had a result above the prespecified seropositivity cutoff of 607 EU/mL and we considered these samples to be seropositive for EBOV GP in our study.

There were fewer seropositive participants among children <5 years compared with older age groups (Appendix Table 1). However, we found no statistical evidence of an association between seropositivity and age. We also saw no statistically significant difference in the percentage of seropositive samples by sex. In univariable analyses, we noted some evidence of an association between seropositivity and living in a village or town with EVD cases (Table 1), or in a household compound with ≥1 pigs at the time of the outbreak (Table 2). After adjusting for age and sex, only having ≥1 pigs in the household compound at the time of the outbreak remained associated with EBOV seropositivity (adjusted OR 4.5, 95% CI 1.6–13.0, p = 0.01) (Table 2). A post-hoc analysis with an alternative cutoff calculated by using serum samples from 388 EBOV-naive persons from the United Kingdom, showed similar results (see Alternative Cutoff Analysis results).

We noted a statistically significant increase in EBOV GP binding antibody GMC with age and GMC was higher in participants ≥5 years of age than in younger children (Appendix Table 1). This association remained after adjusting for year of recruitment, which suggested that it was not due to the age-staggered recruitment process (Appendix Table 2). Male persons had a slightly higher GMC than female persons but we saw no evidence of a difference after adjusting for age. Other statistically significant variables associated with EBOV GP binding antibody concentration on univariable analysis were education, frequency of travel outside the place of residence, living in a village or town with EVD cases, and having ≥1 pigs in the household compound at the time of the outbreak (Table 1, Table 2; Appendix Table 1). After adjusting for age and sex, we saw no evidence of an association between antibody concentration and education or travel or residence in a village or town with EVD cases. However, we still saw evidence of an association between antibody concentration and the presence of ≥1 pigs in the household compound at the time of the outbreak (adjusted GMC ratio 3.0, 95% CI 1.5%–5.9%, p < 0.01) (Table 2).

Alternative Cutoff Analysis Results

Because the assay has no established diagnostic serostatus threshold, we calculated a range of seropositivity estimates by using different cut-off values and the prespecified cutoff used in our study (Appendix Table 3). We also conducted a post-hoc analysis with an alternative cutoff calculated by using serum samples from EBOV-naive persons from the United Kingdom (see Alternative Cutoff Calculation). Overall, 411 participants (32.3%, 95% CI 29.7%–34.9%)

had a result above the seropositivity cutoff of 99.03 EU/mL and we considered these samples to be seropositive for EBOV GP in our supplementary analysis.

The number of seropositive participants increased with age and fewer children <5 years of age were seropostive compared with persons in older age groups (Appendix Table 4). We saw no statistically significant difference in the percentage of seropositive participants by sex. In univariable analyses, we noted some evidence of an association between seropositivity and education and living in a household compound that kept ≥1 pigs at the time of the outbreak (Appendix Tables 4–6). After adjusting for age and sex, only having ≥1 pigs in the household compound at the time of the outbreak remained associated with EBOV seropositivity (adjusted OR 4.1, 95% CI 1.5–11.4, p < 0.01) (Appendix Table 6).

Discussion

FANG ELISA Uses and Limitations

The FANG ELISA used in our study has been proven to be more precise and accurate than a commercial alternative for the assessment of immune response after Ebola vaccination (*8*). Despite being the best option available at the time, the assay has some limitations. Positivity has been observed in samples from countries that have never experienced EBOV outbreaks, which indicates that the assay might not have a high specificity (*10–13*). For this reason, we adopted a seropositivity cutoff that has been calculated in EBOV-naive persons from West Africa, although this analysis was not done in the same laboratory where our study samples were analyzed (*8*). Another limitation of the FANG ELISA is that it only detects IgG against the EBOV GP, but a concomitant test to detect IgG against the EBOV nucleoprotein could have enabled a better identification of previous EBOV infections, as noted in another study (*14*). A seropositive cutoff of >607 EU/mL could be considered high for a seroepidemiologic study, considering that in some Ebola vaccine trials the antibody concentration that was achieved post vaccination was sometimes below this threshold, even in participants considered as vaccine responders (*10–13*). However, we believe that this cutoff is suitable to provide a conservative estimate of the prevalence of IgG to EBOV GP in West Africa but it would not be appropriate to use this cutoff for the interpretation of post-vaccination results in a clinical trial. Most Ebola vaccine trials that used the FANG ELISA for the measurement of postvaccination antibody response have adopted

a vaccine responder definition that was based on an x-fold increase over prevaccination baseline values, instead of using a predefined cutoff (*10–13*). We are aware that, without an established diagnostic serostatus threshold, the choice of a cutoff can be arbitrary. Thus, we also analyzed the data as a continuous variable, i.e., EBOV IgG concentration and we conducted a post-hoc analysis using an alternative cutoff calculated in EBOV-naive persons from the United Kingdom and these analyses showed similar results.

References

1. World Health Organiztion. Ebola maps from 30 March 2016: Geographical distribution of new and total confirmed cases [cited 2021 Mar 1]. http://apps.who.int/ebola/sites/default/files/thumbnails/image/sitrep_casecount_40.png?ua=1

2. Allela L, Bourry O, Pouillot R, Délicat A, Yaba P, Kumulungui B, et al. Ebola virus antibody prevalence in dogs and human risk. Emerg Infect Dis. 2005;11:385–90.

3. Haun BK, Kamara V, Dweh AS, Garalde-Machida K, Forkay SSE, Takaaze M, et al. Serological evidence of Ebola virus exposure in dogs from affected communities in Liberia: A preliminary report. PLoS Negl Trop Dis. 2019;13(7):e0007614.

4. Gumusova S, Sunbul M, Leblebicioglu H. Ebola virus disease and the veterinary perspective. Ann Clin Microbiol Antimicrob. 2015;14:30.

5. Fischer K, Camara A, Troupin C, Fehling SK, Strecker T, Groschup MH, et al. Serological evidence of exposure to ebolaviruses in domestic pigs from Guinea. Transbound Emerg Dis. 2020;67:724–32.

6. Fischer K, Jabaty J, Suluku R, Strecker T, Groseth A, Fehling SK, et al. Serological evidence for the circulation of ebolaviruses in pigs from Sierra Leone. J Infect Dis. 2018;218:S305–11.

7. Weingartl HM, Embury-Hyatt C, Nfon C, Leung A, Smith G, Kobinger G. Transmission of Ebola virus from pigs to non-human primates. Sci Rep. 2012;2:811.

8. Logue J, Tuznik K, Follmann D, Grandits G, Marchand J, Reilly C, et al. Use of the Filovirus Animal Non-Clinical Group (FANG) Ebola virus immuno-assay requires fewer study participants to power a study than the Alpha Diagnostic International assay. J Virol Methods. 2018;255:84–90.

9. Pollard AJ, Launay O, Lelievre JD, Lacabaratz C, Grande S, Goldstein N, et al.; EBOVAC2 EBL2001 Study Group. Safety and immunogenicity of a two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in adults in Europe (EBOVAC2): a randomised, observer-blind, participant-blind, placebo-controlled, phase 2 trial. Lancet Infect Dis. 2021;21:493–506.

10. Mutua G, Anzala O, Luhn K, Robinson C, Bockstal V, Anumendem D, et al. Safety and immunogenicity of a 2-dose heterologous vaccination regimen with Ad26.ZEBOV and MVA-BN-Filo Ebola vaccines: 12-month data from a phase 1 randomized clinical trial in Nairobi, Kenya. J Infect Dis. 2019;220:57–67.

11. Anywaine Z, Whitworth H, Kaleebu P, Praygod G, Shukarev G, Manno D, et al. Safety and immunogenicity of a 2-dose heterologous vaccination regimen with Ad26.ZEBOV and MVA-BN-Filo Ebola vaccines: 12-month data from a phase 1 randomized clinical trial in Uganda and Tanzania. J Infect Dis. 2019;220:46–56.

12. 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;22:110–22.

13. Ishola D, Manno D, Afolabi MO, Keshinro B, Bockstal V, Rogers B, et al. Safety and long-term immunogenicity of the two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in adults in Sierra Leone: a combined open-label, non-randomised stage 1, and a randomised, double-blind, controlled stage 2 trial. Lancet Infect Dis. 2022;22:97–109.

14. Richardson E, Kelly J, Barrie M, Mesman A, Karku S, Quiwa K, et al. Minimally symptomatic infection in an Ebola 'hotspot': a cross-sectional serosurvey. PLoS Negl Trop Dis. 2016;10(11):e0005087.

Appendix Table 1. Sociodemographic characteristics, Ebola virus glicoprotein-specific binding antibody seropositivity, and geometric mean concentration among participants in a study of EBOV GP–specific binding antibody seropositivity, Sierra Leone*

Characteristics	No. (%), n = 1,282	No. seropositive/no. tested (%)	OR (95% CI)	Adjusted OR (95% CI)†	GMC, EU/mL (95% CI)	GMC ratio (95% CI)	Adjusted GMC ratio (95% CI)†
Age group, y							
1–4	243 (19.0)	14/240 (5.8)	Referent, 1.0 (p = 0.184)	Referent, 1.0 (p = 0.165)‡	32 (26–38)	Referent, 1.0 (p<0.001)	Referent, 1.0 (p<0.001)‡
5–9	170 (13.3)	18/168 (10.7)	1.9 (0.9–4.0)	1.9 (0.9–4.0)	69 (54–88)	2.2 (1.6–2.9)	2.2 (1.6–2.9)
10–19	354 (27.6)	24/353 (6.8)	1.2 (0.6–2.3)	1.2 (0.6–2.3)	71 (61–82)	2.2 (1.8–2.8)	2.2 (1.8–2.8)
20–39	390 (30.4)	39/387 (10.1)	1.8 (1.0–3.4)	1.9 (1.0–3.6)	77 (66–90)	2.4 (1.9–3.0)	2.3 (1.9–2.9)
≥40	125 (9.7)	12/124 (9.7)	1.7 (0.8–3.9)	1.8 (0.8–3.9)	72 (56–92)	2.3 (1.7–3.1)	2.2 (1.7–3.0)
Sex							
F	455 (35.5)	39/451 (8.7)	Referent, 1.0 (p = 0.823)	Referent, 1.0 (p = 0.560)‡	54 (48–62)	Referent, 1.0 (p = 0.018)	Referent, 1.0 (p = 0.125)‡
M	827 (64.5)	68/821 (8.3)	1.0 (0.6–1.4)	0.9 (0.6–1.3)	67 (61–74)	1.2 (1.0–1.5)	1.1 (1.0–1.4)
Highest education level							
No education	362 (28.2)	25/360 (6.9)	Referent, 1.0 (p = 0.572)		42 (37–49)	Referent, 1.0 (p<0.001)	Referent, 1.0 (p = 0.653)
Primary, grades 1–6	378 (29.5)	35/374 (9.4)	1.4 (0.8–2.4)		74 (63–86)	1.7 (1.4–2.2)	1.1 (0.8–1.5)
Secondary school	480 (37.4)	43/477 (9.0)	1.3 (0.8–2.2)		72 (63–82)	1.7 (1.4–2.1)	1.0 (0.7–1.3)
College, university	62 (4.9)	4/61 (6.6)	0.9 (0.3–2.8)		73 (54–100)	1.7 (1.2–2.4)	0.9 (0.6–1.5)
Household socioeconomic status							
Low	470 (36.7)	36/464 (7.8)	Referent, 1.0 (p = 0.805)		57 (50–66)	Referent, 1.0 (p = 0.294)	
Middle	396 (30.9)	34/394 (8.6)	1.1 (0.7–1.8)		66 (57–76)	1.1 (0.9–1.4)	
High	416 (32.5)	37/414 (8.9)	1.2 (0.7–1.9)		66 (57–75)	1.1 (0.9–1.4)	
Number of persons (adults and children) in the household, n = 1,276							
<5	274 (21.5)	21/270 (7.8)	Referent, 1.0 (p = 0.769)		59 (49–70)	Referent, 1.0 (p = 0.300)	
5–9	529 (41.4)	48/527 (9.1)	1.2 (0.7–2.0)		68 (59–77)	1.2 (0.9–1.4)	
≥10	473 (37.1)	38/469 (8.1)	1.0 (0.6–1.8)		60 (53–68)	1.0 (0.8–1.3)	
Number of children in the household, n = 1,274							
0–2	466 (36.6)	41/463 (8.9)	Referent, 1.0 (p = 0.646)		64 (56–73)	Referent, 1.0 (p = 0.854)	
3–5	536 (42.1)	40/530 (7.6)	0.8 (0.5–1.3)		61 (54–69)	1.0 (0.8–1.1)	
>5	272 (21.3)	25/271 (9.2)	1.0 (0.6–1.8)		64 (54–75)	1.0 (0.8–1.2)	
Frequency of travel outside of village or town of residence, n = 1,276							
Never traveled	510 (40.0)	33/507 (6.5)	Referent, 1.0 (p = 0.186)		55 (49–63)	Referent, 1.0 (p = 0.042)	Referent, 1.0 (p = 0.578)
Every day	19 (1.5)	2/19 (10.5)	1.7 (0.4–7.6)		110 (59–204)	2.0 (1.0–3.7)	1.5 (0.8–3.0)
≥1×/wk	58 (4.5)	9/58 (15.5)	2.6 (1.2–5.8)		90 (57–142)	1.6 (1.0–2.6)	1.3 (0.8–1.9)
≥1×/mo.	235 (18.4)	21/232 (9.1)	1.4 (0.8–2.5)		68 (56–82)	1.2 (1.0–1.5)	1.0 (0.8–1.3)
<1×/mo.	454 (35.6)	41/450 (9.1)	1.4 (0.9–2.3)		63 (55–72)	1.1 (0.9–1.4)	1.0 (0.8–1.3)

*Seropositivity defined as >607 EU/mL. p values calculated by using likelihood ratio test. EBOV–GP, Ebola virus glycoprotein; EVD, Ebola virus disease; GMC, geometric mean concentration; OR, odds ratio. EBOV, Ebola virus; EU, ELISA units;.
†Adjusted for age and sex.
‡Age adjusted for sex. Sex adjusted for age.

Appendix Table 2. Association between antibody concentration and age at recruitment, before and after adjusting for year of recruitment, among participants in a study of EBOV GP–specific binding antibody seropositivity, Sierra Leone*

Characteristics	No. (%), n = 1,282	GMC, EU/mL (95% CI)	GMC ratio (95% CI)	Adjusted GMC ratio (95% CI)
Age group, y				
1–4	243 (19.0)	32 (26–38)	Referent, 1.0 (p<0.001)	Referent, 1.0 (p<0.001)†
5–9	170 (13.3)	69 (54–88)	2.2 (1.6–2.9)	2.1 (1.5–2.9)
10–19	354 (27.6)	71 (61–82)	2.2 (1.8–2.8)	2.1 (1.5–2.9)
20–39	390 (30.4)	77 (66–90)	2.4 (1.9–3.0)	2.2 (1.4–3.5)
≥40	125 (9.7)	72 (56–92)	2.3 (1.7–3.1)	2.1 (1.3–3.4)
Year of recruitment				
2016	595 (46.4)	75 (67–85)	Referent, 1.0 (p<0.001)	Referent, 1.0 (p = 0.856)‡
2017	401 (31.3)	68 (58–78)	0.9 (0.7–1.1)	1.0 (0.7–1.4)
2018	286 (22.3)	38 (32–44)	0.5 (0.4–0.6)	0.9 (0.6–1.4)

*p values calculated by using likelihood ratio test. EU, ELISA units; GMC, geometric mean concentration; EBOV–GP, Ebola virus glycoprotein
†Adjusted for year of recruitment.
‡Adjusted for age at recruitment.

Appendix Table 3. Distribution of EBOV GP–specific binding antibody seroprevalence estimates by using different cut-offs, Sierra Leone*

Cutoff, EU/mL	No. seropositive/no. tested (%), n = 1,272	95% CI
>LLOQ (36.11)	684 (53.8)	51.0–56.5
>100	409 (32.2)	29.6–34.8
>200	274 (21.5)	19.4–23.9
>300	199 (15.6)	13.7–17.7
>400	158 (12.4)	10.7–14.4
>500	127 (10.0)	8.5–11.8
>607†	107 (8.4)	7.0–10.0

*EU, ELISA units; LLOQ, lower limit of quantification.
†Seroprevalence cutoff used for the main analysis in this study and calculated in a previous study in persons from West Africa (*8*).

Appendix Table 4. Sociodemographic characteristics and EBOV GP–specific binding antibody seropositivity among participants, Sierra Leone*

Characteristics	No. (%); n = 1,282	No. seropositive/no. tested (%)	OR (95% CI)	Adjusted OR (95% CI)†
Age group, y				
1–4	243 (19.0)	34/240 (14.2)	Referent, 1.0 (p<0.001)	1 (p<0.001)‡
5–9	170 (13.3)	61/168 (36.3)	3.5 (2.1–5.6)	3.5 (2.1–5.6)
10–19	354 (27.6)	126/353 (35.7)	3.4 (2.2–5.1)	3.4 (2.2–5.1)
20–39	390 (30.4)	145/387 (37.5)	3.6 (2.4–5.5)	3.6 (2.4–5.5)
≥40	125 (9.7)	45/124 (36.3)	3.5 (2.1–5.8)	3.4 (2.1–5.8)
Sex				
F	455 (35.5)	138/451 (30.6)	Referent, 1.0 (p = 0.332)	1 (p = 0.777)‡
M	827 (64.5)	273/821 (33.3)	1.1 (0.9–1.4)	1.0 (0.8–1.3)
Highest education level completed				
No education	362 (28.2)	82/360 (22.8)	Referent, 1.0 (p<0.001)	Referent, 1.0 (p = 0.888)
Primary, grades 1–6	378 (29.5)	135/374 (36.1)	1.9 (1.4–2.6)	0.9 (0.6–1.5)
Secondary school	480 (37.4)	170/477 (35.6)	1.9 (1.4–2.6)	0.9 (0.6–1.3)
College, university	62 (4.9)	24/61 (39.3)	2.2 (1.3–3.4)	1.0 (0.5–1.9)
Socioeconomic status of household				
Low	470 (36.7)	134/464 (28.9)	Referent, 1.0 (p = 0.104)	
Middle	396 (30.9)	130/394 (33.0)	1.2 (0.9–1.6)	
High	416 (32.5)	147/414 (35.5)	1.4 (1.0–1.8)	
No. persons in the household, adults and children, n = 1,276				
<5	274 (21.5)	83/270 (30.7)	Referent, 1.0 (p = 0.192)	
5–9	529 (41.4)	186/527 (35.3)	1.2 (0.9–1.7)	
≥10	473 (37.1)	142/469 (30.3)	1.0 (0.7–1.4)	
Number of children in the household, n = 1,274				
0–2	466 (36.6)	156/463 (33.7)	Referent, 1.0 (p = 0.725)	
3–5	536 (42.1)	166/530 (31.3)	0.9 (0.5–1.3)	
>5	272 (21.3)	87/271 (32.1)	0.9 (0.7–1.3)	
Frequency of travel out of village or town of residence, n = 1,276				
Never traveled	510 (40.0)	153/507 (30.2)	Referent, 1.0 (p = 0.252)	
Every day	19 (1.5)	10/19 (52.6)	2.6 (1.0–6.5)	
≥1×/wk	58 (4.5)	22/58 (37.9)	1.4 (0.8–2.5)	
≥1×/mo.	235 (18.4)	77/232 (33.2)	1.1 (0.8–1.6)	
<1×/mo.	454 (35.6)	148/450 (32.9)	1.1 (0.9–1.5)	

*Seropositivity defined as >99.03 ELISA Units/mL. Alternative cutoff calculated in EBOV-naive persons from the United Kingdom. EBOV GP–specific binding antibodies were indeterminate in 10 participants. p values calculated by using likelihood ratio test. EBOV–GP, Ebola virus glycoprotein; EVD, Ebola virus disease; OR, odds ratio.
†Adjusted for age and sex.
‡Age adjusted for sex. Sex adjusted for age.

Appendix Table 5. Potential EVD exposure in community or at work during the 2014–2016 Ebola outbreak and EBOV GP–specific binding antibody seropositivity among participants, Sierra Leone*

Risk factors	No. (%), n = 1,282	No. seropositive/no. tested (%)	Odds ratio (95% CI)
Living in a village/town with Ebola cases, n = 1,281			
N	199 (15.5)	57/198 (28.8)	Referent, 1.0 (p = 0.252)
Y	1,082 (84.5)	353/1,073 (32.9)	1.2 (0.9–1.7)
Knowing someone who had Ebola			
No, don't know	1,044 (81.4)	331/1,036 (32.0)	Referent, 1.0 (p = 0.565)
Y	238 (18.6)	80/236 (33.9)	1.1 (0.8–1.5)
No. EVD cases known by participant			
0	1,044 (81.4)	331/1,036 (31.9)	Referent, 1.0 (p = 0.608)
1	125 (9.8)	39/125 (31.2)	1.0 (0.6–1.4)
2–3	66 (5.2)	26/65 (40.0)	1.4 (0.9–2.4)
>3	47 (3.7)	15/46 (32.6)	1.0 (0.5–1.9)
Closest relationship with an EVD case, n = 1,280			
No relationship†	1,044 (81.5)	331/1,036 (32.0)	Referent, 1.0 (p = 0.500)
Close family‡	27 (2.1)	7/27 (25.9)	0.7 (0.3–1.8)
Other relative	52 (4.1)	16/51 (31.4)	1.0 (0.5–1.8)
Friend	59 (4.6)	18/59 (30.5)	0.9 (0.5–1.7)
Community member	98 (7.7)	39/97 (40.2)	1.4 (0.9–2.2)
Living in the same household with an EVD case, n = 1,280			
N	1,269 (99.1)	407/1,260 (32.3)	Referent, 1.0 (p = 0.876)
Y	11 (0.9)	3/10 (30.0)	0.9 (0.2–3.5)
Caring for an EVD case, n = 1,281			
N	1,272 (99.3)	408/1,262 (32.3)	Referent, 1.0 (p = 0.504)

Risk factors	No. (%), n = 1,282	No. seropositive/no. tested (%)	Odds ratio (95% CI)
Y	9 (0.7)	2/9 (22.2)	0.6 (0.1–2.9)
Direct body contact with an EVD case, n = 1,281			
N	1,275 (99.5)	408/1,265 (32.3)	Referent, 1.0 (p = 0.955)
Y	6 (0.5)	2/6 (33.3)	1.1 (0.2–5.8)
Attending a funeral of an EVD case			
N	1,263 (98.5)	404/1,254 (32.2)	Referent, 1.0 (p = 0.554)
Y	19 (1.5)	7/18 (38.9)	1.3 (0.5–3.5)
Health care frontline worker during EVD outbreak			
No, NA§	1,254 (97.8)	403/1,244 (32.4)	Referent, 1.0 (p = 0.665)
Y	28 (2.2)	8/28 (28.6)	0.8 (0.4–1.9)

*Seropositivity defined as >99.03 ELISA units/mL. Alternative cutoff calculated in EBOV-naive persons from the United Kingdom. EBOV GP–specific binding antibodies were indeterminate in 10 participants. p values calculated by using likelihood ratio test. Because none of the variables was associated with seropositivity in univariable analysis, the adjusted odds ratio column is omitted from the table. EBOV GP, Ebola virus glycoprotein; EVD, Ebola virus disease; NA, not applicable.
†No relationship; participant did not know anyone with Ebola.
‡Participant was the parent or child or spouse or sibling of an EVD case.
§Not applicable; participant was a child or did not have a job.

Appendix Table 6. Potential risk factors for transmission of Ebola virus from animals during the 2014–2016 Ebola outbreak and EBOV GP–specific binding antibody seropositivity among participants, Sierra Leone*

Risk Factors	No. (%), n = 1,282	No. seropositive/no. tested (%)	OR (95% CI)	Adjusted OR (95% CI)†
Number of domestic animals in the participant's compound				
0	503 (39.2)	150/498 (30.1)	Referent, 1.0 (p = 0.362)	
1–5	374 (29.2)	122/371 (32.8)	1.1 (0.9–1.5)	
>5	405 (31.6)	139/403 (34.5)	1.2 (0.9–1.6)	
Having the following domestic animals in the compound, n = 1,281‡				
Dog				
N	1,116 (87.1)	353/1,107 (31.9)	Referent, 1.0 (p = 0.377)	
Y	165 (12.9)	58/164 (35.4)	1.2 (0.8–1.6)	
Cat				
N	951 (74.2)	304/943 (32.2)	Referent, 1.0 (p = 0.898)	
Y	330 (25.8)	107/328 (32.6)	1.0 (0.8–1.3)	
Goat, sheep				
N	870 (67.9)	277/863 (32.1)	Referent, 1.0 (p = 0.790)	
Y	411 (32.1)	134/408 (32.8)	1.0 (0.8–1.3)	
Pig				
N	1,263 (98.6)	399/1,253 (31.8)	Referent, 1.0 (p = 0.003)	Referent, 1.0 (p = 0.004)
Y	18 (1.4)	12/18 (66.7)	4.3 (1.6–11.5)	4.1 (1.5–11.4)
Other				
N	825 (64.4)	258/817 (31.6)	Referent, 1.0 (p = 0.439)	
Y	456 (35.6)	153/454 (33.7)	1.1 (0.9–1.4)	
Touching sick or dead domestic animals				
N	1,253 (97.7)	400/1,243 (32.2)	Referent, 1.0 (p = 0.518)	
Y	29 (2.3)	11/29 (37.9)	1.3 (0.6–2.8)	
Hunting for wild animals§				
N	1,261 (99.3)	404/1,251 (32.3)	Referent, 1.0 (p = 0.947)	
Y	9 (0.7)	3/9 (33.3)	1.0 (0.3–4.2)	
Touching sick or dead wild animals				
N	1,277 (99.6)	410/1,267 (32.4)	Referent, 1.0 (p = 0.538)	
Y	5 (0.4)	1/5 (20.0)	0.5 (0.1–4.7)	
Consumption of bush meat				
N	1,275 (99.4)	409/1,265 (32.3)	Referent, 1.0 (p = 0.830)	
Y	7 (0.6)	2/7(28.6)	0.8 (0.2–4.3)	

*Seropositivity defined as >99.03 ELISA units/mL. Alternative cutoff calculated in EBOV-naive persons from the United Kingdom. EBOV GP–specific binding antibodies were indeterminate in 10 participants. p values calculated by using likelihood ratio test. EBOV–GP, Ebola virus glycoprotein.
†Adjusted for age and sex.
‡Participants could indicate >1 type of domestic animal.
§Types of wild animals hunted by participants who answered yes included monkeys, duiker antelopes, bats, and rodents.

Appendix Table 7. Additional sociodemographic characteristics of the study population not included in the risk factor analysis of Ebola virus IgG seroprevalence, Sierra Leone

Characteristics	No. (%); n = 1,282
Occupation	
Salaried employment	74 (5.8)
Self-employed, e.g., trader or farmer	211 (16.5)
Housewife	18 (1.4)
Unemployed	78 (6.1)
Student or apprentice	635 (49.5)
Preschool child	259 (20.2)
Other	7 (0.5)
Religion*	
Muslim	1,062 (82.9)
Christian	217 (16.9)
None	2 (0.2)
Tribe	
Themne	861 (67.2)
Limba	159 (12.4)
Soso	115 (9.0)
Fula	36 (2.8)
Mende	44 (3.4)
Other	67 (5.2)

*Religion not available for 1 participant.

Appendix Table 8. Additional travel information for persons reporting travel outside their village or city of residence during the Ebola virus disease outbreak, Sierra Leone, March 2014–January 2016

Characteristics	No. (%); n = 770*
Destination of most recent journey†	
Major cities, i.e., Freetown	361 (46.9)
Village in the same chiefdom	172 (22.3)
Different chiefdom within same district	136 (17.7)
Another district within Sierra Leone	43 (5.6)
Guinea	49 (6.4)
Traveling time to the farthest destination‡	
<1 h	148 (19.4)
1–2 h	251 (32.9)
3–6 h	344 (45.2)
All day, >1 d	19 (2.5)
Purpose of the trip	
Visiting someone	498 (64.7)
Work, business	141 (18.3)
Attending a funeral	22 (2.8)
Attending another event§	36 (4.7)
Seeking healthcare	9 (1.2)
Accompanying somebody	13 (1.7)
Study or holiday	16 (2.1)
Other reasons	35 (4.5)

*N = 770 correspond to 766 participants who reported a travel frequency in Appendix Table 1 plus 4 participants with missing data on travel frequency but who reported a travel destination for their most recent journey outside their village/town of residence.
†Participants could indicate more than one destination; information not available for 40 participants.
‡Information not available for 8 participants.
§Other events included weddings, feasts, football matches, and religious ceremonies.

Appendix Table 9. Information on illness or medical issues during the Ebola virus disease outbreak, Sierra Leone, March 2014–January 2016*

Characteristics	No. (%); n = 1,282
Being unwell during the EVD outbreak	
Y	219 (17.1)
N	1,051 (82.0)
Don't know, don't remember	11 (0.9)
Participants who reported being unwell during the EVD outbreak, n = 219†	
Medical issues or symptoms	
Headache	169 (77.2)
Fever	111 (50.7)
Vomiting	25 (11.4)
Diarrhea	18 (8.2)
Joint and muscle pain	73 (33.3)
Rash	17 (7.8)
Muscle weakness	39 (17.8)
Other symptoms	30 (13.7)
Duration of symptoms	
Few hours	51 (23.3)
1–2 d	96 (43.8)
About 1 week	47 (21.5)
>1 week	22 (10.0)
Don't know	3 (1.4)
Seen by a doctor or nurse, n = 216	
Y	97 (44.9)
N	119 (55.1)
Any condition diagnosed, n = 216	
Y‡	80 (37.0)
N	11 (5.1)
Don't know, don't remember	6 (2.8)
Not applicable§	119 (55.1)
Given any treatment, n = 216	
Y	94 (43.5)
N	2 (0.9)
Don't know, don't remember	1 (0.5)
Not applicable§	119 (55.1)
Female participants of childbearing potential, aged 16–50 y, n = 157	
Experienced a miscarriage during the EVD outbreak	
Y	2 (1.6)
N	125 (98.4)
Experienced a stillbirth during the EVD outbreak	
Y	1 (0.8)
N	126 (99.2)

*EVD, Ebola virus disease.
†Percentages calculated only among the participant who reported being unwell during the EVD outbreak, n = 219. Information not available in 3 participants.
‡Diagnoses: malaria (n = 45); typhoid fever/diarrhea with or without concomitant malaria infection (n = 9); pneumonia (n = 1); pulmonary tuberculosis (n = 1); other conditions (n = 14); no diagnosis available (n = 14).
§Not applicable participants were not seen by a doctor.

Paper 2: Safety and long-term immunogenicity of the two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in adults in Sierra Leone: a combined open-label, non-randomised stage 1, and a randomised, double-blind, controlled stage 2 trial

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	150330	**Title**	Dr
First Name(s)	Daniela		
Surname/Family Name	Manno		
Thesis Title	Testing a prophylactic vaccine regimen against Ebola virus disease in Sierra Leone: vaccine safety, immunogenicity and factors affecting immunogenicity		
Primary Supervisor	Professor Deborah Watson Jones		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	Lancet Infectious Diseases		
When was the work published?	January 2022		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	PhD by Prior Publication		
Have you retained the copyright for the work?*	**No**	Was the work subject to academic peer review?	**Yes**

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	

Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I was one of the coordinators of the EBOVAC-Salone trial and was involved in the conduct of the study, collection of data and interpretation of results. I am the co-first author and corresponding author of this paper. I drafted the manuscript with the other co-first author, Dr David Ishola, and I did the literature search for and drafted the 'research in context' section. As the corresponding author, I was responsible for communication with the journal during the manuscript submission, peer-review and publication process.

SECTION E

Student Signature	
Date	

Supervisor Signature	
Date	

EVIDENCE OF PERMISSION FROM THE COPYRIGHT HOLDER

Paper 2: Safety and long-term immunogenicity of the two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in adults in Sierra Leone: a combined open-label, non-randomised stage 1, and a randomised, double-blind, controlled stage 2 trial

Lancet Infectious Disease Journal:

Elsevier copyright policy

https://www.elsevier.com/about/policies-and-standards/copyright#4-find-out-more

Sample publishing agreement for articles financed by journal subscriptions

https://assets.ctfassets.net/o78em1y1w4i4/6N5X1SSw2m4L16unMQBez0/48e9195b92fe4fcce63be69e8da5724b/2023_Standard_JPA_updated_August_2023.pdf

Author Rights for Scholarly Purposes:

The Author Rights include the right to use the Preprint, Accepted Manuscript and **the Published Journal Article for Personal Use.**

Personal Use:

Use by an author in the author's classroom teaching (including distribution of copies, paper or electronic) or presentation by an author at a meeting or conference (including distribution of copies to the delegates attending such meeting), distribution of copies (including through e-mail) to known research colleagues for their personal use, use in a subsequent compilation of the author's works, **inclusion in a thesis or dissertation**, preparation of other derivative works such as extending the Article to book-length form, or otherwise using or re-using portions or excerpts in other works (with full acknowledgment of the original publication of the Article).

Safety and long-term immunogenicity of the two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in adults in Sierra Leone: a combined open-label, non-randomised stage 1, and a randomised, double-blind, controlled stage 2 trial

David Ishola, Daniela Manno*, Muhammed O Afolabi, Babajide Keshinro, Viki Bockstal, Baimba Rogers, Kwabena Owusu-Kyei, Alimamy Serry-Bangura, Ibrahim Swaray, Brett Lowe, Dickens Kowuor, Frank Baiden, Thomas Mooney, Elizabeth Smout, Brian Köhn, Godfrey T Otieno, Morrison Jusu, Julie Foster, Mohamed Samai, Gibrilla Fadlu Deen, Heidi Larson, Shelley Lees, Neil Goldstein, Katherine E Gallagher, Auguste Gaddah, Dirk Heerwegh, Benoit Callendret, Kerstin Luhn, Cynthia Robinson, Maarten Leyssen, Brian Greenwood, Macaya Douoguih, Bailah Leigh, Deborah Watson-Jones, on behalf of the EBL3001 study group*

Summary

Background The Ebola epidemics in west Africa and the Democratic Republic of the Congo highlight an urgent need for safe and effective vaccines to prevent Ebola virus disease. We aimed to assess the safety and long-term immunogenicity of a two-dose heterologous vaccine regimen, comprising the adenovirus type 26 vector-based vaccine encoding the Ebola virus glycoprotein (Ad26.ZEBOV) and the modified vaccinia Ankara vector-based vaccine, encoding glycoproteins from Ebola virus, Sudan virus, and Marburg virus, and the nucleoprotein from the Tai Forest virus (MVA-BN-Filo), in Sierra Leone, a country previously affected by Ebola.

Methods The trial comprised two stages: an open-label, non-randomised stage 1, and a randomised, double-blind, controlled stage 2. The study was done at three clinics in Kambia district, Sierra Leone. In stage 1, healthy adults (aged ≥18 years) residing in or near Kambia district, received an intramuscular injection of Ad26.ZEBOV (5×10^{10} viral particles) on day 1 (first dose) followed by an intramuscular injection of MVA-BN-Filo (1×10^{8} infectious units) on day 57 (second dose). An Ad26.ZEBOV booster vaccination was offered at 2 years after the first dose to stage 1 participants. The eligibility criteria for adult participants in stage 2 were consistent with stage 1 eligibility criteria. Stage 2 participants were randomly assigned (3:1), by computer-generated block randomisation (block size of eight) via an interactive web-response system, to receive either the Ebola vaccine regimen (Ad26.ZEBOV followed by MVA-BN-Filo) or an intramuscular injection of a single dose of meningococcal quadrivalent (serogroups A, C, W135, and Y) conjugate vaccine (MenACWY; first dose) followed by placebo on day 57 (second dose; control group). Study team personnel, except those with primary responsibility for study vaccine preparation, and participants were masked to study vaccine allocation. The primary outcome was the safety of the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen, which was assessed in all participants who had received at least one dose of study vaccine. Safety was assessed as solicited local and systemic adverse events occurring in the first 7 days after each vaccination, unsolicited adverse events occurring in the first 28 days after each vaccination, and serious adverse events or immediate reportable events occurring up to each participant's last study visit. Secondary outcomes were to assess Ebola virus glycoprotein-specific binding antibody responses at 21 days after the second vaccine in a per-protocol set of participants (ie, those who had received both vaccinations within the protocol-defined time window, had at least one evaluable post-vaccination sample, and had no major protocol deviations that could have influenced the immune response) and to assess the safety and tolerability of the Ad26.ZEBOV booster vaccination in stage 1 participants who had received the booster dose. This study is registered at ClinicalTrials.gov, NCT02509494.

Findings Between Sept 30, 2015, and Oct 19, 2016, 443 participants (43 in stage 1 and 400 in stage 2) were enrolled; 341 participants assigned to receive the Ad26.ZEBOV and MVA-BN-Filo regimen and 102 participants assigned to receive the MenACWY and placebo regimen received at least one dose of study vaccine. Both regimens were well tolerated with no safety concerns. In stage 1, solicited local adverse events (mostly mild or moderate injection-site pain) were reported in 12 (28%) of 43 participants after Ad26.ZEBOV vaccination and in six (14%) participants after MVA-BN-Filo vaccination. In stage 2, solicited local adverse events were reported in 51 (17%) of 298 participants after Ad26.ZEBOV vaccination, in 58 (24%) of 246 after MVA-BN-Filo vaccination, in 17 (17%) of 102 after MenACWY vaccination, and in eight (9%) of 86 after placebo injection. In stage 1, solicited systemic adverse events were reported in 18 (42%) of 43 participants after Ad26.ZEBOV vaccination and in 17 (40%) after MVA-BN-Filo vaccination. In stage 2, solicited systemic adverse events were reported in 161 (54%) of 298 participants after Ad26.ZEBOV vaccination, in 107 (43%) of 246 after MVA-BN-Filo vaccination, in 51 (50%) of 102 after MenACWY vaccination, and in 39 (45%) of 86 after placebo injection. Solicited systemic adverse events in both stage 1 and 2 participants included mostly mild or moderate headache, myalgia, fatigue,

Lancet Infect Dis 2022; 22: 97–109

Published Online September 13, 2021 https://doi.org/10.1016/S1473-3099(21)00125-0

This online publication has been corrected. The corrected version first appeared at thelancet.com/infection on July 25, 2023

See Comment page 8

*Joint first authors

London School of Hygiene & Tropical Medicine, London, UK (D Ishola PhD, D Manno MD, M O Afolabi PhD, K Owusu-Kyei MSc, B Lowe MSc, D Kowuor MSc, F Baiden PhD, T Mooney MA, E Smout MSc, B Köhn BSc, G T Otieno BSc, M Jusu MPH, J Foster MSc, Prof H Larson PhD, S Lees PhD, K E Gallagher PhD, Prof B Greenwood MD, Prof D Watson-Jones PhD); **EBOVAC Project, Kambia, Kambia district, Sierra Leone** (D Ishola, M O Afolabi, B Rogers MSc, K Owusu-Kyei, A Serry-Bangura MD, I Swaray MSc, D Kowuor, F Baiden, T Mooney, E Smout, B Köhn, G T Otieno, M Jusu); **Janssen Vaccines and Prevention BV, Leiden, Netherlands** (B Keshinro FWACP, V Bockstal PhD, N Goldstein MD, B Callendret PhD, K Luhn PhD, C Robinson MD, M Leyssen MD, M Douoguih MD); **College of Medicine and Allied Health Sciences, University of Sierra Leone, Freetown, Sierra Leone** (B Rogers, A Serry-Bangura, I Swaray, M Jusu, M Samai PhD, G F Deen FWACP, B Leigh MD); **KEMRI-Wellcome Trust**

and arthralgia. The most frequent unsolicited adverse event after the first dose was headache in stage 1 and malaria in stage 2. Malaria was the most frequent unsolicited adverse event after the second dose in both stage 1 and 2. No serious adverse event was considered related to the study vaccine, and no immediate reportable events were observed. In stage 1, the safety profile after the booster vaccination was not notably different to that observed after the first dose. Vaccine-induced humoral immune responses were observed in 41 (98%) of 42 stage 1 participants (geometric mean binding antibody concentration 4784 ELISA units [EU]/mL [95% CI 3736–6125]) and in 176 (98%) of 179 stage 2 participants (3810 EU/mL [3312–4383]) at 21 days after the second vaccination.

Interpretation The Ad26.ZEBOV and MVA-BN-Filo vaccine regimen was well tolerated and immunogenic, with persistent humoral immune responses. These data support the use of this vaccine regimen for Ebola virus disease prophylaxis in adults.

Funding Innovative Medicines Initiative 2 Joint Undertaking and Janssen Vaccines & Prevention BV.

Research Programme, Kilifi, Kenya (B Lowe); Centre for Tropical Medicine and Global Health, University of Oxford,Oxford, UK (B Lowe); Department of Health Metrics Sciences, University of Washington, Seattle, WA, USA (Prof H Larson); Janssen Research & Development, Beerse, Belgium (A Gaddah PhD, D Heerwegh PhD); Mwanza Intervention Trials Unit, National Institute for Medical Research, Mwanza, Tanzania (Prof D Watson-Jones)

Correspondence to:
Dr Daniela Manno, London School of Hygiene & Tropical Medicine, London WC1E 7HT, UK
daniela.manno@lshtm.ac.uk

Introduction

The magnitude of the Ebola virus outbreak in 2014–16 in west Africa was unprecedented, with more than 28 600 cases reported and 11 300 deaths.[1] The second largest outbreak began in 2018 in the Democratic Republic of the Congo and lasted for nearly 2 years, with more than 3400 cases and 2200 deaths reported.[2] Other small Ebola virus disease outbreaks have occurred since

Research in context

Evidence before this study

We searched PubMed on Feb 20, 2020, using the search terms "ebola" AND "vaccin* OR immunis* OR immuniz*" AND "trial* OR study". We searched for articles published between database inception up to Feb 20, 2020, with no language restrictions. We identified 733 articles. After screening the titles and abstracts, we identified 40 publications reporting on the immunogenicity or safety, or both, of Ebola vaccine candidates across 34 clinical trials. We also consulted a WHO overview of candidate Ebola virus vaccines dated Aug 19, 2019. Several vaccine candidates have been tested in phase 1 and 2 clinical trials (eg, the recombinant vesicular stomatitis virus-vectored vaccine expressing the glycoprotein of Ebola virus [rVSV-ZEBOV-GP]; the recombinant chimpanzee adenovirus type 3 virus-vectored vaccine encoding the glycoprotein of Ebola virus [ChAd3-EBO-Z]; the recombinant adenovirus type-5 vectored vaccine encoding the glycoprotein of Ebola virus [Ad5-EBOV]; and a vaccine consisting of rVSV and Ad5 encoding the glycoprotein of Ebola virus [GamEvac Combi]), with acceptable safety profiles and promising immunogenicity results. Data on the effectiveness against Ebola virus disease were available for only the rVSV-ZEBOV-GP vaccine, with an estimated effectiveness of 100% in a ring vaccination trial done in Guinea during the 2014–16 outbreak, and of 97·5% in a ring vaccination strategy to control the 2018–20 Ebola virus disease outbreak in the Democratic Republic of the Congo. The two-dose heterologous vaccination regimen with a replication deficient adenovirus type 26 vector-based vaccine encoding the Ebola virus glycoprotein (Ad26.ZEBOV), and a non-replicating, recombinant, modified vaccinia Ankara vector-based vaccine, encoding glycoproteins from the Ebola virus, Sudan virus, and Marburg virus, and the nucleoprotein from the Tai Forest virus (MVA-BN-Filo) was shown to have a good safety profile in three phase 1 studies in European and healthy African individuals living in areas unaffected by the Ebola virus. The most common adverse events were pain at the injection site and headache. No vaccine-related serious adverse events were reported. The Ad26.ZEBOV and MVA-BN-Filo vaccine regimen induced durable immune responses for at least 1 year in healthy adults.

Added value of this study

To our knowledge, this is the first study to provide data on the safety, long-term immunogenicity, and humoral immune memory responses induced by the Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in healthy adults from a population severely affected by the 2014–16 Ebola virus disease epidemic in west Africa. The vaccine regimen was well tolerated and induced humoral immune responses that persisted for at least 2 years. Booster vaccination with Ad26.ZEBOV, given 2 years after the initial vaccination, induced a strong anamnestic response within 7 days. These findings will inform the future use of this vaccine regimen; for instance, by justifying the strategy of providing a booster to previously immunised individuals at the start of an Ebola virus disease outbreak. Our findings also supported the decision of the European Commission to grant marketing authorisations for the Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in the EU.

Implications of all the available evidence

Several vaccines against Ebola virus disease have been shown to be safe and immunogenic in clinical trials. One vaccine, rVSV-ZEBOV-GP, has also been shown to be highly effective in preventing Ebola virus disease. Vaccine research should continue to ascertain the long-term immunogenicity of these vaccines, and to assess different options for prophylactic vaccination in populations at potential risk of Ebola virus disease or for reactive vaccination during Ebola virus disease outbreaks.

then in the DR Congo and Guinea, and new outbreaks are likely to occur in the future.[3] Therefore, finding safe and effective vaccines against Ebola virus disease that can be used in combination with other outbreak control measures remains a priority. The recombinant vesicular stomatitis virus-vectored vaccine expressing the Ebola virus glycoprotein (rVSV-ZEBOV-GP) of the Kikwit strain, which showed effectiveness in a ring-vaccination trial done in Guinea during the 2014–16 outbreak,[4] was recommended by WHO for use in emergency situations, and was deployed widely as part of the outbreak control response in DR Congo.[5,6] This vaccine received conditional marketing authorisation in the EU, and was approved for use in adults in the USA and in several African countries.[7–9] However, as part of the preparedness measures for future outbreaks, the Strategic Advisory Group of Experts on Immunization recommended to WHO that urgent consideration should be given to the development of additional vaccines against Ebola, with a focus on safety and induction of appropriate immune responses.[6]

A heterologous, two-dose regimen, comprising the monovalent, recombinant, replication-incompetent, adenovirus type 26 (Ad26) vector-based vaccine, encoding the Ebola virus glycoprotein of the Mayinga variant (Ad26.ZEBOV) as the first vaccine, and the recombinant, non-replicating, modified vaccinia Ankara (MVA) vector-based vaccine, encoding glycoproteins from the Ebola virus Mayinga variant, Sudan virus Gulu variant, and Marburg virus Musoke variant, and the nucleoprotein from the Tai Forest virus (MVA-BN-Filo) administered 56 days after the first vaccine, has received marketing authorisation for prophylactic use, under exceptional circumstances, in adults and children aged 1 year and older in the EU.[10] This vaccine regimen provided protection against Ebola virus challenge in macaques and had a good safety profile, with strong and durable immune responses observed for at least 1 year in European and healthy African adults living in areas unaffected by Ebola.[11–15] In this study, we aimed to evaluate the safety, long-term immunogenicity, and humoral immune memory induced by the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen, administered with a 56-day interval between the two doses, in healthy adults in Kambia district, an area of Sierra Leone affected by the 2014–16 Ebola virus disease epidemic and, therefore, at potential risk for future outbreaks.[16]

Methods

Study design

The trial comprised two stages: an open-label, non-randomised stage 1, and a randomised, double-blind, controlled stage 2. The trial was done at three clinics in Kambia district. The rationale for an open-label stage 1 trial was to obtain initial safety data, as it was the first time that the experimental Ad26.ZEBOV and MVA-BN-Filo vaccine regimen was used in Sierra Leone, and the national health authority requested the inclusion of this initial stage in the study design. Enrolment of stage 1 participants was followed by initiation of stage 2 after review of stage 1 safety data by an independent data monitoring committee. The study was approved by the Sierra Leone Ethics and Scientific Review Committee, the Pharmacy Board of Sierra Leone, and the London School of Hygiene & Tropical Medicine ethics committee. The study protocol is available in the appendix (pp 25–154).

See Online for appendix

Participants

Eligible stage 1 participants were healthy adults aged 18 years or older residing in or near Kambia district, with no intention of leaving the area within the next 5 months, and who were considered healthy on the basis of physical examination and the absence of laboratory abnormalities at screening. Women of childbearing age were required to use adequate birth control measures (ie, contraceptive injection, oral contraception, or barrier methods) from at least 14 days before receiving the first vaccine, and to have a negative urine β-human chorionic gonadotropin pregnancy test at screening and immediately before each vaccination. Male participants who were sexually active were asked to use condoms, starting before enrolment. Exclusion criteria included breast feeding or pregnancy; previous Ebola virus disease or vaccination with a candidate Ebola vaccine; previous vaccination with a live-attenuated vaccine within 30 days before each dose, or with an inactivated vaccine within 15 days before each dose; or a previous severe adverse reaction to a vaccine. Extensive social science research was done before the start of the trial to ensure effective community engagement and the use of appropriate recruitment strategies.[17,18] Written informed consent from a community leader was required before the study start. Participants provided informed consent after passing a test of understanding. If the participant was unable to read or write, the procedures were explained, and informed consent was witnessed by a literate third person not involved in the study. Inclusion and exclusion criteria, and the procedures for obtaining written informed consent for stage 2 adult participants were similar to those for stage 1 participants. Stage 2 also enrolled children aged 1–17 years, and data from these paediatric cohorts are presented in a separate publication.[19] The full list of inclusion and exclusion criteria is provided in the study protocol (appendix pp 84–88).

Randomisation and masking

There was no randomisation in stage 1. Stage 2 participants were randomly assigned (3:1) to receive either Ad26.ZEBOV and MVA-BN-Filo (Ebola vaccine group) or the meningococcal quadrivalent (serogroups A, C, W135, and Y) conjugate vaccine (MenACWY) and placebo (control group). Randomisation was done

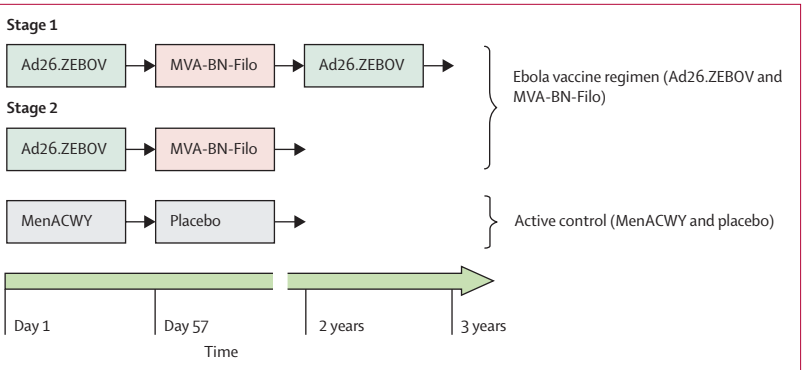


Figure 1: **Study design**
Vaccine doses were 5×10^{10} viral particles for Ad26.ZEBOV, 1×10^{8} infectious units for MVA-BN-Filo, 0·5 mL reconstituted vaccine solution for MenACWY, and 0·5 mL of 0·9% sodium chloride solution for the placebo. Ad26.ZEBOV=adenovirus type 26 vector-based vaccine encoding the Ebola virus glycoprotein. MenACWY=meningococcal quadrivalent (serogroups A, C, W135, and Y) conjugate vaccine. MVA-BN-Filo=modified vaccinia Ankara vector-based vaccine, encoding glycoproteins from the Ebola virus, Sudan virus, and Marburg virus, and the nucleoprotein from the Tai Forest virus.

centrally by computer-generated block randomisation (block size of eight) via an interactive web response system, which was operated by a study pharmacist. Study team personnel (except those with primary responsibility for study vaccine preparation) and participants were masked to study vaccine allocation until all participants had completed the last follow-up visit and the database was locked. Masking was achieved by use of syringes of identical volume, which were taped to conceal the colour of the liquid inside.

Procedures

In stage 1, all participants received Ad26.ZEBOV (Janssen Vaccines & Prevention BV, Leiden, Netherlands; first dose) followed by MVA-BN-Filo (Bavarian Nordic, Planegg, Germany; second dose) 56 days after the first dose. An Ad26.ZEBOV booster vaccination was also offered to stage 1 participants at 2 years (720 days) after the first dose (figure 1). Stage 2 adult participants in the Ebola vaccine group received the Ebola vaccine regimen (Ad26.ZEBOV followed by MVA-BN-Filo), and those in the control group received one dose of the MenACWY vaccine (Menveo [GSK Vaccines, Brentford, UK]; or Nimenrix [Pfizer, New York, NY, USA; first dose]) followed by a saline placebo (second dose) at 56 days after the first dose (figure 1). All vaccines were administered as a single 0·5 mL intramuscular injection into the deltoid muscle at a dose of 5×10^{10} viral particles for Ad26.ZEBOV, 1×10^{8} infectious units for MVA-BN-Filo, 0·5 mL reconstituted vaccine solution for MenACWY, and 0·5 mL sodium chloride solution (0·9%) as the placebo.

To record any immediate adverse events, participants were observed for at least 30 min after each vaccination. Participants recorded any solicited local and systemic adverse events using diary cards for 7 days following each vaccination. Clinical laboratory tests were done at 7 days after each vaccination, comprising a haematology panel (haemoglobin, haematocrit, red blood cell count, platelet count, and white blood cell count with differential) and a serum chemistry panel (alanine aminotransferase, aspartate aminotransferase, and creatinine) to check if there were any clinically relevant laboratory abnormalities that were reported as adverse events (appendix p 10). All participants received a 24-h telephone number to contact an on-call study physician in case of any medical problems. In stage 1, all adverse events were recorded from the first dose until 56 days after the second dose, and then again from the day of the booster vaccination until 28 days after the booster vaccination. In stage 2, adverse events were recorded for 28 days after each vaccination. In both stages 1 and 2, serious adverse events were recorded from the first dose until each participant's last study visit (ie, up to 3 years after the first dose in stage 1, and up to 2 years after the first dose in stage 2). Further information on the grading of adverse events is presented in the appendix (pp 119, 143–148).

In stage 1, immunological assays were done on blood samples taken immediately before the first and second doses, then at 21 days after the second dose, 155 and 359 days after the first dose, and, thereafter, once every 6 months up to 3 years after the first dose. In participants who agreed to the booster vaccination, additional immunogenicity samples were collected immediately before the booster vaccination and at 4 days, 7 days, 21 days, 6 months, and 1 year after the booster vaccination. After initial results from the phase 1 studies[12–14] were obtained, some timepoints were considered less relevant and the samples were not analysed. In stage 2, immunogenicity samples were collected immediately before the first dose, 28 days after the first dose, immediately before the second dose, 21 days and 6 months after the second dose, and 1 year and 2 years after the first dose.

Binding antibody responses against Ebola virus glycoprotein were analysed by use of the Ebola virus glycoprotein (Kikwit strain) Filovirus Animal Non-Clinical Group ELISA (validated by and done at Q² Solutions Vaccine Testing Laboratory [San Juan Capistrano, CA, USA]) using the methods described in previous studies.[12–15] In a randomly selected subset of stage 2 participants, the Ebola virus glycoprotein-specific neutralising antibody response was assessed by use of an Ebola virus glycoprotein (Makona strain) pseudovirion neutralisation assay, which was developed and validated by Monogram Biosciences (San Francisco, CA, USA), where this analysis was done (appendix pp 2). The presence of neutralising antibodies against the Ad26 and MVA vector backbones were measured at baseline by use of an Ad26-specific virus neutralisation assay, which was developed and qualified by Janssen Vaccines & Prevention BV, where this analysis was done, and a plaque reduction neutralisation test, which was developed and validated by Bavarian Nordic (Planegg, Germany), where this analysis was also done.

Outcomes

For stage 1 and 2, the primary study outcome was to assess the safety of the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen, defined as the occurrence of participants with solicited local and systemic adverse events in the first 7 days after each vaccination, unsolicited adverse events in the first 28 days after each vaccination, and serious adverse events or immediate reportable events up to the final study visit. The secondary outcomes were to assess Ebola virus glycoprotein-specific binding IgG antibody responses, as measured by ELISA at 21 days after the second dose in stage 1 and 2 participants; and to assess the safety and tolerability of the Ad26.ZEBOV booster vaccination administered at least 2 years after the first dose in stage 1 participants. Participants were considered as responders by ELISA if samples were negative at baseline and positive post-baseline with a value that was greater than 2·5 times the lower limit of quantification (LLOQ; 36·11 ELISA units [EU] per mL), or if a sample was positive both at baseline and post-baseline and there was a greater than 2·5-times increase from baseline.

The exploratory outcomes were to assess Ebola virus glycoprotein-specific binding antibody responses at other relevant timepoints (at 56, 155, 359, 539, and 719 days after the first dose, and at 4, 7, 21, and 359 days after the booster dose for stage 1; and at 56, 359 and 719 days after the first dose for stage 2) and to assess the neutralising activity of vaccine-induced antibody responses directed against Ebola virus glycoprotein and against the Ad26 and MVA vectors. Participants were considered as responders for the pseudovirion neutralisation assay if a sample was negative at baseline and positive post-baseline and the post-baseline value was greater than two times the LLOQ (a half maximal inhibitory concentration [IC50] titre of 120), or samples were positive both at baseline and post-baseline and there was a greater than two-times increase from baseline. Participants were considered as positive for the Ad26-specific virus neutralisation assay if a sample was greater than the LLOQ (a 90% inhibitory concentration titre of 17), and positive for the plaque reduction neutralisation test if the sample was greater than the LLOQ (an IC50 titre of 8). Only data from baseline samples are presented.

Statistical analysis

The planned sample size for stage 1 (n=40) and stage 2 (n=400; 300 receiving Ad26.ZEBOV and MVA-BN-Filo, and 100 receiving MenACWY and placebo) were calculated to provide, when combined, a probability of 99% or higher of observing at least one serious adverse event occurring in at least 10% of participants in each group. The probability of observing at least one serious adverse event occurring in 1% of participants was 95% with a total sample size of 300 participants.

For the analysis of the Ebola virus glycoprotein-specific neutralising antibody response, a subset of 74 (28%) of 260 stage 2 participants were selected at random with SAS (version 9.2) in a 3:1 ratio of Ebola vaccine group participants to control group participants to ensure that the distribution of the selected participants was similar to the overall distribution of participants across the randomised groups in stage 2. The random selection was done before the sample analysis among 260 stage 2 participants with available samples and no protocol deviations that could have influenced the immune response. Ebola virus glycoprotein-specific neutralising antibody responses were not analysed in stage 1 participants. The selection of a subset of 74 participants for this analysis was not based on a separate sample size calculation, but was instead based on the number of samples that could be analysed in a reasonable amount of time, and was considered large enough to provide a representative characterisation of the neutralising antibody response. For the analysis of the neutralising antibodies against the Ad26 (with the virus neutralisation assay) and MVA (with the plaque reduction neutralisation test) vectors, all stage 1 participants and the subset of 74 stage 2 participants were included. We subsequently decided to analyse the neutralising antibody response against the Ad26 vector in all remaining stage 2 participants in the per-protocol analysis set who received the Ebola vaccine regimen.

Analysis of the primary outcome in stage 1 and stage 2 was done when all participants had completed the study or had discontinued early. The primary analysis set for safety (full analysis set) comprised all participants who had received at least one dose of study vaccine. Data are shown by vaccination group (as treated). The primary analysis set for immunogenicity (per-protocol) included all vaccinated participants who received both the first and second doses within the protocol-defined window, had at least one evaluable post-vaccination sample, and had no major protocol deviations that could have influenced the immune response. A sensitivity analysis was done in participants who received the second dose outside the protocol-defined window. Since the main purpose of stages 1 and 2 was to provide preliminary evaluation of safety and immunogenicity without formal hypothesis testing, all data were analysed by use of descriptive statistics.

Binding antibody responses against Ebola virus glycoprotein are shown as geometric mean concentrations (GMCs), and neutralising antibody activity is shown as geometric mean titres (GMTs), both with their associated 95% CIs. All values less than the LLOQ were imputed as half the LLOQ value. We calculated Spearman's correlation coefficients to assess associations between Ebola virus glycoprotein-specific binding antibody concentrations and pseudovirion neutralisation assay titres at 21 days after the second dose. We did a post-hoc correlation analysis between Ad26 neutralising antibody titres before vaccination and Ebola virus glycoprotein-specific binding antibody responses at 21 days after the second dose. In addition, a post-hoc correlation analysis between Ebola virus glycoprotein-specific binding antibody concentrations

measured at baseline and Ebola virus glycoprotein-specific binding antibody concentrations at 21 days after the second dose was done (appendix pp 3, 20).

All statistical analyses were done using SAS, version 9.2. This study is registered with ClinicalTrials.gov, NCT02509494.

Role of the funding source

The Innovative Medicines Initiative 2 Joint Undertaking had no role in study design, data collection, data analysis, data interpretation, or writing of this report. Janssen Vaccines & Prevention BV had a role in study design, data collection, data analysis, data interpretation, and writing of the report.

Results

Between Sept 30, 2015, and Oct 19, 2016, adult participants were recruited, and follow-up was completed on Nov 28, 2018. In stage 1, 106 individuals were screened, of whom 43 received at least the first dose of the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen and were included in the full analysis set (figure 2A). Of 769 screened individuals

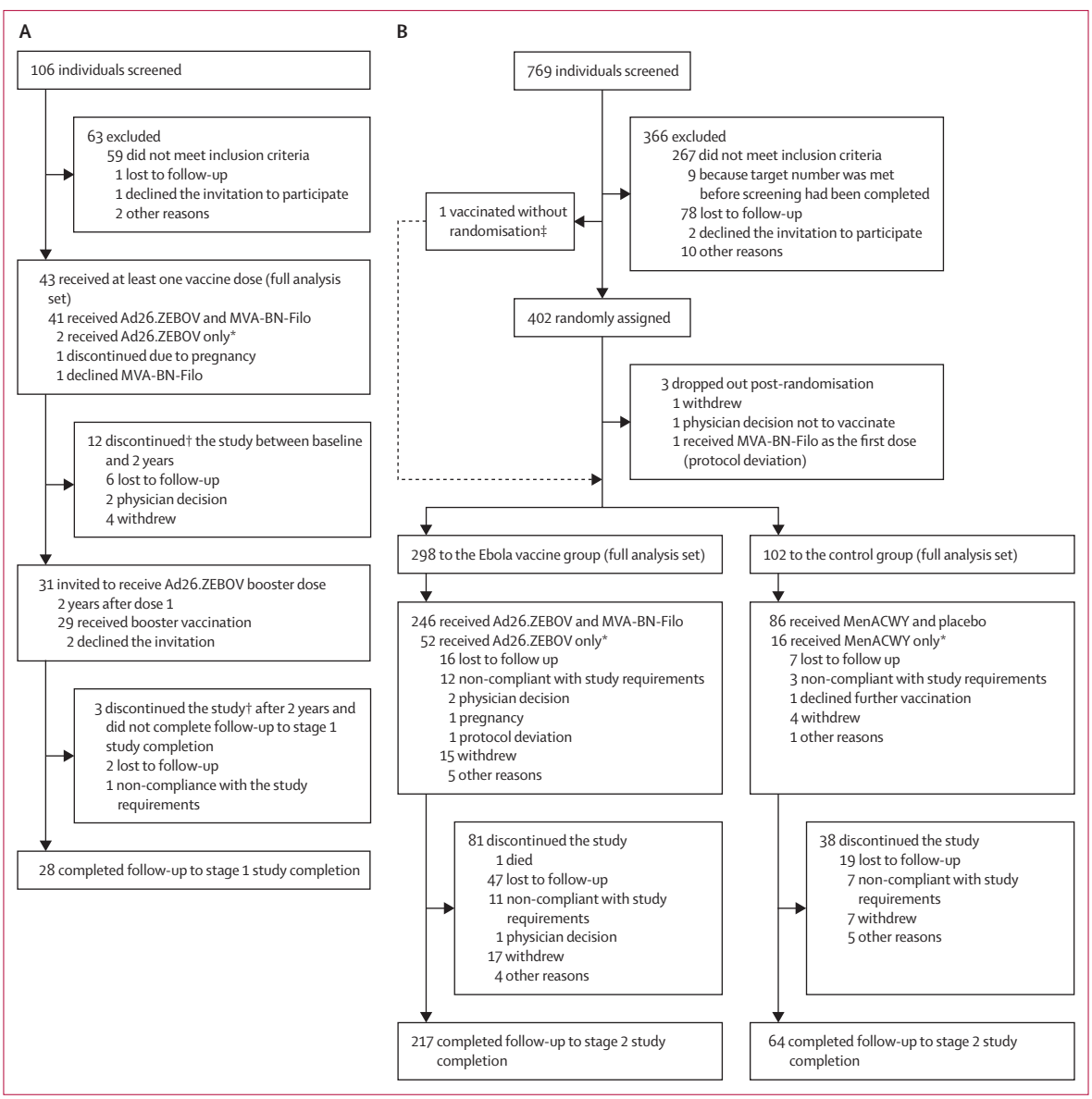


Figure 2: **Stage 1 (A) and stage 2 (B) trial profiles**

Ad26.ZEBOV=adenovirus type 26 vector-based vaccine encoding the Ebola virus glycoprotein. MenACWY=meningococcal quadrivalent (serogroups A, C, W135, and Y) conjugate vaccine. MVA-BN-Filo=modified vaccinia Ankara vector-based vaccine, encoding glycoproteins from the Ebola virus, Sudan virus, and Marburg virus, and the nucleoprotein from the Tai Forest virus. *Participants did not receive the second vaccine irrespective of whether follow-up continued to study completion. †Follow-up did not continue to the end of the study, irrespective of the number of doses received. ‡This individual was properly screened and found to be eligible, but received the Ad26.ZEBOV vaccine before randomisation due to a protocol deviation.

in stage 2, 402 were randomly assigned and 400 received at least the first dose of the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen (Ebola vaccine group; n=298) or the MenACWY and placebo regimen (control group; n=102; figure 2B) and were included in the full analysis set. The baseline characteristics of all participants are shown in table 1. 29 (94%) of 31 stage 1 participants invited to receive the booster vaccination received the booster at 2 years after the first dose.

Solicited adverse events were mostly mild to moderate (grade 1 and 2) in severity (figure 3; appendix pp 4–8). In stage 1, at least one solicited local adverse event was reported in 12 (28%) of 43 participants after Ad26.ZEBOV vaccination and in six (14%) participants after MVA-BN-Filo vaccination (figure 3A, C; appendix pp 4–5). In stage 2, at least one solicited local adverse event was reported in 51 (17%) of 298 participants after Ad26.ZEBOV vaccination and in 58 (24%) of 246 participants after MVA-BN-Filo vaccination. In the control group, at least one solicited local adverse event was reported in 17 (17%) of 102 participants after MenACWY vaccination and in eight (9%) of 86 participants after placebo injection (figures 3A, C; appendix pp 4–5). The most frequent solicited local adverse event was injection-site pain after any vaccination (figure 3A, C; appendix pp 4–5). One (<1%) stage 2 participant had a grade 3 solicited local adverse event of injection-site pain after MVA-BN-Filo vaccination (figure 3C; appendix pp 4–5).

Solicited systemic adverse events in stage 1 were reported by 18 (42%) participants after Ad26.ZEBOV vaccination and by 17 (40%) after MVA-BN-Filo vaccination (figure 3B, D; appendix pp 6–8). In stage 2, at least one solicited systemic adverse event was reported in 161 (54%) participants after Ad26.ZEBOV vaccination, in 107 (43%) after MVA-BN-Filo vaccination, in 51 (50%) after MenACWY vaccination, and in 39 (45%) after placebo injection (figure 3B, 3D; appendix pp 6–8). Headache, myalgia, fatigue, and arthralgia were the most frequently reported solicited systemic adverse events after any vaccination, and grade 3 solicited systemic adverse events were infrequently observed (figure 3B, D; appendix pp 6–8).

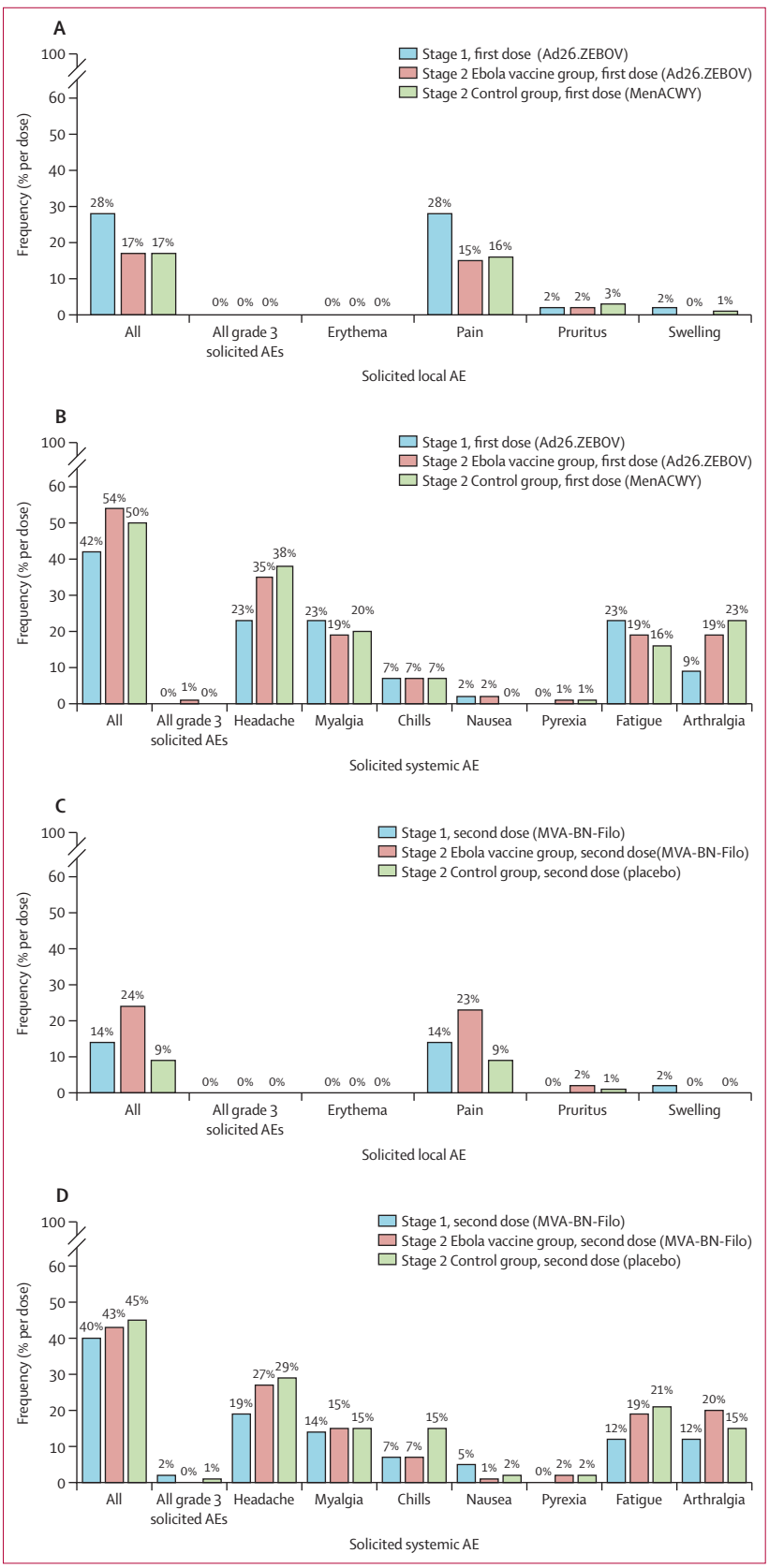
In stage 1, unsolicited adverse events occurred in 17 (40%) of 43 participants after dose 1 and in 17 (40%) after dose 2. In stage 2, unsolicited events were reported in 198 (66%) of 298 participants after Ad26.ZEBOV vaccination, 145 (59%) of 246 after MVA-BN-Filo vaccination, 65 (64%) of 102 after MenACWY vaccination, and 48 (56%) of 86 after placebo injection (appendix p 9). The most frequent unsolicited adverse event after the first dose was headache in stage 1 and malaria in stage 2. Malaria was the most frequent unsolicited adverse event after the second dose in both stage 1 and 2 (appendix p 9). Grade 3 unsolicited adverse events were infrequent; observed in 2% of participants at most, regardless of vaccine received (appendix p 10).

	Stage 1 (n=43)	Stage 2	
		Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine group (n=298)	MenACWY and placebo control group (n=102)
Sex			
Female	1 (2%)	50 (17%)	22 (22%)
Male	42 (98%)	248 (83%)	80 (78%)
Age at screening, years	23 (20–27)	23 (21–31)	25 (21–35)
Height, cm	170 (167–173)	169 (163–173)	166 (162–173)
Weight, kg	63 (58–68)	62 (56–67)	61 (56–67)
Body-mass index, kg/m²	22 (21–23)	22 (20–23)	22 (20–23)

Data are n (%) or median (IQR). Participants in stage 1 were assigned to receive Ad26.ZEBOV, followed by MVA-BN-Filo 56 days later; a subset of these participants received a booster of Ad26.ZEBOV 2 years after the first dose. Ad26.ZEBOV=adenovirus type 26 vector-based vaccine encoding the Ebola virus glycoprotein. MVA-BN-Filo=modified vaccinia Ankara vector-based vaccine, encoding glycoproteins from the Ebola virus, Sudan virus, and Marburg virus, and the nucleoprotein from the Tai Forest virus. MenACWY=meningococcal quadrivalent (serogroups A, C, W135, and Y) conjugate vaccine.

Table 1: **Participant demographic and baseline characteristics**

At least one serious adverse event was reported in 23 (5%) of all 443 stage 1 and stage 2 participants during the study period (appendix pp 11–12); some participants had more than one serious adverse event. In 20 (87%) of 23 participants who reported a serious adverse event during the study period, the event occurred more than 28 days after vaccination, either with the first dose, the second dose, or the booster. In the 28-day period after the first dose, no stage 1 participants and two (<1%) of 298 stage 2 participants in the Ebola vaccine group reported at least one serious adverse event after Ad26.ZEBOV vaccination. One (1%) of 102 stage 2 participants in the control group reported at least one serious adverse event within 28 days of receiving the MenACWY vaccination. No serious adverse events were reported in stage 1 or stage 2 participants in the 28-day period after receiving the second dose. In addition, no stage 1 participants reported a serious adverse event in the 28-day period after receiving the booster dose. No reported serious adverse event was considered related to the study vaccine, and no immediate reportable events were observed. One death occurred in the Ebola vaccine group during the long-term follow-up period at day 197 after the second dose. This individual, who had a history of heavy alcohol consumption and use of unidentified traditional herbal medications, died due to severe dehydration caused by severe vomiting. The most commonly reported laboratory abnormality in stage 1 and 2 participants was a decrease in haemoglobin concentrations from baseline. Only two participants had haemoglobin concentrations less than the local laboratory range of normal, and no laboratory abnormalities were considered clinically significant by the investigator.



The post-booster vaccination adverse event profile in stage 1 participants who received the Ad26.ZEBOV booster vaccination at 2 years after the first dose was not notably different to that observed after the first dose (appendix pp 4–12).

All 43 stage 1 participants and 259 (65%) of 400 stage 2 participants (191 in the Ebola vaccine group and 68 in the control group) fulfilled the criteria for the per-protocol analysis set for the immunogenicity analyses. At 56 days after the first dose, Ebola virus glycoprotein-specific binding antibody responses were observed in 28 (65%) of 43 stage 1 participants (GMC 269 EU/mL [95% CI 208–347]) and 101 (54%) of 187 stage 2 participants (236 EU/mL [206–270]) in the Ebola vaccine group (table 2; figure 4A). At 21 days after the second dose, Ebola virus glycoprotein-specific binding antibody responses were observed in 41 (98%) of 42 stage 1 participants (4784 EU/mL [3736–6125]) and in 176 (98%) of 179 stage 2 participants (3810 EU/mL [3312–4383]).

Due to a study pause, which occurred between April 28 and June 16, 2016 for precautionary reasons during the evaluation of two serious adverse events following the administration of the same Ebola vaccine regimen in a different study,[15] the second dose was delayed in 72 (18%) of 400 stage 2 participants (the time interval between the first and second doses ranged from 96 days to 147 days). This delayed administration of the second dose did not have a negative effect on Ebola virus glycoprotein-specific binding antibody responses. At 21 days after the second dose, antibody responses were observed in 44 (98%) of 45 stage 2 participants in the Ebola vaccine group who received the delayed second dose, with a GMC similar to that observed in participants who received the second dose within the protocol-defined window (delayed second dose GMC 5761 EU/mL [95% CI 3926–8455] *vs* second dose within protocol-defined window 3823 EU/mL [3334–4383]; appendix pp 13–14).

At day 156 (3 months after the second dose), the magnitude of Ebola virus glycoprotein-specific binding antibody concentrations in stage 1 participants had decreased compared with 21 days after the second dose, with a GMC of 544 EU/mL (95% CI 422–701), and remained largely stable until day 720 (table 2; figure 4A). At day 360, persistent Ebola virus glycoprotein-specific binding antibody responses were observed in 24 (77%) of 31 stage 1 participants (GMC 325 EU/mL [95% CI 238–445]) and in 82 (49%) of 166 stage 2 participants

Figure 3: **Solicited AEs after vaccination in stage 1 and stage 2 participants**
Solicited local (A) and systemic (B) AEs after the first dose, and solicited local (C) and systemic (D) AEs after the second dose. Solicited AEs were observed during the period of 7 days after vaccination. Grade 3 solicited AEs were severe AEs requiring medical attention, but which were not immediately life-threatening. Ad26.ZEBOV=adenovirus type 26 vector-based vaccine encoding the Ebola virus glycoprotein. MenACWY=meningococcal quadrivalent (serogroups A, C, W135, and Y) conjugate vaccine. MVA-BN-Filo=modified vaccinia Ankara vector-based vaccine, encoding glycoproteins from the Ebola virus, Sudan virus, and Marburg virus, and the nucleoprotein from the Tai Forest virus.

(259 EU/mL [223–301]). At day 720, a persistent antibody response was observed in 21 (68%) of 31 stage 1 participants (279 EU/mL [201–386]) and in 78 (50%) of 155 stage 2 participants (255 EU/mL [212–306]).

At 7 days after the Ad26.ZEBOV booster vaccination, given to a subset of 29 stage 1 participants 2 years after the first dose, 24 (96%) of 25 showed a strong increase in Ebola virus glycoprotein-specific binding antibody concentrations, with a GMC of 11166 EU/mL (95% CI 5881–21201), which is 40-times higher than the GMC at the pre-booster vaccination timepoint (279 [95% CI 201–386]). At 21 days after the booster vaccination, all 29 participants had an Ebola virus glycoprotein-specific binding antibody response, with a GMC of 30411 EU/mL (21972–42091), which was approximately 110-times higher than the pre-booster vaccination GMC (table 2; figure 4A) and six-times higher than the GMC at 21 days after the second dose. Ebola virus glycoprotein-specific binding antibody concentrations decreased at 1 year after the booster vaccination, with a GMC of 3237 EU/mL (2305–4547); however, persistent responses were observed in all 26 participants still on follow-up at this timepoint, at a level that was at least nine-times higher than that observed at 1 year and 2 years after the first dose.

Ebola virus glycoprotein-specific neutralising antibody titres were measured in a randomly selected subset of 74 stage 2 participants (55 [18%] of 298 in the Ebola vaccine group and 19 [19%] of 102 in the control group; figure 4B; appendix pp 15–16). At 56 days after the first dose, an Ebola virus glycoprotein-specific neutralising antibody response was observed in one (2%) of 51 participants in the Ebola vaccine group, with a GMT less than the LLOQ. At 21 days after the second dose, an Ebola virus glycoprotein-specific neutralising antibody response was observed in 52 (98%) of 53 participants in the Ebola vaccine group, with a GMT of 2199 (95% CI 1634–2960). There was a strong positive correlation between Ebola glycoprotein-specific binding antibody concentrations and neutralising antibody titres at 21 days after the second dose in participants who received both doses of the Ebola vaccine regimen (r=0·751; appendix p 19). At day 360, the neutralising antibody response persisted in three (6%) of 53 participants in the Ebola vaccine group. At approximately 2 years after the first dose, neutralising antibody responses were observed in six (12%) of 51 participants in the Ebola vaccine group.

Pre-existing Ad26-specific neutralising antibody titres were measured in all 43 stage 1 participants, and in 209 (52%) of 400 stage 2 participants (191 [64%] of 298 in the Ebola vaccine group and 18 [18%] of 102 in the control group). Pre-existing Ad26-specific neutralising antibodies were detected in 40 (93%) stage 1 participants, in 177 (93%) stage 2 participants in the Ebola vaccine group, and in 17 (94%) stage 2 participants in the control group, with similar GMTs observed among the three groups (90% inhibitory concentration GMTs of 111 [95% CI 75–163] in stage 1 participants, 124 [101–151] in stage 2 participants in the Ebola vaccine group, and 104 [57–190] in stage 2 participants in the control group; appendix p 16). There was no correlation between baseline Ad26-specific neutralising antibody titres and vaccine-induced Ebola virus glycoprotein-specific binding antibody concentrations at 21 days after the second dose (r=–0·145; appendix p 19).

	Stage 1 (Ad26.ZEBOV and MVA-BN-Filo with an Ad26.ZEBOV booster at 2 years after dose 1)	Stage 2	
		Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine group	MenACWY and placebo control group
Day 1 (baseline)			
Number of participants*	43	188	66
GMC (95% CI), EU/mL	60 (40–90)	69 (56–85)	49 (36–66)
Day 57 (56 days after the first dose)			
Number of participants*	43	190	68
GMC (95% CI), EU/mL	269 (208–347)	236 (206–270)	50 (37–69)
Responders†	28/43 (65%; 49–79)	101/187 (54%; 47–61)	4/66 (6%; 2–15)
Day 78 (21 days after the second dose)			
Number of participants*	42	182	62
GMC (95% CI), EU/mL	4784 (3736–6125)	3810 (3312–4383)	50 (<LLOQ–70)
Responders†	41/42 (98%; 87–100)	176/179 (98%; 95–100)	2/60 (3%; 0–12)
Day 156 (155 days after the first dose)			
Number of participants*	41	..	..
GMC (95% CI), EU/mL	544 (422–701)	..	..
Responders†	32/41 (78%; 62–89)	..	..
Day 360 (359 days after the first dose)			
Number of participants*	31	168	62
GMC (95% CI), EU/mL	325 (238–445)	259 (223–301)	50 (<LLOQ–71)
Responders†	24/31 (77%; 59–90)	82/166 (49%; 42–57)	4/60 (7%; 2–16)
Day 540 (539 days after the first dose)			
Number of participants*	32	..	..
GMC (95% CI), EU/mL	257 (186–356)	..	..
Responders†	23/32 (72%; 53–86)	..	..
Day 720 (719 days after the first dose)			
Number of participants*	31	158	48
GMC (95% CI), EU/mL	279 (201–386)	255 (212–306)	49 (<LLOQ–72)
Responders†	21/31 (68%; 49–83)	78/155 (50%; 42–58)	7/47 (15%; 6–28)
Day 724 (4 days after booster vaccination)			
Number of participants*	27	NA	NA
GMC (95% CI), EU/mL	304 (211–440)	NA	NA
Responders†	19/27 (70%; 50–86)	NA	NA
Day 727 (7 days after booster vaccination)			
Number of participants*	25	NA	NA
GMC (95% CI), EU/mL	11166 (5881–21201)	NA	NA
Responders†	24/25 (96%; 80–100)	NA	NA
Day 741 (21 days after booster vaccination)			
Number of participants*	29	NA	NA
GMC (95% CI), EU/mL	30411 (21972–42091)	NA	NA
Responders†	29/29 (100%; 88–100)	NA	NA

(Table 2 continues on next page)

	Stage 1 (Ad26.ZEBOV and MVA-BN-Filo with an Ad26.ZEBOV booster at 2 years after dose 1)	Stage 2	
		Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine group	MenACWY and placebo control group
(Continued from previous page)			
Day 1080 (359 days after booster vaccination)			
Number of participants*	26	NA	NA
GMC (95% CI), EU/mL	3237 (2305–4547)	NA	NA
Responders†	26/26 (100%; 87–100)	NA	NA

For the proportions of responders, exact (Clopper-Pearson) 95% CIs are shown. A participant was considered a responder at a specific timepoint if either: (1) the sample was negative at baseline and positive post-baseline, and the post-baseline value was more than 2·5-times higher than the LLOQ; or (2) the sample was positive both at baseline and post-baseline, and there was a greater than 2·5-times increase from baseline. Ad26.ZEBOV=adenovirus type 26 vector-based vaccine encoding the Ebola virus glycoprotein. EU=ELISA units. GMC=geometric mean concentration. LLOQ=lower limit of quantification. MenACWY=meningococcal quadrivalent (serogroups A, C, W135, and Y) conjugate vaccine. MVA-BN-Filo=modified vaccinia Ankara vector-based vaccine, encoding glycoproteins from the Ebola virus, Sudan virus, and Marburg virus, and the nucleoprotein from the Tai Forest virus. NA=not appropriate. *Refers to the number with data at that timepoint. †Expressed as n/N (%; 95% CI), where n is the number of responders at that timepoint and N is the total number of participants with data at baseline and at that timepoint.

Table 2: **Ebola glycoprotein-specific binding antibody responses in each study group from baseline to study completion**

Before vaccination, MVA-specific neutralising antibodies were analysed in almost all stage 1 participants (42 [98%] of 43) and in 74 (19%) of 400 stage 2 participants [56 [19%] of 298 in the Ebola vaccine group and 18 (18%) of 102 in the control group). Neutralising antibodies against the MVA vector were observed in only two (5%) stage 1 participants, in three (5%) stage 2 participants in the Ebola vaccine group, and in three (17%) stage 2 participants in the control group. The GMT values for all three groups of participants at baseline were all less than the LLOQ (appendix p 17).

Discussion

To our knowledge, this is the first clinical study to assess the safety and tolerability two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in a region affected by the west African Ebola virus disease outbreak in 2014–16. The results showed that this regimen was well tolerated; injection-site pain was the most frequent solicited local adverse event, and headache, myalgia, fatigue, and arthralgia were the most frequent solicited systemic adverse events. No serious adverse events were considered related to the study vaccine.

The Ad26.ZEBOV and MVA-BN-Filo vaccine regimen induced Ebola virus glycoprotein-specific binding and neutralising antibody responses in 98% of participants at 21 days after the second dose. At this timepoint, a strong positive correlation was observed between binding antibody concentrations and neutralising antibody titres. The magnitude of antibody responses declined over time, although Ebola virus glycoprotein-specific binding antibody responses persisted in 24 (77%) of 31 stage 1 participants and in 82 (49%) of 166 stage 2 participants in the Ebola vaccine group at 1 year after the first dose and in 21 (68%) of 31 stage 1 participants and 78 (50%) of 155 stage 2 participants at 2 years after the first dose. In a randomly selected subset of stage 2 participants in the Ebola vaccine group, neutralising antibody responses persisted in three (6%) of 53 participants at 1 year after the first dose and in six (12%) of 51 participants at 2 years after the first dose.

Although more than 90% of participants had pre-existing neutralising antibodies specific for the Ad26 vector, correlation analyses indicated that there was no association between pre-existing Ad26-specific immunity and the vaccine-induced Ebola virus glycoprotein-specific binding antibody responses (appendix p 19).

The immunogenicity findings described in this report are consistent with data observed in previous studies showing the safety and immunogenicity of the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in a European population[13,15,20] and in east African populations that were not affected by the 2014–16 Ebola outbreak.[12,14] The kinetics of the humoral responses observed in phase 1 and 2 clinical studies are supported by the results of our study.[12–15,20]

A small proportion (18%) of stage 2 participants received their second dose outside the protocol-defined window. A sensitivity analysis showed that extension of the time interval between Ad26.ZEBOV and MVA-BN-Filo doses did not have a negative effect on vaccine-induced immune responses at 21 days after the second dose, as 44 (98%) of 45 participants who received a delayed second dose had Ebola virus glycoprotein-specific binding antibody responses, with a GMC similar to that observed in participants who received the second dose within the protocol-defined window. Our study also showed that a booster vaccination with Ad26.ZEBOV given at 2 years after the first dose was well tolerated and induced a strong anamnestic response, as evidenced by Ebola virus glycoprotein-specific binding antibody concentrations that were approximately 40-times higher at 7 days after the booster vaccination and approximately 110-times higher at 21 days after the booster vaccination than immediately before the booster. Ebola virus glycoprotein-specific binding antibody concentrations decreased at 1 year after the booster vaccination; however, responses were observed in all participants at that timepoint, at a concentration that was at least nine-times higher than that observed at 1 year and 2 years after the first dose. This finding indicates that the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen had induced humoral immune memory, which we believe can be triggered by future natural infections and is important for subsequent considerations of the deployment of this vaccine. Prophylactic vaccination with the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen could be considered as a medium-term to long-term strategy. In addition, as a precautionary measure, a booster vaccination with Ad26.

ZEBOV could be considered in anticipation of an imminent exposure to Ebola virus.

This study has some limitations, including the imbalance in the numbers of men and women in the study population (most participants were men because of local socioeconomic and cultural factors); the exclusion of pregnant women, as is generally conventional during trials of new investigational products (with the related requirement for contraception in those of childbearing potential);[21] the measurement of Ebola virus glycoprotein-specific neutralising antibody titres in only a subset of participants; and the offer of a booster dose to only stage 1 participants. The study was initially planned as a large cluster-randomised trial, with vaccine effectiveness as the primary endpoint; however, the study design and outcomes were changed after the Ebola virus disease outbreak in Sierra Leone declined (ie, the cluster-randomised trial component was removed, the follow-up period was extended, and the booster dose in stage 1 participants was included). The addition of the booster dose was to ascertain whether the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen could establish a memory response. As stage 1 participants were the first to be vaccinated in the study, they were also the first group to reach the follow-up timepoint of 2 years after the first dose, when the booster dose was due to be administered and when the collection of data at 1 year after the booster dose in this group would not have delayed the reporting of the results of the overall study.

Aside from these limitations, the study has many strengths, including the enrolment of participants in a country previously affected by Ebola virus; a 2-year follow-up period, which provided the opportunity to assess the durability of immune responses; and the inclusion of a booster vaccination given 2 years after the initial vaccination. Starting the study during the Ebola outbreak, in a largely rural setting, and in a research-naive population, has provided valuable lessons regarding clinical trial implementation and conduct under difficult conditions.[18] Participant retention was challenging, especially in the aftermath of the outbreak, as some individuals relocated outside the study area for work, business, or study. Despite this challenge, reasonable long-term retention rates were achieved due to concerted community trust-building and participant follow-up arrangements.[17,18,22]

The Ad26.ZEBOV and MVA-BN-Filo vaccine regimen, with a 56-day interval between the two doses, assessed in this study, received marketing authorisation on July 1, 2020, for prophylactic use, under exceptional circumstances, in adults and children aged 1 year or older in the EU.[10] This vaccine regimen was previously shown to provide protection in vaccinated non-human primates against an Ebola virus challenge, which is fully lethal in unvaccinated control animals.[11] In the absence of clinical efficacy data in humans, a statistical approach referred to as immunobridging using data from this study and other clinical studies was used to infer the likelihood of protection induced by vaccination by correlating the magnitude of vaccine-elicited immune parameters in non-human primates with those observed in vaccinated humans.[23] Although a mechanistic correlate of protection has not yet been identified, the Ebola virus glycoprotein-specific binding antibody GMCs observed 21 days after the second dose in participants who received the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen were similar to the GMC of 1262 EU/mL (95% CI 1169–1363) reported

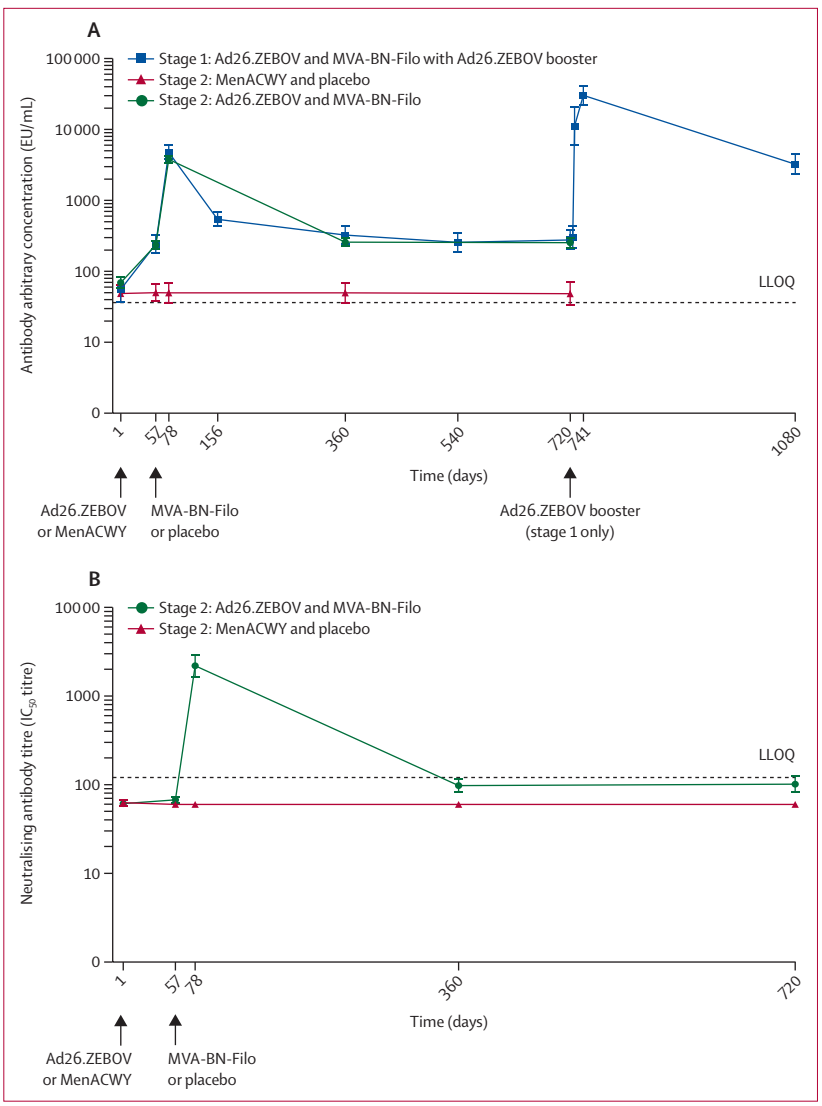


Figure 4: **Ebola virus glycoprotein-specific binding antibody responses in stage 1 and 2 participants (A) and Ebola virus glycoprotein-specific neutralising antibody responses in stage 2 participants (B)**
In (A), the response profile for each study group is shown as geometric mean concentrations of anti-Ebola virus glycoprotein IgG. The error bars show the 95% CIs. Labels for day 724 (4 days after the booster vaccination) and day 727 (7 days after the booster vaccination) have been omitted. In (B), the response profile for each study group is shown as geometric mean titres. The error bars show the 95% CIs. Ad26.ZEBOV=adenovirus type 26 vector-based vaccine encoding the Ebola virus glycoprotein. EU=ELISA units. IC_{50}=half maximal inhibitory concentration. LLOQ=lower limit of quantification. MenACWY=meningococcal quadrivalent (serogroups A, C, W135, and Y) conjugate vaccine. MVA-BN-Filo=modified vaccinia Ankara vector-based vaccine, encoding glycoproteins from the Ebola virus, Sudan virus, and Marburg virus, and the nucleoprotein from the Tai Forest virus.

at 28 days after rVSV-ZEBOV-GP vaccination by use of the same assay in the same laboratory.[24] rVSV-ZEBOV-GP, which was the first Ebola virus vaccine to receive conditional marketing authorisation in Europe and approval for use in adults in the USA and several African countries,[7–9] is the only vaccine for which data on vaccine effectiveness are currently available (ie, estimated vaccine effectiveness of 100% from 10 days after vaccination onwards in a phase 3 trial in Guinea during the west African outbreak,[4] and an estimated vaccine effectiveness of 97·5% in DR Congo).[6]

Recognising the threat of unpredictable future Ebola virus disease outbreaks, further vaccine development work is crucial to strengthen international health security by diversifying vaccination strategy options. Additional studies are in progress, such as PREVAC (NCT02876328), a randomised trial currently underway in Sierra Leone, Guinea, Liberia, and Mali assessing three vaccine strategies in adults and children, including the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen, the single-dose rVSV-ZEBOV-GP vaccine, and a two-dose rVSV-ZEBOV-GP vaccine regimen.[25] Another study, DRC-EB-001 (NCT04152486), is currently underway in North Kivu, DR Congo, to assess the feasibility and safety of the two-dose Ad26.ZEBOV and MVA-BN-Filo regimen at the population level. EBL2007 (NCT0418600) in DR Congo and EBL2009 (NCT04028349) in Uganda are two ongoing open-label trials that will provide additional information on the immunogenicity and safety of the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen.

In conclusion, our findings show that in healthy African adult volunteers living in a region previously affected by Ebola virus disease, the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen administered with a 56-day interval between the two doses is well tolerated and induces humoral immune responses that persist for at least 2 years, as well as humoral immune memory. Booster vaccination with Ad26.ZEBOV administered 2 years after the first dose induces a strong anamnestic response within 7 days, which could be valuable for populations at imminent risk of exposure to Ebola virus, such as health workers in Ebola-endemic settings.

Contributors

DI and DM drafted the manuscript. DM did the literature search for and drafted the research in context section. DI, DM, MOA, FB, KO-K, BLo, TM, ES, JF, KEG, MS, GFD, BKe, HL, SL, NG, ML, VB, AG, DH, BC, KL, CR, BG, MD, BLe, and DW-J were involved in study conceptualisation, design, and conduct, and the interpretation of results. DW-J was the lead scientist for the programme (EBOVAC1) at the London School of Hygiene & Tropical Medicine. MD was the lead scientist for the programme at Janssen Vaccines & Prevention BV. BLe was the principal investigator of the clinical trial in Sierra Leone. GFD, BR, AS-B, and IS contributed to the enrolment and clinical care of participants and to data collection. DK was responsible for data management. BLo, BKö, GTO, VB, and KL were responsible for the management and analysis of laboratory samples, and the interpretation of laboratory results. TM, ES, and SL were responsible for community engagement activities. MJ was the clinical trial pharmacist and was responsible for study vaccine preparation and dispensing. AG and DH performed the statistical analyses. AG, CR, and DM have accessed and verified the study data. All authors reviewed and approved the final manuscript. All authors had full access to all the data in the study and the corresponding author had final responsibility for the decision to submit for publication.

Declaration of interests

BKe was a full-time employee of Janssen at the time of the study. NG, ML, AG, DH, VB, KL, BC, CR, and MD were full-time employees of Janssen at the time of the study, and declare ownership of shares in Janssen. DW-J reports grants from the Innovative Medicines Initiative and non-financial support from Janssen Vaccines & Prevention BV during the conduct of the study; and grants from the Coalition for Epidemic Preparedness Innovations and non-financial support from Janssen Vaccines & Prevention BV outside the submitted work. HL reports grants from GSK and Merck outside the submitted work. All other authors declare no competing interests.

Data sharing

Janssen has an agreement with the Yale Open Data Access (YODA) Project to serve as the independent review panel for evaluation of requests for clinical study reports and participant-level data from investigators and physicians for scientific research that will advance medical knowledge and public health. Data will be made available following publication and approval by YODA of any formal requests with a defined analysis plan. For more information on this process, or to make a request, please visit The YODA Project website at http://yoda.yale.edu. The data sharing policy of Janssen is available at https://www.janssen.com/clinical-trials/transparency. We consider that the study methods and results in adult participants are clearly documented in this Article. Study methods for enrolment of children and their results will be presented in a separate publication. The clinical study protocol is available in the appendix (pp 25–154).

Acknowledgments

This work was supported by the Innovative Medicines Initiative 2 Joint Undertaking (grants 115854 [to the EBOVAC 1 project], 115850 [to the EBOMAN project via the Janssen Ebola vaccine programme], and 115847 [to the EBODAC project via the Janssen Ebola vaccine programme]), Janssen Vaccines and Prevention BV, the EU's Horizon 2020 research and innovation programme (to the Innovative Medicines Initiative 2 Joint Undertaking), the European Federation of Pharmaceutical Industries and Association (to the Innovative Medicines Initiative 2 Joint Undertaking), and the National Institute of Allergy and Infectious Diseases, National Institutes of Health (contract HHSN272200800056C to the Janssen Filovirus project). We thank the staff and management of Kambia Government Hospital; the Kambia district health management team; the site study team, including study physicians, pharmacists, and other clinicians; the site-visiting paediatricians; expert trainers who supported the site team; the Janssen trial management, data management, quality assurance, and clinical quality teams; the clinical trial monitors; the College of Medicine and Allied Health Sciences, University of Sierra Leone, and the London School of Hygiene & Tropical Medicine project management and administrative teams; the social science team; the community engagement team; and colleagues from World Vision, GOAL, and the Ebola Vaccine Deployment, Acceptance and Compliance project. We thank all EBL3001 study participants and their families and the EBL3001 study group. We also thank Marialuisa Quadri (Janssen Vaccines and Prevention, Leiden, Netherlands) and Vikki Clayton (Zoetic Science, an Ashfield company, part of UDG Healthcare, Macclesfield, UK) for medical writing assistance and publication coordination. Funding for Zoetic Science medical writing assistance and publication coordination was provided by Janssen Vaccines and Prevention. We also thank Yvonne Salzgeber (Janssen Vaccines and Prevention, Leiden, Netherlands) for publication coordination.

References

1 Centers for Disease Control and Prevention. 2014–2016 Ebola outbreak in west Africa 2019. https://www.cdc.gov/vhf/ebola/history/2014-2016-outbreak (accessed July 9, 2020).

2 WHO. Resurgence of Ebola in north Kivu in the Democratic Republic of the Congo. 2021. https://www.afro.who.int/news/resurgence-ebola-north-kivu-democratic-republic-congo (accessed Aug 23, 2021).

3 Centers for Disease Control and Prevention. History of Ebola virus disease (EVD) outbreaks. 2021. https://www.cdc.gov/vhf/ebola/history/chronology.html (accessed Aug 2, 2021).

4 Henao-Restrepo AM, Camacho A, Longini IM, 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 Ca Suffit!). *Lancet* 2017; **389:** 505–18.

5 Walldorf JA, Cloessner EA, Hyde TB, MacNeil A, CDC Emergency Ebola Vaccine Taskforce. Considerations for use of Ebola vaccine during an emergency response. *Vaccine* 2019; **37:** 7190–200.

6 WHO. Preliminary results on the efficacy of rVSV-ZEBOV-GP Ebola vaccine using the ring vaccination strategy in the control of an Ebola outbreak in the Democratic Republic of the Congo: an example of integration of research into epidemic response. 2019. https://reliefweb.int/report/democratic-republic-congo/preliminary-results-efficacy-rvsv-zebov-gp-ebola-vaccine-using-ring (accessed July 9, 2020).

7 European Commission. Vaccine against Ebola: commission grants first-ever market authorisation. 2019. https://ec.europa.eu/commission/presscorner/detail/en/ip_19_6246 (accessed Aug 12, 2021).

8 US Food and Drug Administration. First FDA-approved vaccine for the prevention of Ebola virus disease, marking a critical milestone in public health preparedness and response. 2019. https://www.fda.gov/news-events/press-announcements/first-fda-approved-vaccine-prevention-ebola-virus-disease-marking-critical-milestone-public-health (accessed Aug 12, 2021).

9 Merck. ERVEBO (Ebola Zaire vaccine, live) now registered in four African countries, within 90 days of reference country approval and WHO prequalification. 2020. https://www.merck.com/news/ervebo-ebola-zaire-vaccine-live-now-registered-in-four-african-countries-within-90-days-of-reference-country-approval-and-who-prequalification/ (accessed July 9, 2020).

10 European Commission. Vaccine against Ebola: commission grants new market authorisations. 2020. https://ec.europa.eu/commission/presscorner/detail/en/ip_20_1248 (accessed July 9, 2020).

11 Callendret B, Vellinga J, Wunderlich K, et al. A prophylactic multivalent vaccine against different filovirus species is immunogenic and provides protection from lethal infections with Ebolavirus and Marburgvirus species in non-human primates. *PLoS One* 2018; **13:** e0192312.

12 Anywaine Z, Whitworth H, Kaleebu P, et al. Safety and immunogenicity of a 2-dose heterologous vaccination regimen with Ad26.ZEBOV and MVA-BN-Filo Ebola vaccines: 12-month data from a phase 1 randomized clinical trial in Uganda and Tanzania. *J Infect Dis* 2019; **220:** 46–56.

13 Milligan ID, Gibani MM, Sewell R, et al. Safety and immunogenicity of novel denovirus type 26- and modified vaccinia Ankara-vectored Ebola vaccines: a randomized clinical trial. *JAMA* 2016; **315:** 1610–23.

14 Mutua G, Anzala O, Luhn K, et al. Safety and immunogenicity of a 2-dose heterologous vaccine regimen with Ad26.ZEBOV and MVA-BN-Filo Ebola vaccines: 12-month data from a phase 1 randomized clinical trial in Nairobi, Kenya. *J Infect Dis* 2019; **220:** 57–67.

15 Pollard AJ, Launay O, Lelievre JD, et al. Safety and immunogenicity of a two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in adults in Europe (EBOVAC2): a randomised, observer-blind, participant-blind, placebo-controlled, phase 2 trial. *Lancet Infect Dis* 2020; **21:** 493–506.

16 WHO. Maps from 30 March 2016: geographical distribution of new and total confirmed cases. 2021. https://apps.who.int/ebola/current-situation/ebola-situation-report-30-march-2016 (accessed Aug 4, 2021).

17 Enria L, Lees S, Smout E, et al. Power, fairness and trust: understanding and engaging with vaccine trial participants and communities in the setting up the EBOVAC-Salone vaccine trial in Sierra Leone. *BMC Public Health* 2016; **16:** 1140.

18 Mooney T, Smout E, Leigh B, et al. EBOVAC-Salone: lessons learned from implementing an Ebola vaccine trial in an Ebola-affected country. *Clin Trials* 2018; **15:** 436–43.

19 Afolabi MO, Ishola D, Manno D, 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* 2021; published online Sept 13. https://doi.org/10.1016/S1473-3099(21)00128-6.

20 Winslow RL, Milligan ID, Voysey M, et al. Immune responses to novel adenovirus type 26 and modified vaccinia virus Ankara-vectored Ebola vaccines at 1 year. *JAMA* 2017; **317:** 1075–77.

21 Gomes MF, de la Fuente-Nunez V, Saxena A, Kuesel AC. Protected to death: systematic exclusion of pregnant women from Ebola virus disease trials. *Reprod Health* 2017; **14** (suppl 3)**:** 172.

22 Tengbeh AF, Enria L, Smout E, et al. "We are the heroes because we are ready to die for this country": participants' decision-making and grounded ethics in an Ebola vaccine clinical trial. *Soc Sci Med* 2018; **203:** 35–42.

23 Roozendaal R, Hendriks J, van Effelterre T, et al. Nonhuman primate to human immunobridging to infer the protective effect of an Ebola virus vaccine candidate. *NPJ Vaccines* 2020; **5:** 112.

24 Halperin S, Das R, Onorato MT, et al. Immunogenicity, lot consistency, and extended safety of rVSVΔG-ZEBOV-GP vaccine: a phase 3 randomized, double-blind, placebo-controlled study in healthy adults. *J Infect Dis* 2019; **220:** 1127–35.

25 Levy Y, Lane C, Piot P, et al. Prevention of Ebola virus disease through vaccination: where we are in 2018. *Lancet* 2018; **392:** 787–90.

THE LANCET
Infectious Diseases

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Ishola D, Manno D, Afolabi MO, et al. Safety and long-term immunogenicity of the two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in adults in Sierra Leone: a combined open-label, non-randomised stage 1, and a randomised, double-blind, controlled stage 2 trial. *Lancet Infect Dis* 2021; published online Sept 13. https://doi.org/10.1016/S1473-3099(21)00125-0.

Safety and long-term immunogenicity of a two-dose Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen in adults: a randomised, double-blind, controlled trial in Sierra Leone

Supplementary material

Table of contents

1. SUPPLEMENTARY METHODS 2
1.1. Data management 2
1.2. Determination of neutralising antibody activity in an EBOV GP pseudovirion neutralisation assay 2

2. SUPPLEMENTARY RESULTS 3
2.1. *Table S1*: Solicited adverse events: Solicited local adverse events by worst severity grade 4
2.2. *Table S2*: Solicited adverse events: Solicited systemic adverse events by worst severity grade. 6
2.3. *Table S3*: Unsolicited adverse events: Most frequent (at least 10% of participants in any vaccination schedule) unsolicited adverse events by system organ class and dictionary-derived term 9
2.4. *Table S4*: Grade 3 unsolicited adverse events reported after each dose (up to 28 days post dose) 10
2.5. *Table S5*: Serious adverse events reported during the study. 11
2.6. *Table S6*: Ebola glycoprotein-specific binding antibody, stratified by the timing of dose 2 vaccination (dose 2 vaccination received at the protocol planned time, or delayed). 13
2.7. *Table S7*: Ebola glycoprotein-specific neutralising antibody concentrations, as measured in a subset of Stage 2 participants 15
2.8. *Table S8*: Ad26 neutralizing antibodies (Ad26 VNA, IC_{90} titre): Geometric mean and sample interpretation: per protocol analysis set. 16
2.9. *Table S9*: MVA neutralizing antibodies (MVA PRNT, IC_{50} titre): Geometric mean and sample interpretation: per protocol analysis set. 17
2.10. *Figure S1*: Correlation between Ebola glycoprotein-specific binding antibody concentrations and Ebola glycoprotein-specific neutralizing antibody titres at 21 days post dose 2 in participants who received Ebola vaccine regimen 18
2.11. *Figure S2*: Correlation between baseline Ad26-specific nAb titres and the vaccine-induced EBOV GP-specific binding antibody concentrations at 21 days post-dose 2 19
2.12. *Figure S3*: Correlation between pre-existing Ebola glycoprotein-binding antibody at baseline and at 21 days post dose 2 in participants who received Ebola vaccine regimen 20

3. EBL3001 STUDY GROUP 21

4. STUDY PROTOCOL 25

1. SUPPLEMENTARY METHODS

1.1. Data management

Paper source document data were entered onto electronic data collection forms in Medidata Rave Clinical Cloud™. Data were subjected to visual (ie, completeness of source documents) and electronic validation to check for discrepancies, missing data and protocol deviations in the database. Issues were notified to the site as queries for resolution. Data were source-verified, and all queries were resolved before data lock for interim and final analyses.

1.2. Determination of neutralising antibody activity in an Ebola Virus Glycoprotein (EBOV GP) pseudovirion neutralisation assay

In order to assess the functionality of vaccine-induced antibody responses, a pseudovirion neutralisation assay (psVNA) was developed at Monogram Biosciences (San Francisco, CA, USA) and was qualified with human serum. Samples for the current report were assayed at Monogram Biosciences according to the standard operating procedure 'Crucell EBOV Neutralization Assay'.

Pseudovirions expressing the glycoprotein of an EBOV isolate from the 2014 outbreak (Makona variant) were produced in human embryonic kidney 293 (HEK293) cells by transfection with a GP expression plasmid and a vector encoding a firefly luciferase gene and all of the human immunodeficiency virus (HIV) type 1 genes required for viral replication, except for the envelope gene. Sera were pretreated to remove any nonspecific neutralising factors. A fixed amount of pseudovirions was mixed with a series of serial dilutions of serum samples. Following incubation, the samples were transferred to a HEK293 cell monolayer. The inhibition of pseudovirion infection was measured by luciferase reporter gene expression. The assay responses of the serially diluted samples were plotted in a 4-parameter logistic regression curve and the 50% inhibitory concentration (IC_{50}) of each curve was reported as each a neutralisation titre for each serum sample.

A psVNA result (IC_{50} titre) was considered positive if the specific IC_{50} titre was more than three times amphotropic murine leukaemia virus (aMLV) and above the assay-specific lower limit of quantitation (LLOQ). Values that were less than three times aMLV or below the LLOQ were imputed with LLOQ/2 (120/2). For the calculation of fold increases, values that were less than three times aMLV or below the LLOQ were imputed with the LLOQ. The psVNA values were log_{10}-transformed before further handling. The log_{10}-transformed values were used throughout the entire analysis.

For psVNA, a participant was a responder at a considered time point if the sample interpretation was negative at baseline and positive post baseline and the post-baseline value was greater than twice the LLOQ, or sample interpretation was positive both at baseline and post baseline and there was a greater than two-fold increase from baseline.

2. SUPPLEMENTARY RESULTS

In 142 participants who received the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen (25/43 [58%] participants in Stage 1 and 117/188 [62%] participants in Stage 2), the baseline sample interpretation was positive for EBOV GP-specific binding antibodies (ie, the sample value was above the LLOQ). Yet, the GMC values at baseline were low (60 ELISA units/mL in Stage 1 and 69 ELISA units/mL in Stage 2). To evaluate the potential impact of EBOV GP-specific binding antibody concentrations detected by ELISA at baseline on the 21 days post dose 2 ELISA values, a correlation analysis was performed (Figure S3). If baseline ELISA concentrations were an indication of priming of the immune system triggered by previous natural Ebola virus infection, one would expect a positive (anamnestic response) or a negative (immune interference) correlation between the baseline and the post-dose 2 ELISA concentrations. However, a negligible positive correlation was observed, indicating that there is no apparent influence of the observed baseline EBOV GP FANG ELISA positivity on the binding antibody concentrations following Ad26.ZEBOV, MVA-BN-Filo vaccination (Spearman correlation coefficient: 0·104).

2.1. ***Table S1*: Solicited adverse events: Solicited local adverse events by worst severity grade**

		Stage 1	Stage 2	
Post dose 1		**Ad26.ZEBOV**	**Ad26.ZEBOV**	**MenACWY**
n		**43**	**298**	**102**
Any solicited local event, n (%)	Any	12 (28)	51 (17)	17 (17)
	Grade 1	11 (26)	50 (17)	14 (14)
	Grade 2	1 (2)	1 (0)	3 (3)
Injection site erythema	Any	0	1 (0)	0
	Grade 2	0	1 (0)	0
Injection site pain	Any	12 (28)	44 (15)	16 (16)
	Grade 1	11 (26)	44 (15)	13 (13)
	Grade 2	1 (2)	0	3 (3)
Injection site pruritus	Any	1 (2)	6 (2)	3 (3)
	Grade 1	1 (2)	6 (2)	2 (2)
	Grade 2	0	0	1 (1)
Injection site swelling	Any	1 (2)	1 (0)	1 (1)
	Grade 1	1 (2)	1 (0)	0
	Grade 2	0	0	1 (1)
Post dose 2		**MVA-BN-Filo**	**MVA-BN-Filo**	**Placebo**
n		**43**	**246**	**86**
Any solicited local event, n (%)	Any	6 (14)	58 (24)	8 (9)
	Grade 1	6 (14)	47 (19)	8 (9)
	Grade 2	0	10 (4)	0
	Grade 3	0	1 (0)	0
Injection site erythema	Any	0	0	0
Injection site pain	Any	6 (14)	57 (23)	8 (9)
	Grade 1	6 (14)	47 (19)	8 (9)
	Grade 2	0	9 (4)	0
	Grade 3	0	1 (0)	0
Injection site pruritus	Any	0	5 (2)	1 (1)
	Grade 1	0	4 (2)	1 (1)
	Grade 2	0	1 (0)	0
Injection site swelling	Any	1 (2)	0	0
	Grade 1	1 (2)	0	0
Post booster dose		**Ad26.ZEBOV**		
n		**29**	–	–
Any solicited local event, n (%)	Any	5 (17)		
	Grade 1	5 (17)		
Injection site erythema	Any	0		
Injection site pain	Any	5 (17)		
	Grade 1	5 (17)		
Injection site pruritus	Any	1 (3)		
	Grade 1	1 (3)		
Injection site swelling	Any	0		

Vaccines: Ad26.ZEBOV; MVA-BN-Filo. Control: Meningococcal quadrivalent (serogroups A, C, W135 and Y) conjugate vaccine (MenACWY; dose 1), Placebo (dose 2). n=number of participants with data.

2.2. ***Table S2*: Solicited adverse events: Solicited systemic adverse events by worst severity grade**

		Stage 1	Stage 2	
Post dose 1		**Ad26.ZEBOV**	**Ad26.ZEBOV**	**MenACWY**
n		**43**	**298**	**102**
Any solicited systemic event, n (%)	Any	18 (42)	161 (54)	51 (50)
	Grade 1	15 (35)	144 (48)	45 (44)
	Grade 2	3 (7)	14 (5)	6 (6)
	Grade 3	0	3 (1)	0
Arthralgia	Any	4 (9)	57 (19)	23 (23)
	Grade 1	4 (9)	54 (18)	20 (20)
	Grade 2	0	2 (1)	3 (3)
	Grade 3	0	1 (0)	0
Chills	Any	3 (7)	22 (7)	7 (7)
	Grade 1	3 (7)	20 (7)	7 (7)
	Grade 2	0	2 (1)	0
Fatigue	Any	10 (23)	57 (19)	16 (16)
	Grade 1	9 (21)	50 (17)	15 (15)
	Grade 2	1 (2)	7 (2)	1 (1)
Headache	Any	10 (23)	105 (35)	39 (38)
	Grade 1	7 (16)	95 (32)	38 (37)
	Grade 2	3 (7)	9 (3)	1 (1)
	Grade 3	0	1 (0)	0
Myalgia	Any	10 (23)	57 (19)	20 (20)
	Grade 1	9 (21)	54 (18)	19 (19)
	Grade 2	1 (2)	3 (1)	1 (1)
Nausea	Any	1 (2)	6 (2)	0
	Grade 1	1 (2)	5 (2)	0
	Grade 3	0	1 (0)	0
Pyrexia	Any	0	2 (1)	1 (1)
	Grade 1	0	0	1 (1)
	Grade 2	0	1 (0)	0
	Grade 3	0	1 (0)	0

Post dose 2		**MVA-BN-Filo**	**MVA-BN-Filo**	**Placebo**
n		**43**	**246**	**86**
Any solicited systemic event, n (%)	Any	17 (40)	107 (43)	39 (45)
	Grade 1	13 (30)	90 (37)	32 (37)
	Grade 2	3 (7)	17 (7)	6 (7)
	Grade 3	1 (2)	0	1 (1)
Arthralgia	Any	5 (12)	48 (20)	13 (15)
	Grade 1	4 (9)	42 (17)	10 (12)
	Grade 2	1 (2)	6 (2)	3 (3)
Chills	Any	3 (7)	17 (7)	13 (15)
	Grade 1	2 (5)	14 (6)	13 (15)
	Grade 2	1 (2)	3 (1)	0
Fatigue	Any	5 (12)	47 (19)	18 (21)
	Grade 1	2 (5)	43 (17)	15 (17)
	Grade 2	2 (5)	4 (2)	3 (3)
	Grade 3	1 (2)	0	0
Headache	Any	8 (19)	66 (27)	25 (29)
	Grade 1	8 (19)	57 (23)	21 (24)
	Grade 2	0	9 (4)	4 (5)
Myalgia	Any	6 (14)	37 (15)	13 (15)
	Grade 1	6 (14)	34 (14)	10 (12)
	Grade 2	0	3 (1)	3 (3)
Nausea	Any	2 (5)	2 (1)	2 (2)
	Grade 1	1 (2)	2 (1)	2 (2)
	Grade 3	1 (2)	0	0
Pyrexia	Any	0	5 (2)	2 (2)
	Grade 1	0	4 (2)	0
	Grade 2	0	1 (0)	1 (1)
	Grade 3	0	0	1 (1)

Post booster dose		**Ad26.ZEBOV**
n		**29**
Any solicited systemic event, n (%)	Any	9 (31)
	Grade 1	7 (24)

	Grade 2	2 (7)
Arthralgia	Any	2 (7)
	Grade 1	2 (7)
Chills	Any	1 (3)
	Grade 2	1 (3)
Fatigue	Any	5 (17)
	Grade 1	4 (14)
	Grade 2	1 (3)
Headache	Any	6 (21)
	Grade 1	6 (21)
Myalgia	Any	2 (7)
	Grade 1	2 (7)
Nausea	Any	0
Pyrexia	Any	0

Vaccines: Ad26.ZEBOV; MVA-BN-Filo. Control: Meningococcal quadrivalent (serogroups A, C, W135 and Y) conjugate vaccine (MenACWY; dose 1), Placebo (dose 2). n=number of participants with data.

2.3. ***Table S3*: Unsolicited adverse events: Most frequent (at least 10% of participants in any vaccination schedule) unsolicited adverse events by system organ class and dictionary-derived term**

	Stage 1	Stage 2	
Post dose 1	**Ad26.ZEBOV**	**Ad26.ZEBOV**	**MenACWY**
n	**43**	**298**	**102**
Any event, n (%)	17 (40)	198 (66)	65 (64)
Infections and infestations	7 (16)	129 (43)	40 (39)
Malaria	1 (2)	88 (30)	26 (26)
Nervous system disorders	7 (16)	33 (11)	10 (10)
Headache	7 (16)	32 (11)	10 (10)
Musculoskeletal and connective tissue disorders	4 (9)	24 (8)	14 (14)
Post dose 2	**MVA-BN-Filo**	**MVA-BN-Filo**	**Placebo**
n	**43**	**246**	**86**
Any event, n (%)	17 (40)	145 (59)	48 (56)
Infections and infestations	11 (26)	96 (39)	35 (41)
Malaria	4 (9)	58 (24)	22 (26)
Musculoskeletal and connective tissue disorders	2 (5)	27 (11)	5 (6)
Post booster dose	**Ad26.ZEBOV**	-	-
n	**29**		
Any event, n (%)	5 (17)		

Vaccines: Ad26.ZEBOV; MVA-BN-Filo. Control: Meningococcal quadrivalent (serogroups A, C, W135 and Y) conjugate vaccine (MenACWY; dose 1), Placebo (dose 2). n=number of participants with data.

This table only includes adverse events that were reported between dose 1 vaccination and 28 days post dose 1, between dose 2 vaccination and 28 days post dose 2, and between Ad26.ZEBOV booster dose vaccination and 28 days post Ad26.ZEBOV booster dose. Adverse events are coded using MedDRA version 21.1.

2.4. ***Table S4***: **Grade 3 unsolicited adverse events reported after each dose (up to 28 days post dose)**

	Stage 1	Stage 2	
Post dose 1	**Ad26.ZEBOV**	**Ad26.ZEBOV**	**MenACWY**
n	**43**	**298**	**102**
Any event, n (%)	1 (2)	5 (2)	2 (2)
Investigations	0	3 (1)	1 (1)
Haemoglobin decreased	0	3 (1)	1 (1)
Blood and lymphatic system disorders	1 (2)	1 (0)	0
Thrombocytopenia	1 (2)	1 (0)	0
Infections and infestations	0	1 (0)	1 (1)
Brain abscess	0	1 (0)	0
Gastroenteritis	0	0	1 (1)

Post dose 2	**MVA-BN-Filo**	**MVA-BN-Filo**	**Placebo**
n	**43**	**246**	**86**
Any event, n (%)	1 (2)	5 (2)	1 (1)
Investigations	0	5 (2)	1 (1)
Haemoglobin decreased	0	4 (2)	1 (1)
Aspartate aminotransferase increased	0	1 (0)	0
Blood and lymphatic system disorders	1 (2)	0	0
Anaemia	1 (2)	0	0

Post booster dose	**Ad26.ZEBOV**		
n	**29**	-	-
Any event, n (%)	0		

Vaccines: Ad26.ZEBOV; MVA-BN-Filo. Control: Meningococcal quadrivalent (serogroups A, C, W135 and Y) conjugate vaccine (MenACWY; dose 1), Placebo (dose 2). n=number of participants with data.

MedDRA SOC term=MedDRA System Organ Class Dictionary-derived Term.
Events are coded using MedDRA version 21.1.

2.5. ***Table S5***: **Serious adverse events reported during the study**

MedDRA SOC term	Stage 1	Stage 2	
	Ad26.ZEBOV, MVA-BN-Filo with an Ad26.ZEBOV booster at 2 years post dose 1	Ad26.ZEBOV, MVA-BN-Filo	MenACWY, Placebo
Serious adverse events across the entire study, n	**43**	**298**	**102**
Any event, n (%)	3 (7)	16 (5)	4 (4)
28-day post dose 1, n	**43**	**298**	**102**
Any event, n (%)	0	2 (1)	1 (1)
Infections and infestations	0	1 (0)	1 (1)
Brain abscess	0	1 (0)	0
Gastroenteritis	0	0	1 (1)
Subcutaneous abscess	0	1 (0)	0
Injury, poisoning and procedural complications	0	1 (0)	0
Ligament sprain	0	1 (0)	0
Skin laceration	0	1 (0)	0
Further post-dose 1 FU, n	**43**	**280**	**96**
Any event, n (%)	0	1 (0)	2 (2)
Infections and infestations	0	1 (0)	2 (2)
Gastroenteritis	0	1 (0)	2 (2)
Nervous system disorders	0	0	1 (1)
Syncope	0	0	1 (1)
28-day post dose 2, n	**43**	**246**	**86**
Any event, n (%)	0	0	0
Further post-dose 2 FU, n	**43**	**244**	**81**
Any event, n (%)	2 (5)	13 (5)	1 (1)
Infections and infestations	1 (2)	5 (2)	0
Malaria	0	3 (1)	0
Appendicitis	0	2 (1)	0
Gastroenteritis	0	1 (0)	0
Helminthic infection	0	1 (0)	0
Orchitis	1 (2)	0	0
Sepsis	0	1 (0)	0
Injury, poisoning and procedural complications	0	5 (2)	0
Head injury	0	2 (1)	0

Abortion induced incomplete	0	1 (0)	0
Chest injury	0	1 (0)	0
Multiple injuries	0	1 (0)	0
Open globe injury	0	1 (0)	0
Radius fracture	0	1 (0)	0
Blood and lymphatic system disorders	0	2 (1)	0
Anaemia	0	1 (0)	0
Anaemia of pregnancy	0	1 (0)	0
Gastrointestinal disorders	1 (2)	1 (0)	0
Abdominal pain	0	1 (0)	0
Peptic ulcer	1 (2)	0	0
Metabolism and nutrition disorders	0	1 (0)	0
Dehydration*	0	1 (0)	0
Pregnancy, puerperium and perinatal conditions	0	1 (0)	0
Abortion threatened	0	1 (0)	0
Haemorrhage in pregnancy	0	1 (0)	0
Placenta praevia	0	1 (0)	0
Premature labour	0	1 (0)	0
Renal and urinary disorders	0	0	1 (1)
Renal haematoma	0	0	1 (1)
Vascular disorders	0	1 (0)	0
Hypovolaemic shock	0	1 (0)	0
28-day post booster dose (given at 2 years post dose 1), n	**29**	**-**	**-**
Any event, n (%)	0		
Further post booster dose FU, n	**28**	**-**	**-**
Any event, n (%)	1 (4)		
Eye disorders	1 (4)		
Retinal detachment	1 (4)		
Infections and infestations	1 (4)		
Chorioretinitis	1 (4)		

Vaccines: Ad26.ZEBOV; MVA-BN-Filo. Control: Meningococcal quadrivalent (serogroups A, C, W135 and Y) conjugate vaccine (MenACWY; dose 1), Placebo (dose 2). FU=follow up. Further post-dose 1 FU: from 29 days post dose 1 to dose 2 or date of last contact if lost to follow-up. Further post-dose 2 FU: from 29 days post dose 2 to booster dose (only in stage 1) or date of last visit or date of last contact if lost to follow-up. Further post-booster dose FU: from 29 days post-booster dose to date of last visit or date of last contact if lost to follow-up; n=number of participants with data.

*Fatal case

2.6. *Table S6:* Ebola glycoprotein-specific binding antibody, stratified by the timing of dose 2 vaccination (dose 2 vaccination received at the protocol planned time, or delayed*)

	Stage 2	
	Ad26.ZEBOV, MVA-BN-Filo	**MenACWY, Placebo**
Second dose timing: within protocol-defined window		
Day 1 (Baseline)		
n	189	66
GMC (95% CI)	69 (56–85)	49 (36–66)
Day 57 (56 days post dose 1)		
n	191	68
GMC (95% CI)	235 (205–269)	50 (37–69)
Responder (n/N*, %)	102/188 (54)	4/66 (6)
(95% CI)	(47–62)	(2–15)
Day 78 (21 days post dose 2)		
n	187	64
GMC (95% CI)	3823 (3334–4383)	48 (<LLOQ–67)
Responder (n/N*, %)	181/184 (98)	2/62 (3)
(95% CI)	(95–100)	(0–11)
Day 360 (359 days post dose 1)		
n	171	62
GMC (95% CI)	258 (222–300)	50 (<LLOQ–71)
Responder (n/N*, %)	84/169 (50)	4/60 (7)
(95% CI)	(42–58)	(2–16)
Day 720 (719 days post dose 1)		
n	159	49
GMC (95% CI)	255 (213–306)	52 (<LLOQ–78)
Responder (n/N*, %)	79/156 (51)	7/48 (15)
(95% CI)	(43–59)	(6–28)
Second dose timing: Delayed* dose 2		
Day 1 (Baseline)		
n	53	17
GMC (95% CI)	71 (50–100)	56 (<LLOQ–123)
Day 57 (56 days post dose 1)		
n	54	18
GMC (95% CI)	241 (192–302)	94 (37–238)
Responder (n/N*, %)	34/53 (64)	1/17 (6)
(95% CI)	(50–77)	(0–29)

	Stage 2	
	Ad26.ZEBOV, MVA-BN-Filo	**MenACWY, Placebo**
Delayed* dose 2 (21 days post dose 2)		
n	46	15
GMC (95% CI)	5761 (3926–8455)	93 (<LLOQ–268)
Responder (n/N*, %)	44/45 (98)	3/14 (21)
(95% CI)	(88–100)	(5–51)
Day 360 (359 days post dose 1)		
n	48	11
GMC (95% CI)	390 (313–486)	45 (<LLOQ–126)
Responder (n/N*, %)	29/47 (62)	2/11 (18)
(95% CI)	(47–76)	(2–52)

Vaccines: Ad26.ZEBOV; MVA-BN-Filo. Control: Meningococcal quadrivalent (serogroups A, C, W135 and Y) conjugate vaccine (MenACWY; dose 1), Placebo (dose 2). n=number of participants with data. CI= confidence interval; GMC=geometric mean concentration; LLOQ=lower limit of quantification; N*=number of participants with data at baseline and at that time point.

GMCs and corresponding CIs are shown as ELISA units/mL.
For the responder rates, Exact Clopper-Pearson CI are shown. A participant was a responder at a considered time point if either: (i) the sample interpretation was negative at baseline and positive post baseline and the post-baseline value was greater than 2·5 x LLOQ; or (ii) if the sample interpretation was positive both at baseline and post baseline and there was a greater than 2·5-fold increase from baseline.

Only Stage 2 participants who received dose 2 are shown.

* Due to a study pause (for precautionary reasons during the evaluation of two SAEs in a different study), in 72 Stage 2 participants dose 2 was delayed (with the time interval between dose 1 and dose 2 ranging from 96 to 147 days)

2.7. ***Table S7***: **Ebola glycoprotein-specific neutralising antibody concentrations, as measured in a subset of Stage 2 participants**

	Stage 2	
	Ad26.ZEBOV, MVA-BN-Filo	**MenACWY, placebo**
Day 1 (Baseline)		
n	53	19
GMT (95% CI)	<LLOQ (<LLOQ–<LLOQ)	<LLOQ (<LLOQ–<LLOQ)
Day 57 (56 days post dose 1)		
n	53	19
GMT (95% CI)	<LLOQ (<LLOQ–<LLOQ)	<LLOQ
Responder (n/N*, %)	1/51 (2)	0/19 (0)
(95% CI)	(0–10)	(0–18)
Day 78 (21 days post dose 2)		
n	55	19
GMT (95% CI)	2199 (1634–2960)	<LLOQ
Responder (n/N*, %)	52/53 (98)	0/19 (0)
(95% CI)	(90–100)	(0–18)
Day 360 (359 days post dose 1)		
n	55	19
GMT (95% CI)	<LLOQ (<LLOQ–<LLOQ)	<LLOQ
Responder (n/N*, %)	3/53 (6)	0/19 (0)
(95% CI)	(1–16)	(0–18)
Day 720 (719 days post dose 1)		
n	53	17
GMT (95% CI)	<LLOQ (<LLOQ–126)	<LLOQ
Responder (n/N*, %)	6/51 (12)	0/17 (0)
(95% CI)	(4–24)	(0–20)

Vaccines: Ad26.ZEBOV; MVA-BN-Filo. Control: Meningococcal quadrivalent (serogroups A, C, W135 and Y) conjugate vaccine (MenACWY; dose 1), Placebo (dose 2). n=number of participants with data.
CI=confidence interval; GMC=geometric mean concentration; LLOQ=lower limit of quantification;
N*=number of participants with data at baseline and at that time point.

GMTs and their corresponding CIs are shown on the reported scale (psVNA IC_{50} titer).
For the responder rates, Exact Clopper-Pearson Cis are shown. A participant was a responder at a considered time point either (i) if the sample interpretation was negative at baseline and positive post baseline and the post-

baseline value was greater than 2x LLOQ; or (ii) if the sample interpretation was positive both at baseline and post baseline and there was a greater than 2-fold increase from baseline.

2.8. *Table S8*: Ad26 neutralizing antibodies (Ad26 VNA, IC_{90} titre): Geometric mean and sample interpretation: per protocol analysis set

	Stage 1	Stage 2	
	Ad26.ZEBOV, MVA-BN-Filo	Ad26.ZEBOV, MVA-BN-Filo	Control
Day 1 (Baseline)			
n	43	191	18
GMT (95% CI)	111 (75–163)	124 (101–151)	104 (57–190)
Positive Sample (n (%))	40 (93)	177 (93)	17 (94)
(95% CI)	(81–99)	(88–96)	(73–100)

Vaccines: Ad26.ZEBOV; MVA-BN-Filo. Control: Meningococcal quadrivalent (serogroups A, C, W135 and Y) conjugate vaccine (MenACWY; dose 1), Placebo (dose 2). CI=confidence interval; GMC=geometric mean concentration; n=number of participants with data.

The geometric mean titre and corresponding confidence interval are shown on the reported scale (IC_{90} titre). Exact Clopper-Pearson Confidence Interval is shown for the corresponding sample interpretation rate.

2.9. ***Table S9***: **MVA neutralizing antibodies (MVA PRNT, IC_{50} titre): Geometric mean and sample interpretation: per protocol analysis set**

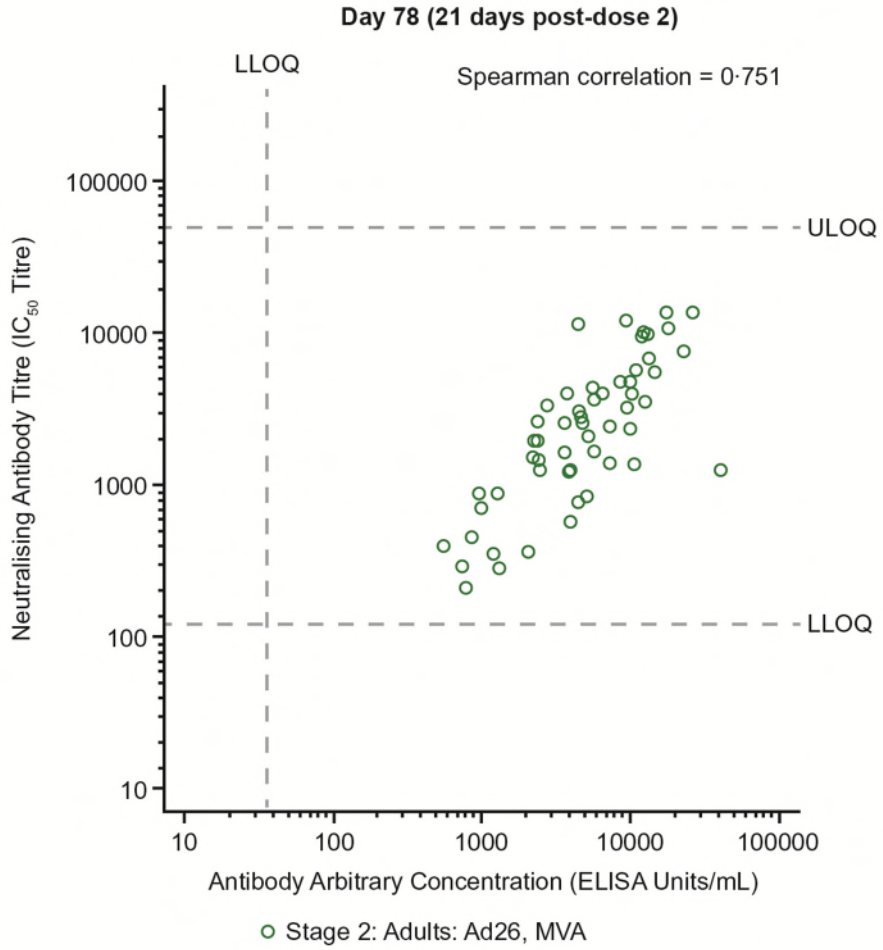
	Stage 1	Stage 2	
	Ad26.ZEBOV, MVA-BN-Filo	Ad26.ZEBOV, MVA-BN-Filo	Control
Day 1 (Baseline)			
n	42	56	18
GMT (95% CI)	<LLOQ (<LLOQ–<LLOQ)	<LLOQ (<LLOQ–<LLOQ)	<LLOQ (<LLOQ–8)
Positive Sample (n (%))	2 (5)	3 (5)	3 (17)
(95% CI)	(1–16)	(1–15)	(4–41)

Vaccines: Ad26.ZEBOV; MVA-BN-Filo. Control: Meningococcal quadrivalent (serogroups A, C, W135 and Y) conjugate vaccine (MenACWY; dose 1), Placebo (dose 2). CI=confidence interval; GMC=geometric mean concentration; n=number of participants with data.

The geometric mean titre and corresponding confidence interval are shown on the reported scale (MVA IC_{50} titre).

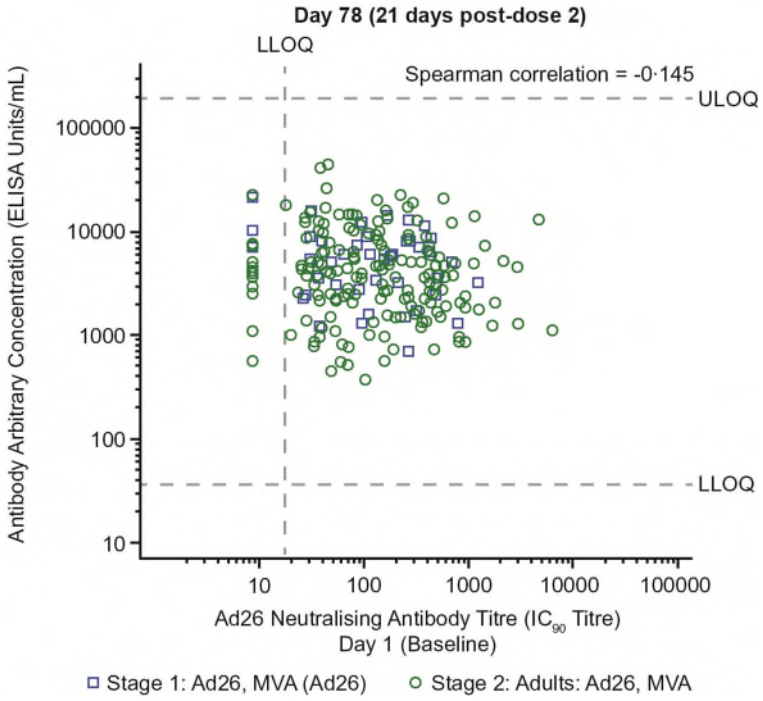
Exact Clopper-Pearson confidence interval is shown for the corresponding sample interpretation rate.

2.10. ***Figure S1***: **Correlation between Ebola glycoprotein-specific binding antibody concentrations and Ebola glycoprotein-specific neutralizing antibody titres at 21 days post dose 2 in participants who received Ebola vaccine regimen**



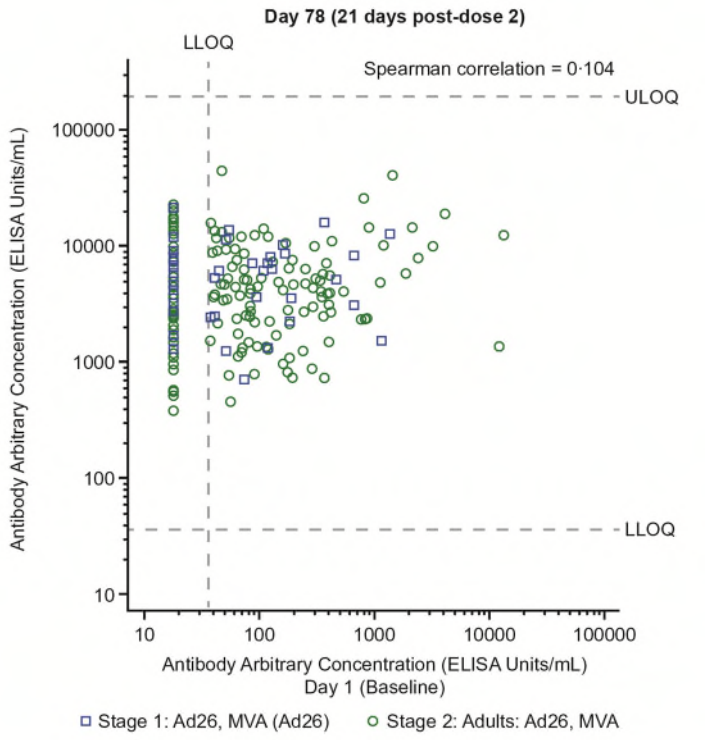
Vaccines: Ad26.ZEBOV (Ad26); MVA-BN-Filo (MVA).
LLOQ=lower limit of quantification; ULOQ=upper limit of quantification. Control participants are not included in this scatter diagram.

2.11. ***Figure S2:*** **Correlation between baseline Ad26-specific nAb titres and the vaccine-induced EBOV GP-specific binding antibody concentrations at 21 days post-dose 2**



Vaccines: Ad26.ZEBOV (Ad26); MVA-BN-Filo (MVA).
LLOQ=lower limit of quantification; ULOQ=upper limit of quantification. Control participants are not included in this scatter diagram.

2.12. ***Figure S3***: **Correlation between pre-existing Ebola glycoprotein-binding antibody at baseline and at 21 days post dose 2 in participants who received Ebola vaccine regimen**



Vaccines: Ad26.ZEBOV (Ad26); MVA-BN-Filo (MVA).
LLOQ=lower limit of quantification; ULOQ=upper limit of quantification. Control participants are not included in this scatter diagram.

Note: the EBL3001 study group and protocol have not been included in this PhD thesis due to word count constraints. They can be accessed in the supplemental material of the paper available online at https://researchonline.lshtm.ac.uk/id/eprint/4659920/ (access date 15 December 2023).

Paper 3: 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

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	150330	**Title**	Dr
First Name(s)	Daniela		
Surname/Family Name	Manno		
Thesis Title	Testing a prophylactic vaccine regimen against Ebola virus disease in Sierra Leone: vaccine safety, immunogenicity and factors affecting immunogenicity		
Primary Supervisor	Professor Deborah Watson Jones		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	Lancet Infectious Diseases		
When was the work published?	October 2022		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	PhD by Prior Publication		
Have you retained the copyright for the work?*	**Yes**	Was the work subject to academic peer review?	**Yes**

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	

Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I was the coordinator and responsible physician for the study. I drafted the protocol, led the conduct of the study and supervised the collection of data and interpretation of results. I am the first and corresponding author of the paper. I drafted the manuscript, including the 'research in context' section. As the corresponding author, I was responsible for the submission of the manuscript and replying to the editorial and reviewers' comments during the peer-review process.

SECTION E

[redacted]

Student Signature	
Date	

Supervisor Signature	
Date	

RETENTION OF COPYRIGHT EVIDENCE

Paper 3. 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 Infectious Diseases: copyright policy

https://s100.copyright.com/AppDispatchServlet?publisherName=ELS&contentID=S1473309922005941&orderBeanReset=true&orderSource=Phoenix

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

Daniela Manno, Agnes Bangura, Frank Baiden, Abu Bakarr Kamara, Philip Ayieko, Joseph Kallon, Julie Foster, Musa Conteh, Nicholas Edward Connor, Bockarie Koroma, Yusupha Njie, Paul Borboh, Babajide Keshinro, Bolarinde Joseph Lawal, Mattu Tehtor Kroma, Godfrey Tuda Otieno, Abdul Tejan Deen, Edward Man-Lik Choi, Ahmed Dahiru Balami, Auguste Gaddah, Chelsea McLean, Kerstin Luhn, Hammed Hassan Adetola, Gibrilla Fadlu Deen, Mohamed Samai, Brett Lowe, Cynthia Robinson, Bailah Leigh, Brian Greenwood, Deborah Watson-Jones

Summary

Background Children account for a substantial proportion of cases and deaths during Ebola virus disease outbreaks. We aimed to evaluate the safety and immunogenicity of a booster dose of the Ad26.ZEBOV vaccine in children who had been vaccinated with a two-dose regimen comprising Ad26.ZEBOV as dose one and MVA-BN-Filo as dose two.

Methods We conducted an open-label, non-randomised, phase 2 trial at one clinic in Kambia Town, Sierra Leone. Healthy children, excluding pregnant or breastfeeding girls, who had received the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in a previous study, and were aged 1–11 years at the time of their first vaccine dose, received an intramuscular injection of Ad26.ZEBOV (5×10^{10} viral particles) and were followed up for 28 days. Primary outcomes were safety (measured by adverse events) and immunogenicity (measured by Ebola virus glycoprotein-specific IgG binding antibody geometric mean concentration) of the booster vaccine dose. Safety was assessed in all participants who received the booster vaccination; immunogenicity was assessed in all participants who received the booster vaccination, had at least one evaluable sample after the booster, and had no major protocol deviations that could have influenced the immune response. This trial is registered with ClinicalTrials.gov, NCT04711356.

Findings Between July 8 and Aug 18, 2021, 58 children were assessed for eligibility and 50 (27 aged 4–7 years and 23 aged 9–15 years) were enrolled and received an Ad26.ZEBOV booster vaccination, more than 3 years after receiving dose one of the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen. The booster was well tolerated. The most common solicited local adverse event during the 7 days after vaccination was injection site pain, reported in 18 (36%, 95% CI 23–51) of 50 participants. The most common solicited systemic adverse event during the 7 days after vaccination was headache, reported in 11 (22%, 12–36) of 50 participants. Malaria was the most common unsolicited adverse event during the 28 days after vaccination, reported in 25 (50%, 36–64) of 50 participants. No serious adverse events were observed during the study period. 7 days after vaccination, the Ebola virus glycoprotein-specific IgG binding antibody geometric mean concentration was 28 561 ELISA units per mL (95% CI 20 255–40 272), which was 44 times higher than the geometric mean concentration before the booster dose. 21 days after vaccination, the geometric mean concentration reached 64 690 ELISA units per mL (95% CI 48 356–86 541), which was 101 times higher than the geometric mean concentration before the booster dose.

Interpretation A booster dose of Ad26.ZEBOV in children who had received the two-dose Ad26.ZEBOV and MVA-BN-Filo vaccine regimen more than 3 years earlier was well tolerated and induced a rapid and robust increase in binding antibodies against Ebola virus. These findings could inform Ebola vaccination strategies in paediatric populations.

Funding Innovative Medicines Initiative 2 Joint Undertaking.

Lancet Infect Dis 2022

Published **Online** October 20, 2022 https://doi.org/10.1016/S1473-3099(22)00594-1

For the French translation of the abstract see **Online** for appendix 1

Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK (D Manno MD, A Bangura MSc, F Baiden PhD, P Ayieko PhD, J Foster MSc, N E Connor MSc, Y Njie MSc, B J Lawal MSc, G T Otieno BSc, E M-L Choi PhD, A D Balami PhD, H H Adetola FWACP, B Lowe MSc, Prof B Greenwood MD, Prof D Watson-Jones PhD); **EBOVAC Project, Kambia Town, Kambia District, Sierra Leone** (A Bangura, F Baiden, Y Njie, B J Lawal, G T Otieno, A D Balami, H H Adetola); **College of Medicine and Allied Health Sciences, University of Sierra Leone, Freetown, Sierra Leone** (A B Kamara MBChB, J Kallon MBChB, M Conteh MBChB, B Koroma BPharm Hons, P Borboh BPharm Hons, M T Kroma MSc, A T Deen BSc, G F Deen FWACP, Prof M Samai PhD, B Leigh FWACS); **Mwanza Intervention Trials Unit, National Institute for Medical Research, Mwanza, Tanzania** (P Ayieko, Prof D Watson-Jones); **Janssen Vaccines and Prevention, Leiden, Netherlands** (B Keshinro FWACP, C McLean PhD, K Luhn PhD, C Robinson MD); **Janssen Research and Development, Beerse, Belgium** (A Gaddah PhD)

Introduction

Children account for approximately 20% of Ebola virus disease cases during outbreaks.[1] Ebola virus disease affects children in many ways: young children (aged <5 years) have a more rapid disease progression and a higher risk of dying than adults,[2] and those children who survive Ebola virus disease can have major psychological trauma, having been separated from their parents and family throughout their disease, having lost time from school, and because they are often stigmatised when they return to their community.[3,4] For these reasons, an effective Ebola prevention strategy for

Correspondence to:
Dr Daniela Manno, Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London WC1E 7HT, UK
daniela.manno@lshtm.ac.uk

Research in context

Evidence before this study

We searched PubMed on June 30, 2022, using the terms "Ad26.ZEBOV" AND "booster", for articles published since database inception, with no language restrictions. We identified eight citations. After screening the full texts, we identified three studies that reported results on the safety and immunogenicity of a booster dose of Ad26.ZEBOV in previously vaccinated participants, and one study protocol.

An article by Goldstein and colleagues (2022) described results from a randomised, placebo-controlled, phase 1 trial that assessed the safety and immunogenicity of different regimens of the Ad26.ZEBOV and MVA-BN-Filo vaccines in healthy adults from the USA. A subgroup of participants received a booster vaccination 1 year after their first vaccine dose. The study found that an Ad26.ZEBOV booster vaccination was safe and elicited an anamnestic response in all participants.

An article by Ishola and colleagues (2022) reported results from a study conducted in Sierra Leone and had an open-label, non-randomised stage followed by a randomised, double-blind, controlled stage. Healthy adults who received the two-dose Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in the open-label stage were offered an Ad26.ZEBOV booster dose 2 years after their first vaccine dose. The study showed that the booster vaccination was well tolerated and induced a strong anamnestic response, as evidenced by a rapid increase in Ebola virus glycoprotein-specific binding antibody concentrations, which were approximately 40 times higher at 7 days after the booster vaccination and approximately 110 times higher at 21 days after the booster vaccination than before the booster.

An article by Barry and colleagues (2021) reported results from a randomised, placebo-controlled trial conducted in Burkina Faso, Cote d'Ivoire, Kenya, and Uganda. An Ad26.ZEBOV booster, in healthy adults 1 year after their first vaccine dose, was well tolerated and induced a rapid and robust increase in Ebola virus glycoprotein-specific binding antibody concentrations. In those who received the same Ebola vaccine regimen as in our study with a 56-day interval between doses, the binding antibody concentrations were approximately 59 times higher at 7 days after the booster vaccination and approximately 121 times higher at 21 days after the booster vaccination than before the booster.

Larivière and colleagues (2021) described the protocol of an open-label, randomised trial to evaluate the immunogenicity and safety of the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in health-care providers in the Democratic Republic of the Congo. In this study, participants were to be randomised to receive an Ad26.ZEBOV booster dose at either 1 year or 2 years after their first vaccine dose; the study is ongoing and the results are not available yet.

Added value of this study

To our knowledge, this is the first study to evaluate the safety and immunogenicity of a booster dose of Ad26.ZEBOV in children aged 4–15 years who had received the two-dose Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen more than 3 years earlier. We found that the Ad26.ZEBOV booster was well tolerated by the study participants, with no safety concerns. The booster vaccination elicited a robust anamnestic response, as shown by a rapid increase in Ebola virus glycoprotein-specific IgG binding antibody concentrations, which were approximately 44 times higher at 7 days after the booster vaccination and approximately 101 times higher at 21 days after the booster vaccination than immediately before the booster.

Implications of all the available evidence

To protect people from Ebola virus disease, effective interventions are needed. Three studies have shown that, in adults who have had previous vaccination, an Ad26.ZEBOV booster is safe and able to produce a rapid and robust increase of binding antibodies against Ebola virus. Our study shows that these findings apply to children, with a very similar extent of increase in antibody concentrations after the booster dose. Our results therefore support the strategy of providing vaccination to children with an additional Ad26.ZEBOV booster to be given at the start of an Ebola virus disease outbreak.

children living in areas at risk of Ebola virus disease outbreaks is crucial.

A heterologous, two-dose vaccine regimen comprising the monovalent, recombinant, replication-incompetent, adenovirus type 26 (Ad26) vector-based vaccine, encoding the Ebola virus glycoprotein of the Mayinga variant (Ad26.ZEBOV) as dose one, and the recombinant, non-replicating, modified vaccinia Ankara (MVA) vector-based vaccine, encoding glycoproteins from the Ebola virus Mayinga variant, Sudan virus Gulu variant, and Marburg virus Musoke variant, and the nucleoprotein from the Tai Forest virus (MVA-BN-Filo) as dose two, administered 56 days apart, is the only vaccine regimen that has received marketing authorisation (under exceptional circumstances) for immunisation of children aged 1 year or older in the EU.[5]

This vaccine regimen, which has previously been shown to provide protection in vaccinated non-human primates against an Ebola virus challenge,[6] had an acceptable safety profile and induced robust humoral immune responses in children participating in two randomised controlled trials, one in Sierra Leone and the other in Burkina Faso, Cote d'Ivoire, Kenya, and Uganda.[7,8]

The trial in Sierra Leone (VAC52150EBL3001, EBOVAC-Salone) was initiated during the 2014–16 Ebola virus disease outbreak in west Africa, with the aim to assess the efficacy of the vaccine regimen in preventing Ebola virus disease; however, it was not able to achieve

this objective because the disease incidence declined during the course of the study, as the outbreak was eventually brought under control.[7,9] In the absence of clinical efficacy data, the likelihood of protection induced by the vaccine regimen was inferred by correlating the magnitude of vaccine-elicited immune responses associated with protection in non-human primates with those observed in vaccinated human participants, a statistical approach referred to as immunobridging.[10]

In previous trials, robust immune responses were observed after dose two of the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in both adults and children, but they were also shown to wane over time.[7–9,11–15] In children, the long-term persistence of an immune response beyond 1 year was not known.[7] Although expected, it was also not known whether the vaccine regimen was able to produce immune memory that could be rapidly reactivated by a vaccine booster in children, as had been observed in adults.[9,14,16]

We aimed to evaluate the safety and immunogenicity of a booster dose of the Ad26.ZEBOV vaccine given more than 3 years after the first dose in children who had been vaccinated with the two-dose Ad26.ZEBOV and MVA-BN-Filo vaccine regimen.

Methods

Study design

We conducted an open-label, non-randomised, phase 2 trial (VAC52150EBL2011) at one clinic in Kambia Town, located in Kambia District in the North West Province of Sierra Leone. The study was approved by the Sierra Leone Ethics and Scientific Review Committee, the Pharmacy Board of Sierra Leone, and the London School of Hygiene & Tropical Medicine Ethics Committee. The protocol is available in appendix 2 (pp 7–57).

Participants

Eligible participants were healthy children who had received the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen at least 2 years earlier in the previous EBOVAC-Salone trial (in Kambia District, Sierra Leone; NCT02509494; figure 1) and who were aged 1–11 years at the time of their first vaccine dose in the earlier trial.[7,9] Participants were enrolled in two cohorts by age at the time of their first vaccine dose in the EBOVAC-Salone trial (1–3 years and 4–11 years), and we planned to enrol approximately equal numbers from each of these two age cohorts. Eligible participants were required to be healthy in the investigator's clinical judgement on the basis of medical history, physical examination, vital signs, and a haematological assessment at screening. Adolescent girls who had started their menstrual periods or were aged 12 years or older were required to have a negative urine β-human chorionic gonadotrophin pregnancy test at screening and immediately before booster vaccination. Exclusion criteria included breastfeeding or pregnancy; previous vaccination with a live-attenuated vaccine within 30 days before booster vaccination, or an inactivated vaccine within 15 days before booster vaccination; and previous severe adverse reaction to a vaccine. Eligiblity criteria are listed in full in appendix 2 (pp 25–27). Community engagement was conducted before commencement of the trial to ensure that effective recruitment strategies were in place. Documented informed consent from a community leader was obtained before the start of the study. Parents or guardians of eligible participants were given information about the trial in a language that they understood and they provided written informed consent after passing a test of understanding. Children aged 7 years or older were asked to provide written assent. If the parent or guardian could not read or write, the study procedures were explained by a study team member in a language that the parent or guardian understood, and informed consent was witnessed by a literate third person not involved in the study.

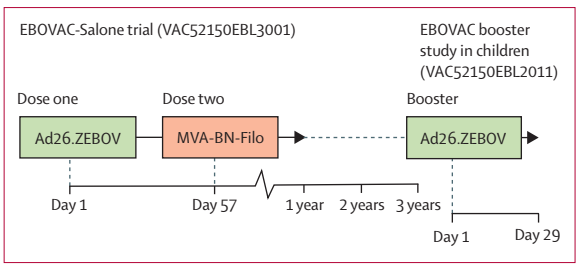


Figure 1: **Study design**
Vaccine doses were 5×10^{10} viral particles for Ad26.ZEBOV (dose one and booster) and 1×10^{8} infectious units for MVA-BN-Filo (dose two).

Procedures

All participants received a booster dose of Ad26.ZEBOV (Janssen Vaccines and Prevention, Dessau-Rosslau, Germany). The booster vaccine was administered as a single 0·5 mL intramuscular injection into the deltoid muscle at a dose of 5×10^{10} viral particles.

See Online for appendix 2

To record any immediate adverse events, participants were observed for at least 30 min after vaccination. During the first 7 days following booster vaccination, trained field workers visited participants at home to record local and systemic solicited adverse events (defined as signs and symptoms that participants' parents or guardians were specifically asked to report) using a diary card. A haematology panel (haemoglobin, white blood cell count with three-part differential, and platelet count) was performed at 7 days and 21 days after booster vaccination. Parents or guardians of participants received a 24-h telephone number to contact in case of a medical problem. Unsolicited adverse events (defined as events that were reported by the participants or their parents or guardians on their initiative or when they were asked about any symptoms or health problems after vaccination) were recorded from the booster vaccination until the end of the study at 28 days after booster

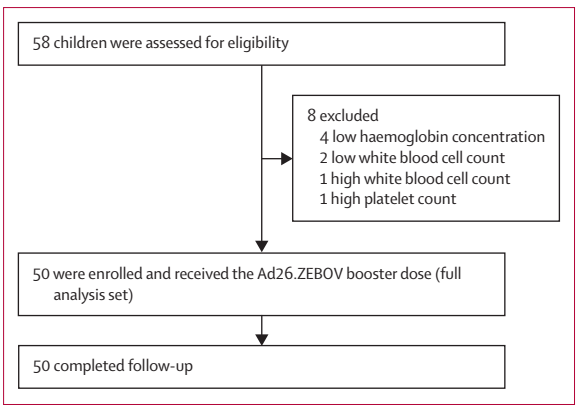


Figure 2: **Study profile**

vaccination. Grade 3 adverse events were defined as severe adverse events that required medical attention but were not immediately life-threatening.

Blood samples for immunogenicity analysis were collected immediately before the booster vaccination and at 7 days and 21 days after the booster vaccination. IgG responses against Ebola virus glycoprotein were analysed using the validated Ebola virus glycoprotein (Kikwit) Filovirus Animal Non-Clinical Group ELISA, as in previous studies.[7–9,11–15] The test has a lower limit of quantification of 36·11 ELISA units per mL and an upper limit of quantification of 194 938·88 ELISA units per mL. The analysis was conducted at Q² Solutions, San Juan Capistrano, CA, USA.

There were no major protocol deviations during the conduct of the study. There were two minor deviations. One was the use of infrared temperature scanning machines instead of using axillary temperature thermometers in all 50 participants. Infrared temperature scanning machines were considered to be more acceptable by study participants and staff during the COVID-19 outbreak. The other minor protocol deviation was a missed day 22 visit in one participant in the age 4–11 years cohort. These two deviations were not considered to have the potential to affect the safety of participants or to influence the immune response.

Outcomes

The primary outcomes were the safety and tolerability of the Ad26.ZEBOV booster vaccination, measured as the number of participants with solicited local and systemic adverse events in the 7 days after vaccination and unsolicited adverse events, including serious adverse events, in the 28 days after vaccination; and the vaccine-induced humoral immune response to the Ebola virus glycoprotein at 7 days and 21 days after vaccination, measured by Ebola virus glycoprotein-specific IgG binding antibody geometric mean concentration.

A planned exploratory outcome was the neutralising antibody response against the Ad26 vector before booster vaccination, as measured by a virus neutralisation assay, but this analysis had not yet been completed and is not reported in this manuscript. Results for this exploratory outcome will be made available on the trial registration page on ClinicalTrials.gov.

Statistical analysis

The study sample size (n=50) was a convenience sample and was not based on formal hypothesis testing considerations. However, using the sample size formula for estimating a population proportion with a given absolute precision, $n=Z^2\times P(1-P)/d^2$,[17] we calculated that this sample size would have allowed an estimation of the proportion of participants with solicited or unsolicited adverse events after booster vaccination with a plus or minus 10% margin of error (ie, an absolute precision within 10 percentage points of an anticipated proportion, with 95% confidence), assuming that approximately 15% of participants had a solicited adverse event or an unsolicited adverse event: $1{\cdot}96^2\times(0{\cdot}15\times0{\cdot}85)/0{\cdot}1^2=49$. A sample size of 50 participants (approximately 25 in each of the two age cohorts) would also allow for adequate characterisation of the humoral immune response after booster vaccination.

The primary analysis was performed when all participants had completed the study. The primary analysis set for safety (full analysis set) comprised all participants who received the booster vaccine. The primary analysis set for immunogenicity (per-protocol set) included all participants who received the booster vaccine, had at least one evaluable immunogenicity serum sample after vaccination, and had no major protocol deviations considered to have an effect on the immune response to the booster vaccination.

We merged the database containing the immunogenicity data from this booster study with the database containing the immunogenicity data from the EBOVAC-Salone trial, in particular the antibody concentration before the first dose (Ad26.ZEBOV) measured in the same participants using the same assay in the same laboratory. Thus, we were able to calculate the percentage of participants with an immunogenic response in the booster study with respect to the baseline before the first dose in the EBOVAC-Salone trial. Participants were considered to have a response by ELISA if samples were negative at baseline before the first dose and positive at following evaluations with a value that was greater than 2·5 times the lower limit of quantification of 36·11 ELISA units per mL, or if a sample was positive both at baseline before the first dose and at following evaluations with a greater than 2·5-times increase from baseline. The definition of response is the same as that used in the previous EBOVAC-Salone trial.[7,9] Binding antibody responses against Ebola virus glycoprotein were summarised as geometric mean concentrations. For this calculation, all values of less than the lower limit of quantification were imputed with half the lower limit of

www.thelancet.com/infection **Published online October 20, 2022 https://doi.org/10.1016/S1473-3099(22)00594-1**

	Age 1–3 years cohort (n=27)	Age 4–11 years cohort (n=23)
Age, years		
Median (IQR)	5 (4–5)	13 (11–14)
Range	4–7	9–15
Weight-for-age percentile*		
Number assessed	26	5
Median (IQR)	32% (15–53)	52% (7–65)
Lower than 2nd percentile	2 (8%)	0
Height-for-age percentile*		
Number assessed	26	6
Median (IQR)	42% (12–58)	61% (12–67)
Lower than 2nd percentile	1 (4%)	0
Weight-for-height percentile†		
Number assessed	21	..
Median (IQR)	38% (21–59)	..
Lower than 2nd percentile	2 (10%)	..
BMI, kg/m²‡		
Number assessed	..	17
Median (IQR)	..	22 (6–59)
Lower than 2nd percentile	..	1 (6%)
Sex		
Male	19 (70%)	12 (52%)
Female	8 (30%)	11 (48%)
Duration since first vaccine dose in the EBOVAC-Salone trial, years		
Median (IQR)	3·11 (3·08–3·13)	3·83 (3·82–3·85)
Range	3·04–3·23	3·53–3·93

Data are n or n (%) unless otherwise stated. *Calculated in children aged 11 years or younger (at enrolment in the current study) according to WHO growth charts. †Calculated in children aged 5 years or younger (at enrolment in the current study) according to WHO growth charts. ‡BMI was calculated for older children only (age 12–17 years at enrolment in the current study).

Table 1: **Baseline characteristics at the booster study screening visit, by age cohort at first vaccine dose in the EBOVAC-Salone trial**

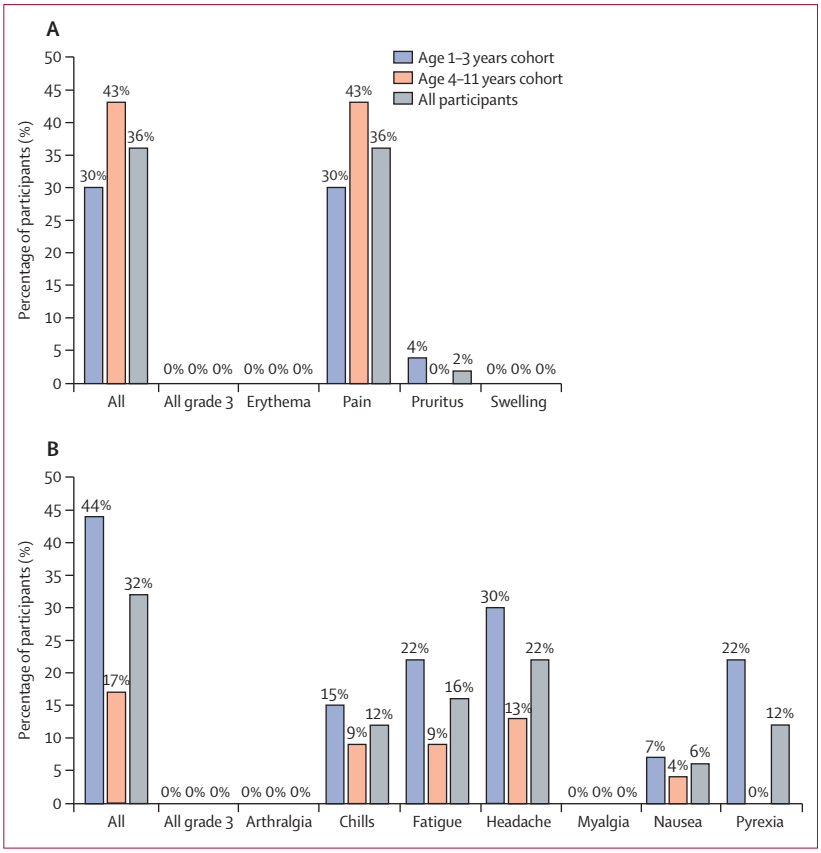


Figure 3: **Solicited adverse events after the Ad26.ZEBOV booster vaccination**
(A) Solicited local adverse events. (B) Solicited systemic adverse events. Solicited adverse events were observed during the period of 7 days after Ad26.ZEBOV booster vaccination.

quantification value. CIs were calculated using the Clopper-Pearson methods for percentages and using linear regression for geometric mean concentrations. We present two-sided 95% CIs for all safety and immunogenicity point estimates that were not 0, except when responder rates were 100%; in this case, we present one-sided 97·5% CIs.

We did a post-hoc analysis to compare antibody concentrations at 7 and 21 days after booster vaccination between participants classified as responders and non-responders at day 1 before the booster administration. For this analysis, geometric mean ratios were used to compare geometric mean concentrations, and p values were calculated using a *t* test.

Stata 16 was used for the statistical analyses. This study is registered with ClinicalTrials.gov, NCT04711356.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between July 8 and Aug 18, 2021, 58 children were assessed for eligibility and 50 were enrolled and received an Ad26.ZEBOV booster vaccination more than 3 years after their first vaccine dose, 27 (54%) of whom were in the original age 1–3 years parent study cohort (aged 4–7 years at the time of screening for this booster study), and 23 (46%) of whom were in the original age 4–11 years parent study cohort (aged 9–15 years at the time of screening for this booster study). Follow-up was completed on Sept 17, 2021. The safety analysis included all 50 study participants (figure 2). Baseline characteristics of the participants are shown in table 1.

Solicited adverse events were all mild (grade 1) and of short duration (≤3 days; figure 3; appendix 2 p 2). At least one solicited local adverse event was reported by 18 (36%, 95% CI 23–51) of 50 participants after booster vaccination: eight (30%, 14–50) of 27 in the age 1–3 years cohort and ten (43%, 23–66) of 23 in the age 4–11 years cohort. All 18 participants who reported at least one solicited local adverse event reported injection site pain and one (2%) participant also reported pruritus at the injection site (figure 3A; appendix 2 p 2). 16 (32%, 95% CI 20–47) of 50 participants reported at least one solicited systemic

	Age 1–3 years cohort (n=27)	Age 4–11 years cohort (n=23)	Overall (n=50)
Day 1 (baseline before booster vaccine)			
Number assessed	26	23	49
Geometric mean concentration, ELISA units per mL (95% CI)	934 (568–1534)	418 (287–608)	640 (461–888)
Participants with response*	23/24 (96%, 79–100)	17/22 (77%, 55–92)	40/46 (87%, 74–95)
Day 8 (7 days after booster vaccine)			
Number assessed	27	23	50
Geometric mean concentration, ELISA units per mL (95% CI)	30 463 (18 087–51 307)	26 478 (16 512–42 461)	28 561 (20 255–40 272)
Participants with response*	25/25 (100%, 86–100)	22/22 (100%, 85–100)	47/47 (100%, 92–100)
Day 22 (21 days after booster vaccine)			
Number assessed	27	22	49
Geometric mean concentration, ELISA units per mL (95% CI)	71 143 (47 819–105 844)	57 564 (36 375–91 095)	64 690 (48 356–86 541)
Participants with response*	25/25 (100%, 86–100)	22/22 (100%, 85–100)	47/47 (100%, 92–100)

Data are n unless otherwise stated. *Expressed as n/N (%, two-sided 95% CI or, when 100%, one-sided 97·5% CI), where n is the number of participants with response at that timepoint and N is the total number of participants with baseline data at first vaccine dose in the EBOVAC-Salone trial and at that timepoint. Participants were considered as having a response by ELISA if samples were negative at baseline before the first vaccine dose and positive at following evaluations with a value that was greater than 2·5 times the lower limit of quantification (36·11 ELISA units per mL), or if a sample was positive both at baseline before the first vaccine dose and at following evaluations and there was a greater than 2·5-times increase from baseline.

Table 2: **Ebola glycoprotein-specific binding antibody concentrations by age cohort at first vaccine dose in the EBOVAC-Salone trial and overall**

adverse event after booster vaccination (figure 3B; appendix 2 p 2): 12 (44%, 25–65) of 27 in the age 1–3 years cohort and four (17%, 5–39) of 23 in the age 4–11 years cohort. Headache was the most frequently reported solicited systemic adverse event, followed by fatigue, chills, and pyrexia (figure 3B; appendix 2 p 2). The most frequent unsolicited adverse event after booster vaccination was malaria, reported by 25 (50%, 95% CI 36–64) of 50 participants: 19 (70%, 50–86) of 27 in the age 1–3 years cohort and six (26%, 10–48) of 23 in the age 4–11 years cohort (appendix 2 p 3). No grade 3 adverse events and no serious adverse events were reported throughout the study (appendix 2 p 3).

After booster vaccination, the most commonly reported laboratory abnormalities were low haemoglobin concentration and low white blood cell count (appendix 2 pp 4–5). Two (4%, 95% CI 0–14) of 50 participants had haemoglobin concentrations of less than the local normal laboratory range at 7 days after the booster vaccination and three (6%, 1–17) of 49 had haemoglobin concentrations of less than the local normal laboratory range at 21 days after the booster. One (2%, 95% CI 0–11) of 50 participants had low white blood cell count at 7 days after the booster and two (4%, 0–14) of 49 had low white blood cell count at 21 days after the booster. None of these abnormalities were considered clinically relevant by the investigator. One participant in the youngest age cohort had a low platelet count ($80·0 \times 10^9$ cells per L) at 21 days after the booster, which was considered clinically relevant and was reported as an adverse event (appendix 2 pp 3, 5). The participant was asymptomatic and a repeated haematology assessment 10 days later showed a normal platelet count ($150·0 \times 10^9$ cells per L).

All 50 participants in the study fulfilled the criteria for the per-protocol analysis set for immunogenicity and the results of this analysis are presented in table 2 and figure 4.

Before the booster vaccination, participants' geometric mean concentration of binding antibodies against the Ebola virus glycoprotein was 640 ELISA units per mL (95% CI 461–888) overall, 934 ELISA units per mL (568–1534) in the age 1–3 years cohort, and 418 ELISA units per mL (287–608) in the age 4–11 years cohort (table 2). When compared with the binding antibody geometric mean concentration at baseline before their first vaccine dose, 40 (87%, 95% CI 74–95) of 46 participants still had a response at a median of 3·2 years from the time of dose one vaccination with the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in the EBOVAC-Salone trial. In the age 1–3 years cohort, 23 (96%, 95% CI 79–100) of 24 participants still had a response at a median of 3·1 years from the time of dose one vaccination in the EBOVAC-Salone trial. In the age 4–11 years cohort, 17 (77%, 95% CI 55–92) of 22 participants still had a response at a median of 3·8 years from the time of dose one vaccination.

7 days after the booster vaccination, participants' Ebola virus glycoprotein binding antibody geometric mean concentration increased to 28 561 ELISA units per mL (95% CI 20 255–40 272) overall, 30 463 ELISA units per mL (18 087–51 307) in the age 1–3 years cohort, and 26 478 ELISA units per mL (16 512–42 461) in the age 4–11 years cohort (table 2).

21 days after the booster vaccination, participants' Ebola virus glycoprotein binding antibody geometric mean concentration increased to 64 690 ELISA units per mL (95% CI 48 356–86 541) overall, 71 143 ELISA units per mL (47 819–105 844) in the age 1–3 years cohort, and 57 564 ELISA units per mL (36 375–91 095) in the age 4–11 years cohort (table 2).

When compared with the binding antibody geometric mean concentration before dose one vaccination, 47 (100%, one-sided 97·5% CI 92–100) of 47 participants with available data had a response at both 7 days and 21 days after the booster vaccination (table 2).

The overall binding antibody geometric mean concentration at 7 days after the booster vaccination was approximately 44 times higher than the geometric mean concentration before the booster; 32 times higher in the age 1–3 years cohort and 63 times higher in the age 4–11 years cohort. The overall binding antibody geometric mean concentration at 21 days after the booster vaccination

was approximately 101 times higher than the geometric mean concentration before the booster; 76 times higher in the age 1–3 years cohort and 137 times higher in the age 4–11 years cohort. The comparison of antibody concentrations between responders and non-responders at day 1 after booster vaccination, performed as a post-hoc analysis, showed that responders had higher binding antibody geometric mean concentrations at 21 days after the booster than non-responders (geometric mean ratio 2·39, 95% CI 1·21–4·74; p=0·014; appendix 2 p 6).

Discussion

This is the first clinical study of an Ad26.ZEBOV booster vaccination in children who had previously been vaccinated with the two-dose Ad26.ZEBOV and MVA-BN-Filo vaccine regimen. The booster vaccination was well tolerated, with injection site pain being the most frequent solicited local adverse event, and headache being the most frequent solicited systemic adverse event, followed by fatigue, chills, and pyrexia. No serious adverse events were reported in the 28 days after the booster dose. The Ad26.ZEBOV booster vaccine induced Ebola virus glycoprotein-specific binding antibody responses in all participants at 7 days and 21 days after the booster, with 44-times higher antibody concentration at 7 days and 101-times higher antibody concentration at 21 days for both age cohorts combined compared with concentration before the booster dose.

The safety results after the Ad26.ZEBOV booster dose in our study are consistent with the safety profile of the Ad26.ZEBOV dose one in children of similar age in the EBOVAC-Salone trial.[7]

The immunogenicity findings in children in this study are consistent with the data from adults in the EBOVAC-Salone trial, which showed that an Ad26.ZEBOV booster vaccination given 2 years after initial vaccination was well tolerated and induced a robust increase in binding antibody concentrations.[9] An Ad26.ZEBOV booster given to healthy adults 1 year after initial vaccination with the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen was also shown to be well tolerated and strongly immunogenic in the VAC52150EBL2002 study,[14] which was conducted in Kenya, Burkina Faso, Côte d'Ivoire, and Uganda.

To our knowledge, this is also the first study to collect long-term immunogenicity data in children vaccinated with the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen. The median time from receipt of first vaccine dose with the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in the EBOVAC-Salone study to baseline assessment before booster vaccination in the current study was 3·2 years. At this timepoint, binding antibodies were still detectable and 87% of all participants were classified as still having a response, indicating that the humoral immune response to the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in children is durable to at least 3 years. When stratified by age group, the median time from the first vaccine dose in the EBOVAC-Salone trial to baseline assessment in the current study was 3·1 years in the age 1–3 years cohort (96% still had a response) and 3·8 years in the age 4–11 years cohort (77% still had a response). In a post-hoc analysis, participants who were responders before the booster dose had higher antibody concentrations at 21 days after the booster vaccine than non-responders. However, our results also show that all non-responders had a response after the booster vaccination, suggesting that the booster was also immunogenic in this group.

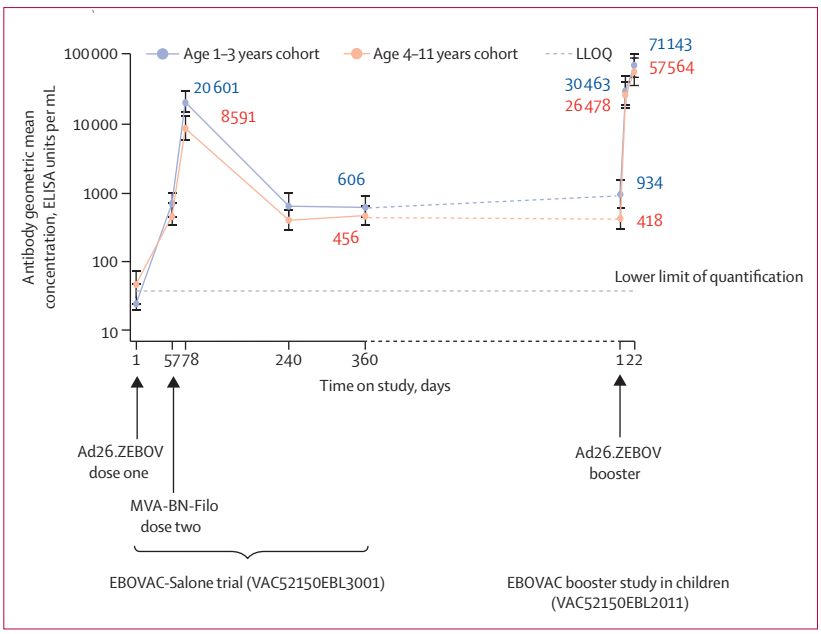


Figure 4: **Ebola virus glycoprotein-specific binding antibody concentrations**
The response profile of each age group is shown as geometric mean concentrations of anti-Ebola virus glycoprotein IgG. Error bars show 95% CIs. Labels for day 8 (7 days after the booster vaccination) and day 29 (28 days after the booster vaccination) in the VAC52150EBL2011 study have been omitted.

This study has some limitations. The follow-up period after booster vaccination was only 28 days, due to the end of the grant that funded this study. Although this did not affect the collection of solicited and unsolicited adverse events after vaccination, which continued up to 7 days for solicited adverse events and 28 days for unsolicited adverse events, as in previous studies, it limited the timeframe for collection of serious adverse events to 28 days after booster vaccination.[9,14] However, in previous studies with longer follow-up periods, none of the serious adverse events reported were considered related to the booster vaccine.[9,14] Therefore, we believe that a 28-day follow-up period was sufficient to characterise the safety of the Ad26.ZEBOV booster dose. In the previous studies, adults who were followed up for 1 year after receiving the Ad26.ZEBOV booster showed binding antibody geometric mean concentrations at this timepoint that were higher than at 1 year after the initial Ad26.ZEBOV and MVA-BN-Filo vaccine regimen administration.[9,14] Because the children in our study showed a binding antibody response similar to that previously observed in adults at 7 days

and 21 days after booster vaccination, it is plausible that their binding antibody kinetics will continue to reflect those of adults at later timepoints, and that the binding antibody geometric mean concentrations will be maintained at levels higher than after the initial Ad26.ZEBOV and MVA-BN-Filo vaccine regimen for at least 1 year after booster vaccine administration. Another limitation of this study is that neutralising antibodies against Ebola virus could not be assessed within the timeframe of the grant that funded the study. However, previous clinical trials and non-human primates' challenge studies have shown that the titres of neutralising antibodies strongly correlated with the concentration of Ebola virus glycoprotein binding antibodies after the initial Ad26.ZEBOV and MVA-BN-Filo vaccine regimen administration, therefore neutralising antibodies are likely to increase similarly to binding antibodies after booster vaccination.[6,7,9,10,15] Binding antibodies were also identified as the immune parameter most highly correlated with non-human primates' survival in challenge studies and were selected for use in the immunobridging analysis.[6,10] The assessment of cellular immune responses after booster vaccination was also not included in the study protocol because the laboratory in Sierra Leone was not capable of processing peripheral blood mononuclear cells at the time when the protocol was written, and we could not have established the technique within the timeframe of the grant. The ability of the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen to induce cellular immune responses has been studied previously in adults and children,[8,11–14] but data in children are scarce;[8] therefore, it would be important in future studies to collect further data in children and also assess cellular immune responses after the booster dose in both adults and children.

Finally, an important limitation of the study is that we do not know if the concentrations of binding antibodies observed after booster vaccination indicate protection against Ebola virus disease because an antibody threshold correlating with protection has not yet been established. However, considering that the clinical benefit of the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen was inferred using the immunobridging model based on the vaccine-induced binding antibody concentrations, and that these were higher after booster vaccination than after the initial vaccine regimen administration, it is plausible that the booster dose is beneficial in providing an increased likelihood of protection against Ebola virus disease.

This study provides valuable data that can inform future Ebola vaccination strategies in paediatric populations. The 56-day interval Ad26.ZEBOV and MVA-BN-Filo vaccine regimen has received marketing authorisation for immunisation of adults and children aged 1 year or older in the EU, with the possibility of an Ad26.ZEBOV booster in previously vaccinated people at imminent risk of infection with Ebola virus.[18] Our results, which show that the Ad26.ZEBOV booster vaccination induces a strong anamnestic response within 7 days in children vaccinated more than 3 years previously, support this recommendation in paediatric populations. Vaccination with the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen could be considered for children in areas with Ebola risk, with an additional Ad26.ZEBOV booster provided if there is an imminent risk of exposure to Ebola virus, such as during an Ebola virus disease outbreak. Modelling studies are needed to evaluate the best administration strategy in these emergency situations (ie, ring vaccination *vs* mass vaccination approach). Outside outbreak situations, whether a booster dose is needed after an interval of time from the first vaccination, and the optimal timing for booster administration, still remain to be established. Results from the ongoing VAC52150EBL2007 study will elucidate if there is any difference in the elicited immune response if the booster dose is given either 1 year or 2 years after the first vaccination,[19] while another booster study, VAC52150EBL2010 (NCT05064956), assessing the safety and immunogenicity of a booster dose in previously vaccinated HIV-positive adults, will also provide long-term immunogenicity data after more than 4 years from initial vaccination with the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in this group. Further research is also needed to define the best approach for the administration of the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in paediatric populations in countries with Ebola risk; for example, whether vaccination should be given through campaigns or integrated within the routine paediatric immunisation schedule.

Contributors

DM drafted the manuscript and conducted the literature search. DM, FB, JF, NEC, BKe, EM-LC, AG, CM, KL, MS, BLo, CR, BLe, BG, and DW-J were involved in the study concept and design, study conduct, and interpretation of results. DW-J was the lead scientist for the programme (EBOVAC1) at the London School of Hygiene & Tropical Medicine. CR was the lead scientist for the programme at Janssen Vaccines and Prevention. BLe was the clinical trial principal investigator in Sierra Leone. AB and ADB were the coordinators of the study in Sierra Leone. ABK, JK, MC, GFD, and HHA contributed to enrolment and clinical care of participants and data collection. YN was responsible for data management. BJL, MTK, GTO, and BLo were responsible for laboratory sample analysis, samples management, and laboratory results interpretation. ATD, AB, and JK were responsible for community engagement activities. BKo and PB were the clinical trial pharmacists and were responsible for study vaccine preparation and dispensing. PA conducted the statistical analysis. PA and DM accessed and verified the data. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

Janssen Vaccines and Prevention was the vaccine manufacturer and donated the vaccine for this study. BKe, AG, CM, KL, and CR were full-time employees of Janssen, Pharmaceutical Companies of Johnson & Johnson, at the time of the study. AG, CM, KL, and CR report ownership of shares in Janssen, Pharmaceutical Companies of Johnson & Johnson. All other authors declare funding from the Innovative Medicines Initiative 2 Joint Undertaking.

Data sharing

Following publication of the primary and exploratory objectives as detailed in the protocol, individual deidentified participants' data and a data dictionary will be made available upon request via the London School of Hygiene & Tropical Medicine research data repository, LSHTM Data Compass at http://datacompass.lshtm.ac.uk. Requests with a defined analysis plan can be sent via LSHTM Data Compass. The clinical study protocol is available in appendix 2.

Acknowledgments

This work was supported by the Innovative Medicines Initiative 2 Joint Undertaking (grant 115854 [to the EBOVAC 1 project]), Janssen Vaccines and Prevention, the EU's Horizon 2020 research and innovation programme (to the Innovative Medicines Initiative 2 Joint Undertaking), and the European Federation of Pharmaceutical Industries and Associations (to the Innovative Medicines Initiative 2 Joint Undertaking). We thank the staff and management of Kambia Government Hospital; the Kambia District health management team; the site study team; the clinical trial monitors; the College of Medicine and Allied Health Sciences, University of Sierra Leone, and the London School of Hygiene & Tropical Medicine project management and administrative teams; the social science team; the community engagement team; and colleagues from GOAL. We thank all the study participants and their families. We also thank Sónia Silva (Janssen Vaccines and Prevention, Leiden, Netherlands) for publication coordination assistance. We thank Valérie Oriol-Mathieu (Janssen Vaccines and Prevention, Leiden, Netherlands) for reviewing the manuscript and assisting with responses to reviewers' and editorial comments.

References

1 US Centers for Disease Control and Prevention. Cost of the Ebola epidemic: impact of Ebola on children. 2019. https://www.cdc.gov/vhf/ebola/history/2014-2016-outbreak/cost-of-ebola.html (accessed Aug 18, 2022).

2 WHO. Ebola virus disease—Democratic Republic of the Congo. May 30, 2019. https://www.who.int/emergencies/disease-outbreak-news/item/30-may-2019-ebola-drc-en (accessed Aug 18, 2022).

3 Vetter P, Kaiser L, Schibler M, Ciglenecki I, Bausch DG. Sequelae of Ebola virus disease: the emergency within the emergency. *Lancet Infect Dis* 2016; **16:** e82–91.

4 UNICEF. Ebola: why Ebola is so devastating for children. 2022. https://www.unicef.org/emergencies/ebola (accessed Aug 18, 2022).

5 European Commission. Vaccine against Ebola: Commission grants new market authorisations. July 1, 2020. https://ec.europa.eu/commission/presscorner/detail/en/IP_20_1248 (accessed Aug 18, 2022).

6 Callendret B, Vellinga J, Wunderlich K, et al. A prophylactic multivalent vaccine against different filovirus species is immunogenic and provides protection from lethal infections with Ebolavirus and Marburgvirus species in non-human primates. *PLoS One* 2018; **13:** e0192312.

7 Afolabi MO, Ishola D, Manno D, 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:** 110–22.

8 Anywaine Z, Barry H, Anzala O, 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.

9 Ishola D, Manno D, Afolabi MO, et al. Safety and long-term immunogenicity of the two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in adults in Sierra Leone: a combined open-label, non-randomised stage 1, and a randomised, double-blind, controlled stage 2 trial. *Lancet Infect Dis* 2022; **22:** 97–109.

10 Roozendaal R, Hendriks J, van Effelterre T, et al. Nonhuman primate to human immunobridging to infer the protective effect of an Ebola virus vaccine candidate. *NPJ Vaccines* 2020; **5:** 112.

11 Milligan ID, Gibani MM, Sewell R, et al. Safety and immunogenicity of novel adenovirus type 26- and modified vaccinia ankara-vectored Ebola vaccines: a randomized clinical trial. *JAMA* 2016; **315:** 1610–23.

12 Mutua G, Anzala O, Luhn K, et al. Safety and immunogenicity of a 2-dose heterologous vaccine regimen with Ad26.ZEBOV and MVA-BN-Filo Ebola vaccines: 12-month data from a phase 1 randomized clinical trial in Nairobi, Kenya. *J Infect Dis* 2019; **220:** 57–67.

13 Anywaine Z, Whitworth H, Kaleebu P, et al. Safety and immunogenicity of a 2-dose heterologous vaccination regimen with Ad26.ZEBOV and MVA-BN-Filo Ebola vaccines: 12-month data from a phase 1 randomized clinical trial in Uganda and Tanzania. *J Infect Dis* 2019; **220:** 46–56.

14 Barry H, Mutua G, Kibuuka H, et al. Safety and immunogenicity of 2-dose heterologous Ad26.ZEBOV, MVA-BN-Filo Ebola vaccination in healthy and HIV-infected adults: a randomised, placebo-controlled phase II clinical trial in Africa. *PLoS Med* 2021; **18:** e1003813.

15 Pollard AJ, Launay O, Lelievre JD, et al. Safety and immunogenicity of a two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in adults in Europe (EBOVAC2): a randomised, observer-blind, participant-blind, placebo-controlled, phase 2 trial. *Lancet Infect Dis* 2021; **21:** 493–506.

16 Goldstein N, Bockstal V, Bart S, et al. Safety and immunogenicity of heterologous and homologous two dose regimens of Ad26- and MVA-vectored Ebola vaccines: a randomized, controlled phase 1 study. *J Infect Dis* 2022; **226:** 595–607.

17 Lwanga SK, Lemeshow S. Sample size determination in health studies: a practical manual. 1991. https://apps.who.int/iris/handle/10665/40062 (accessed Aug 18, 2022).

18 European Medicines Agency. Zabdeno. 2022. https://www.ema.europa.eu/en/medicines/human/EPAR/zabdeno (accessed Aug 18, 2022).

19 Larivière Y, Zola T, Stoppie E, et al. Open-label, randomised, clinical trial to evaluate the immunogenicity and safety of a prophylactic vaccination of healthcare providers by administration of a heterologous vaccine regimen against Ebola in the Democratic Republic of the Congo: the study protocol. *BMJ Open* 2021; **11:** e046835.

THE LANCET
Infectious Diseases

Supplementary appendix 2

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Manno D, Bangura A, Baiden F, 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; published online Oct 20. https://doi.org/10.1016/S1473-3099(22)00594-1.

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

Supplementary material

Table of Contents

1. SUPPLEMENTARY TABLES 2

Table S1. Solicited local adverse events by age cohort and overall: number, duration and time to onset 2

Table S2. Solicited systemic adverse events by age cohort and overall: number, duration and time to onset 2

Table S3. Unsolicited adverse events by age cohort and overall 3

Table S4. Haematology assessment 7 days after booster vaccination, by age cohort and overall 4

Table S5. Haematology assessment 21 days after booster vaccination, by age cohort and overall 5

Table S6. Responder status at day 1 and Ebola glycoprotein-specific binding antibody responses after booster vaccination 6

2. STUDY PROTOCOL 7

1. SUPPLEMENTARY TABLES

Table S1. Solicited local adverse events by age cohort and overall: number, duration and time to onset

	Age 1–3 years cohort (n=27)	Age 4–11 years cohort (n=23)	Overall (n=50)
Any solicited local event*	8 (30; 14-50)	10 (43; 23-66)	18 (36; 23-51)
Any grade 3 solicited local event	0	0	0
Erythema	0	0	0
Pain	8 (30; 14-50)	10 (43; 23-66)	18 (36; 23-51)
Median duration (min; max), days	1 (0·5; 1·6)	0·6 (0·5; 1·0)	0·6 (0·5; 1·6)
Median time to onset (min; max), days	0·8 (0·1; 1·1)	0·2 (0·1; 1·2)	0·3 (0·1; 1·2)
Pruritus	1 (4; 0-19)	0	1 (2; 0-11)
Median duration (min; max), days	†	†	†
Median time to onset (min; max), days	†	†	†
Swelling	0	0	0

Solicited local adverse events (AEs) are reported as number (%; 95%CI) of participants with 1 or more solicited AEs at the injection site. *All solicited local AEs were grade 1. †Data not available.

Table S2. Solicited systemic adverse events by age cohort and overall: number, duration and time to onset

	Age 1–3 years cohort (n=27)	Age 4–11 years cohort (n=23)	Overall (n=50)
Any solicited systemic event*	12 (44; 25-65)	4 (17; 5-39)	16 (32; 20-47)
Any grade 3 solicited systemic event	0	0	0
Arthralgia	0	0	0
Chills	4 (15; 4-34)	2 (9; 1-28)	6 (12; 5-24)
Median duration (min; max), days	0·5 (0·5; 3·0)	0·5 (0·5; 0·5)	1 (0·5; 3·0)
Median time to onset (min; max), days	3·8 (0·9; 6·9)	1·8 (1·8; 1·9)	1·9 (0·9; 6·9)
Fatigue	6 (22; 9-42)	2 (9; 1-28)	8 (16; 7-29)
Median duration (min; max), days	0·5 (0·5; 1·0)	0·5 (0·5; 0·5)	0·5 (0·5; 1·0)
Median time to onset (min; max), days	2·0 (0·3; 6·9)	0·5 (0·1; 1·0)	1·0 (0·1; 6·9)
Headache	8 (30; 14-50)	3 (13; 3-34)	11 (22; 12-36)
Median duration (min; max), days	0·5 (0·5; 0·5)	0·5 (0·5; 0·5)	0·5 (0·5; 0·5)
Median time to onset (min; max), days	3·0 (0·3; 6·9)	1·0 (0·2; 4·9)	2·9 (0·2; 6·9)
Myalgia	0	0	0
Nausea	2 (7; 1-24)	1 (4; 0-22)	3 (6; 1-17)
Median duration (min; max), days	0·5 (0·5; 0·5)	†	0·5 (0·5; 0·5)
Median time to onset (min; max), days	5·4 (3·8; 6·9)	1·9 (1·9; 1·9)	3·8 (1·9; 6·9)
Pyrexia	6 (22; 9-42)	0	6 (12; 5-24)
Median duration (min; max), days	0·5 (0·5; 0·6)	†	0·5 (0·5; 0·6)
Median time to onset (min; max), days	3·6 (0·3; 6·9)	†	3·6 (0·3; 6·9)

Solicited systemic adverse events (AEs) are reported as number (%; 95%CI) of participants with 1 or more solicited systemic AEs. *All solicited systemic AEs were grade 1. †Data not available.

Table S3. Unsolicited adverse events by age cohort and overall

	Age 1–3 years cohort (n=27)	**Age 4–11 years cohort (n=23)**	**Overall (n=50)**
Any unsolicited AE	23 (85; 66-96)	9 (39; 20-61)	32 (64; 49-77)
Any grade 3 unsolicited AE	0	0	0
Infections and infestations	21 (78; 58-91)	7 (30; 13-53)	28 (56; 41-70)
Malaria	19 (70; 50-86)	6 (26; 10-48)	25 (50; 36-64)
Upper respiratory tract infections	6 (22; 9-42)	1 (4; 0-22)	7 (14; 6-27)
Lower respiratory tract infection/pneumonia	3 (11; 2-29)	1 (4; 0-22)	4 (8; 2-19)
Rhinitis	1 (4; 0-19)	0	1 (2; 0-11)
Worm infestation	1 (4; 0-19)	1 (4; 0-22)	2 (4; 0-14)
Acute otitis media	1 (4; 0-19)	0	1 (2; 0-11)
Scabies	1 (4; 0-19)	1 (4; 0-22)	2 (4; 0-14)
Urinary tract infection	0	2 (9; 1-28)	2 (4; 0-14)
Gastroenteritis	0	1 (4; 0-22)	1 (2; 0-11)
Blood and lymphatic system disorders	2 (7; 1-24)	0	2 (4; 0-14)
Thrombocytopaenia	1 (4; 0-19)*	0	1 (2; 0-11)
Thrombocytosis	1 (4; 0-19)	0	1 (2; 0-11)
Vascular disorders	0	1 (4; 0-22)	1 (2; 0-11)
Leg ulcer	0	1 (4; 0-22)	1 (2; 0-11)
Skin and subcutaneous tissue disorders	1 (4; 0-19)	0	1 (2; 0-11)
Rash	1 (4; 0-19)	0	1 (2; 0-11)
Injuries, poisoning and procedural complications	1 (4; 0-19)	0	1 (2; 0-11)
Eye burns	1 (4; 0-19)	0	1 (2; 0-11)
Gastrointestinal disorders	0	1 (4; 0-22)	1 (2; 0-11)
Dental caries	0	1 (4; 0-22)	1 (2; 0-11)
General disorders and administration site conditions	1 (4; 0-19)	0	1 (2; 0-11)
Swollen thumb	1 (4; 0-19)	0	1 (2; 0-11)

Unsolicited adverse events (AEs) are reported as number (%; 95%CI) of participants with 1 or more unsolicited AEs and are coded using MedDRA version 23·1. *Platelet count = $80{\cdot}0 \times 10^9$/L, the participant was asymptomatic; a repeated haematology assessment performed 10 days later showed a normal platelet count ($150{\cdot}0 \times 10^9$/L).

Table S4. Haematology assessment 7 days after booster vaccination, by age cohort and overall

	Age 1–3 years cohort (n=27)	Age 4–11 years cohort (n=23)	Overall (n=50)
HGB, g/dl			
Mean (SD)	10·5 (1·1)	11·1 (0·8)	10·8 (1·0)
Median (Q1; Q3)	10·7 (8·3; 12·2)	11·1 (10·0; 12·4)	10·9 (8·3; 12·4)
Low HGB,* n (%; 95%CI)	1 (4; 0-19)	1 (4; 0-22)	2 (4; 0-14)
WBC, × 10^9/L			
Mean (SD)	8·5 (2·1)	6·1 (1·4)	7·4 (2·2)
Median (Q1; Q3)	8·6 (5·4; 12·9)	6·2 (2·7; 8·3)	7·0 (2·7; 12·9)
Low WBC,* n (%; 95%CI)	0	1 (4; 0-22)	1 (2; 0-11)
LYM, × 10^9/L			
Mean (SD)	4·4 (1·3)	3·1 (0·8)	3·8 (1·3)
Median (Q1; Q3)	4·3 (2·0; 7·2)	3·1 (1·5; 4·8)	3·4 (1·5; 7·2)
Low LYM,* n	0	0	0
MON, × 10^9/L			
Mean (SD)	0·6 (0·2)	0·5 (0·2)	0·5 (0·2)
Median (Q1; Q3)	0·6 (0·3; 1·0)	0·4 (0·2; 0·8)	0·5 (0·2; 1·0)
Low MON,* n	0	0	0
GRA, × 10^9/L			
Mean (SD)	4·4 (0·6)	4·4 (0·4)	4·4 (0·5)
Median (Q1; Q3)	4·4 (3·2; 5·6)	4·4 (3·7; 5·4)	4·4 (3·2; 5·6)
Low GRA,* n	0	0	0
PLT, × 10^9/L			
Mean (SD)	376·6 (115·9)	262·5 (50·7)	324·1 (107·6)
Median (Q1; Q3)	364·0 (211·0; 712·0)	254·0 (202·0; 362·0)	301·0 (202·0; 712·0)
Low PLT,* n	0	0	0
Mean change from baseline (screening)			
HGB, g/dl	0·3	0·1	0·2
WBC, × 10^9/L	-0·1	-0·2	-0·1
LYM, × 10^9/L	-0·1	-0·1	-0·1
MON, × 10^9/L	-0·1	0·0	-0·1
GRA, × 10^9/L	0·1	0·0	0·1
PLT, × 10^9/L	44·8	-11·0	19·1

*According to the following age and sex specific normal ranges of the local laboratory: age 0·5–4·9 years (males and females): haemoglobin (HGB) 8·0–12·7 g/dL, white blood cells (WBC) 5·1–17·6 x 10^9/L, lymphocytes (LYM) 2·3–11·9 x 10^9/L, monocytes (MON) 0·2–1·0 × 10^9/L, granulocytes (GRA) 1·5-8·5 × 10^9/L, platelets (PLT) 110–637 × 10^{12}/L; age 5–12 years (males and females): HGB 9·1–13·5 g/dL, WBC 4·1–11·9 × 10^9/L, LYM 1·6–5·8 × 10^9/L, MON 0·2–1·1 × 10^9/L, GRA 1·6-6·2 × 10^9/L, PLT 117–417 × 10^{12}/L; age 13-17 years (males): HGB 10·4–14·8 g/dL, WBC 3·6–10·3 × 10^9/L, LYM 1·4–4·2 × 10^9/L, MON 0·2–1·0 × 10^9/L, GRA 1·4–5·4 × 10^9/L, PLT 108–326 × 10^{12}/L; age 13-17 years (females): HGB 9·4–14·2 g/dL, WBC 3·8–9·3 × 10^9/L, LYM 1·4–3·9 × 10^9/L, MON 0·2–0·9 × 10^9/L, GRA 1·6–5·2 × 10^9/L, PLT 143–390 × 10^{12}/L.

Table S5. Haematology assessment 21 days after booster vaccination, by age cohort and overall

	Age 1–3 years cohort (n=27)	Age 4–11 years cohort (n=22*)	Overall (n=49*)
HGB, g/dl			
Mean (SD)	10·4 (0·9)	11·0 (0·9)	10·7 (0·9)
Median (Q1; Q3)	10·4 (7·8; 11·8)	11·0 (9·5; 12·5)	10·7 (7·8; 12·5)
Low HGB,† n (%; 95%CI)	1 (4; 0-19)	2 (9; 1-28)	3 (6; 1-17)
WBC, × 10^9/L			
Mean (SD)	9·3 (3·1)	5·9 (1·4)	7·8 (3·0)
Median (Q1; Q3)	8·5 (5·1; 17·5)	5·9 (3·6; 9·4)	6·9 (3·6; 17·5)
Low WBC,† n (%; 95%CI)	0	2 (9; 1-28)	2 (4; 0-14)
LYM, × 10^9/L			
Mean (SD)	4·6 (2·0)	2·6 (0·8)	3·7 (1·9)
Median (Q1; Q3)	4·2 (1·5; 9·8)	2·7 (0·8; 4·1)	3·3 (0·8; 9·8)
Low LYM,† n (%; 95%CI)	1 (4; 0-19)	1 (4; 0-22)	2 (4; 0-14)
MON, × 10^9/L			
Mean (SD)	0·6 (0·3)	0·4 (0·2)	0·5 (0·3)
Median (Q1; Q3)	0·5 (0·3; 1·2)	0·4 (0·2; 1·0)	0·5 (0·2; 1·2)
Low MON,† n	0	0	0
GRA, × 10^9/L			
Mean (SD)	4·5 (0·5)	4·4 (0·4)	4·4 (0·4)
Median (Q1; Q3)	4·5 (3·0; 5·4)	4·4 (3·5; 4·8)	4·4 (3·0; 5·4)
Low GRA,† n	0	0	0
PLT, × 10^9/L			
Mean (SD)	324·9 (106·7)	251·8 (64·5)	292·1 (96·6)
Median (Q1; Q3)	331·0 (80·0; 570·0)	244·0 (147·0; 446·0)	266·0 (80·0; 570·0)
Low PLT,† n (%; 95%CI)	1 (4; 0-19)‡	0	1 (2; 0-11)
Mean change from baseline (screening)			
HGB, g/dl	0·2	0·0	0·1
WBC, × 10^9/L	0·7	-0·4	0·2
LYM, × 10^9/L	0·2	-0·5	-0·2
MON, × 10^9/L	0·0	-0·1	0·0
GRA, × 10^9/L	0·1	0·0	0·0
PLT, × 10^9/L	-7·0	-22·6	-14·0

*One participant missed the day 22 visit. †According to the following age and sex specific normal ranges of the local laboratory: age 0·5–4·9 years (males and females): haemoglobin (HGB) 8·0–12·7 g/dL, white blood cells (WBC) 5·1–17·6 ×10^9/L, lymphocytes (LYM) 2·3–11·9 × 10^9/L, monocytes (MON) 0·2–1·0 × 10^9/L, granulocytes (GRA) 1·5-8·5 × 10^9/L, platelets (PLT) 110–637 × 10^{12}/L; age 5–12 years (males and females): HGB 9·1–13·5 g/dL, WBC 4·1–11·9 × 10^9/L, LYM 1·6–5·8 × 10^9/L, MON 0·2–1·1 × 10^9/L, GRA 1·6-6·2 × 10^9/L, PLT 117–417 × 10^{12}/L; age 13-17 years (males): HGB 10·4–14·8 g/dL, WBC 3·6–10·3 × 10^9/L, LYM 1·4–4·2 × 10^9/L, MON 0·2–1·0 × 10^9/L, GRA 1·4–5·4 × 10^9/L, PLT 108–326 × 10^{12}/L; age 13-17 years (females): HGB 9·4–14·2 g/dL, WBC 3·8–9·3 × 10^9/L, LYM 1·4–3·9 × 10^9/L, MON 0·2–0·9 × 10^9/L, GRA 1·6–5·2 × 10^9/L, PLT 143–390 × 10^{12}/L. ‡Platelet count = 80·0 × 10^9/L, the participant was asymptomatic; a repeated haematology assessment performed 10 days later showed a normal platelet count (150·0 × 10^9/L).

Table S6. Responder status at day 1 and Ebola glycoprotein-specific binding antibody responses after booster vaccination

	Responder status at day 1 (before booster vaccine)*		GMR	P-value
	Responder	Non-responder	(95% CI)	
Day 8 (7 days after booster vaccine)				
Number assessed	40	10		
GMC (95% CI)	31 602 (21767-45880)	19 053 (7213-50325)	1·66 (0·71-3·90)	0·240
Day 22 (21 days after booster vaccine)				
Number assessed	39	10		
GMC (95% CI)	77 282 (57 706-103 500)	32 328 (13 974-74 786)	2·39 (1·21-4·74)	0·014

*Participants were considered as having a response by ELISA if samples were negative at baseline before the first vaccine dose and positive at following evaluations with a value that was greater than 2·5 times the lower limit of quantification (36·11 ELISA units per mL), or if a sample was positive both at baseline before the first vaccine dose and at following evaluations and there was a greater than 2·5-times increase from baseline. GMC=geometric mean concentration in ELISA units per mL; GMR=geometric mean ratio.

2. STUDY PROTOCOL

VAC52150EBL2011

An open label study to evaluate the safety and immunogenicity of an Ad26.ZEBOV booster dose in children previously vaccinated with the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen

Protocol VAC52150EBL2011; Phase 2

Innovative Medicines Initiative

London School of Hygiene and Tropical Medicine and
and Janssen Vaccines and Prevention B.V.

VAC52150 (Ad26.ZEBOV/MVA-BN-Filo [MVA-mBN226B])

Version 2.0, 15 June 2021

	NUMBER	DATE
FINAL VERSION	1.0	30/10/2020
AMENDMENT (if any)	2.0	15/06/2021

Protocol authorised by:

Name:	Professor Deborah Watson-Jones	**Role:**	Chief Investigator
Signature:		**Date:**	
Name:	Dr Bailah Leigh	**Role:**	Principal Investigator
Signature:		**Date:**	
Name:	Mrs Patricia Henley	**Role:**	Sponsor Representative
Signature:		**Date:**	

Sponsor

London School of Hygiene & Tropical Medicine is the main research sponsor for this study. For further information regarding the sponsorship conditions, please contact:

The Research Governance and Integrity Office,
London School of Hygiene & Tropical Medicine
Keppel Street
London WC1E 7HT
Tel: +44 207 927 2626
Email: rgio@lshtm.ac.uk

Funder

This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement EBOVAC1 (grant nr. 115854). This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and the European Federation of Pharmaceutical Industries and Associations.

This study will adhere to the principles outlined in the International Conference on Harmonisation Good Clinical Practice guidelines, protocol and all applicable local regulations.

Table of Contents

ABBREVIATIONS 5

PROTOCOL AMENDMENTS 6

SYNOPSIS 8

TIME AND EVENTS SCHEDULE 11

1 INTRODUCTION 13
1.1 BACKGROUND 13
1.2 BENEFITS/RISKS OF PARTICIPATION 14
1.3 OVERALL RATIONALE FOR THE STUDY 16
2 OBJECTIVES, ENDPOINTS, AND HYPOTHESIS 17
2.1 OBJECTIVES AND ENDPOINTS 17
2.2 HYPOTHESIS 17
3 STUDY DESIGN 17
3.1 OVERVIEW OF STUDY DESIGN 17
4 PARTICIPANT POPULATION 18
4.1 INCLUSION CRITERIA 18
4.2 EXCLUSION CRITERIA 19
4.3 PROHIBITIONS AND RESTRICTIONS 20
5 INTERVENTION ALLOCATION AND BLINDING 20
5.1 BLINDING 20
6 DOSAGE AND ADMINISTRATION 20
6.1 CRITERIA FOR POSTPONEMENT OF VACCINATION 21
7 CONCOMITANT THERAPY 21
8 STUDY EVALUATIONS 21
8.1 STUDY PROCEDURES 22
8.2 PROCEDURES IN CASE OF A STUDY PAUSE 23
8.3 SAFETY EVALUATIONS 23
8.4 IMMUNOGENICITY ASSESSMENTS 26
8.5 SAMPLE COLLECTION AND HANDLING 27
9 PARTICIPANT COMPLETION/WITHDRAWAL FROM THE STUDY 27
9.1 COMPLETION 27
9.2 WITHDRAWAL FROM THE STUDY 27
10 STATISTICAL METHODS 28
10.1 ANALYSIS SETS 28
10.2 SAMPLE SIZE DETERMINATION 28
10.3 PARTICIPANT INFORMATION 28
10.4 SAFETY ANALYSES 28
10.5 IMMUNOGENICITY ANALYSIS 29
10.6 INTERIM ANALYSIS 29
10.7 INDEPENDENT DATA MONITORING COMMITTEE 29
11 ADVERSE EVENT REPORTING 29
11.1 DEFINITIONS 29
11.2 SPECIAL REPORTING SITUATIONS 32
11.3 PROCEDURES 32
11.4 CONTACTING SPONSOR REGARDING SAFETY 34
12 PRODUCT QUALITY COMPLAINT HANDLING 34
12.1 PROCEDURES 34
12.2 CONTACTING SPONSOR REGARDING PRODUCT QUALITY 35
13 STUDY INTERVENTION INFORMATION 35
13.1 PACKAGING 35
13.2 LABELLING 35
13.3 PREPARATION, HANDLING, STORAGE 35
13.4 INTERVENTION ACCOUNTABILITY 35
14 STUDY -SPECIFIC MATERIALS 36
15 ETHICAL ASPECTS 36
15.1 STUDY-SPECIFIC DESIGN CONSIDERATIONS 36
15.2 REGULATORY ETHICS COMPLIANCE 37

16 ADMINISTRATIVE REQUIREMENTS 40
16.1 PROTOCOL AMENDMENTS 40
16.2 REGULATORY DOCUMENTATION 40
16.3 SUBJECT IDENTIFICATION AND ENROLMENT LOGS 41
16.4 SOURCE DOCUMENTATION 41
16.5 CASE REPORT FORM COMPLETION 42
16.6 DATA QUALITY ASSURANCE/QUALITY CONTROL 42
16.7 RECORDS RETENTION 42
16.8 MONITORING 43
16.9 STUDY COMPLETION/TERMINATION 43
16.10 ON-SITE AUDITS 44
16.11 USE OF INFORMATION AND PUBLICATION 44
APPENDIX 1 45
APPENDIX 2: 49
REFERENCES 50

ABBREVIATIONS

Ad26.ZEBOV	adenovirus serotype 26 expressing the Ebola virus Mayinga glycoprotein
AE	adverse event
CRF	case report form
DMID	Division of Microbiology and Infectious Diseases
EBOV	Ebola virus
eDC	electronic data capture
eCRF	electronic case report form
ELISA	enzyme-linked immunosorbent assay
EPI	Expanded Program on Immunisation
EVD	Ebola virus disease
FANG	Filovirus Animal Non-Clinical Group
GCP	Good Clinical Practice
GP	glycoprotein
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICMJE	International Committee of Medical Journal Editors
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
IM	intramuscular
LLOQ	lower limit of quantitation
LSHTM	London School of Hygiene & Tropical Medicine
MedDRA	Medical Dictionary for Regulatory Activities
mL	millilitres
MVA-BN-Filo	Modified Vaccinia Ankara Bavarian Nordic vector expressing multiple filovirus proteins
PCR	polymerase chain reaction
PI	Principal Investigator
PQC	Product Quality Complaint
RBC	red blood cell
SAE	serious adverse event
SUSAR	suspected unexpected serious adverse reaction
TOU	Test of Understanding
VISP	vaccine induced seropositivity
vp	viral particle(s)
WBC	white blood cell
WHO	World Health Organisation

PROTOCOL AMENDMENTS

Protocol Versions	DATE
Original Protocol, Version 1.0	30/10/2020
Amendment 1, Protocol Version 2.0	15/06/2021

Amendments below are listed beginning with the most recent amendment.

Amendment 1 (This document)
The overall reasons for the amendment:

- To include the assessment of neutralising antibody responses directed against the Ad26 vector before booster vaccination as an exploratory outcome of the study.
- To add safety information on the Ad26-based vaccines, following the latest update of the Investigator's Brochure and Addendum.
- To add Adverse Events of Special Interest

The changes made to the clinical protocol VAC52150EBL2011 are listed below, including the rationale of each change and a list of all applicable sections.

Rationale: the assessment of neutralising antibody responses directed against the Ad26 vector will be useful to understand if previous immunity against the Ad26 vector influences the immune response to the Ad26.ZEBOV booster. The assessment of neutralising antibody responses directed against the Ad26 vector before booster vaccination has been included as an exploratory outcome of the study.

SYNOPSIS
Time and Events Schedule
2.1 OBJECTIVES AND ENDPOINTS
8.4 IMMUNOGENICITY ASSESSMENTS

Rationale: new information on the safety of the Ad26-based vaccines, in particular, the risk of thrombosis with thrombocytopenia syndrome (TTS), has been included following the latest update of the Investigator's Brochure Addendum.

Clinical Safety Experience with Ad26-based Vaccines in section 1.2.3 Potential Risks

---

Rationale: history of thrombotic thrombocytopaenia syndrome (TTS) or heparin-induced thrombocytopaenia and thrombosis (HITT) have been mentioned as conditions that would increase the risk of an adverse outcome from participation in the study in exclusion criterion 10.

4.2 EXCLUSION CRITERIA

Rationale: Thrombotic events and symptomatic thrombocytopenia have been added to the protocol as Adverse Events of Special Interest.

Adverse Events of Special Interest in section 11.1.1 Adverse Event Definitions and Classifications APPENDIX 2

Rationale: inconsistent information about retesting of values, rescreening and rescheduling of the vaccination (Day 1) have been corrected.

8.1.3 Vaccination Period

Rationale: Minor editorial changes have been made.

Throughout the document

Rationale: References have been updated.

REFERENCES

SYNOPSIS

TITLE

An open label study to evaluate the safety and immunogenicity of an Ad26.ZEBOV booster dose in children previously vaccinated with the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen.

RATIONALE

Over 600 children have received the adenovirus serotype 26 expressing the Ebola virus Mayinga glycoprotein (Ad26.ZEBOV), modified Vaccinia Ankara Bavarian Nordic vector expressing multiple filovirus proteins (MVA-BN-Filo) Ebola vaccine regimen in the EBL2002 and EBL3001 clinical trials. The vaccine regimen was well-tolerated and highly immunogenic in children; however, the durability of vaccine-induced immune responses is not known. In adults previously vaccinated with the Ad26.ZEBOV and MVA-BN-Filo regimen, a booster vaccination with Ad26.ZEBOV was safe and induced a strong anamnestic response within seven days of the booster vaccination. It is important to establish if a booster dose of Ad26.ZEBOV is safe and immunogenic also in children, as this can guide the clinical use of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen in this age group. For example, it could support the strategy of boosting immunised children at the start of an Ebola outbreak.

STUDY OBJECTIVES AND HYPOTHESIS

This study aims to evaluate the safety and immunogenicity of an Ad26.ZEBOV booster dose in healthy children who were previously (>2 years) vaccinated with the Ad26.ZEBOV (dose 1) followed by MVA-BN-Filo (dose 2) 56 days later, by monitoring adverse events (AEs) following the booster vaccination and by assessing binding antibody responses using the Filovirus Animal Non-Clinical Group (FANG) Enzyme-Linked Immunosorbent Assay (ELISA).

Primary Objectives

- To assess the safety and tolerability of a booster dose of Ad26.ZEBOV at a dose of $5x10^{10}$ viral particles (vp) in children previously vaccinated with the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen with a 56-day interval.

- To assess vaccine-induced humoral immune responses to the Ebola virus glycoprotein (EBOV GP), as measured by FANG ELISA, at 7 and 21 days following a booster dose of Ad26.ZEBOV at a dose of $5x10^{10}$ vp in children previously vaccinated with the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen with a 56-day interval.

Exploratory Objectives

- To assess neutralising antibody responses directed against the Ad26 vector before booster vaccination as measured by a virus neutralization assay (VNA).

Hypothesis

As this study is designed to provide descriptive information regarding safety and immunogenicity without formal treatment comparisons, no formal statistical hypothesis testing is planned.

OVERVIEW OF STUDY DESIGN

This is an open-label study evaluating the immune response to a booster dose of Ad26.ZEBOV administered to children who were previously vaccinated with Ad26.ZEBOV followed by MVA-BN-Filo 56 days later. Only subjects who received the Ad26.ZEBOV and MVA-BN-Filo regimen during their participation in the

VAC52150EBL3001 (EBOVAC-Salone) vaccine trial are eligible for enrolment in this study. Participants will be recruited in two age groups: children aged 4-11 years at the time of dose 1 vaccination and children aged 1-3 years at the time of dose 1 vaccination in the EBOVAC-Salone trial. Approximately 25 subjects will be enrolled in each of these two age groups.

Parents/guardians will be asked to consent for the participation of their children in the study. Children aged 7 years and older at the time of enrolment in this study will be asked to give positive assent for their participation. Participants will be followed up to 28 days after their booster vaccination.

The Principal Investigator, together with the sponsor's medical safety officer, will be responsible for the safety monitoring of the study.

The study will be conducted in Kambia, Sierra Leone.

SUBJECT POPULATION

Potential participants must be healthy children (based on physical examination, medical history, a haematological assessment and clinical judgment) who received the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in the EBOVAC-Salone trial and were aged ≥1 to ≤11 years at the time of dose 1 vaccination. They must also be enrolled in the long-term follow-up study to the EBOVAC-Salone trial, VAC52150EBL3005 (EBOVAC-Salone Extension study) but not in the immunogenicity subset.

DOSAGE AND ADMINISTRATION

A single dose of Ad26.ZEBOV at a dose of $5x10^{10}$ vp administered intramuscularly.

SAFETY EVALUATIONS

Solicited local (at the administration site) and systemic AEs will be assessed on the day of vaccination and using a diary for a period of seven days following the booster vaccination. Unsolicited AEs will be tracked for 28 days following booster vaccination, while serious adverse events will be tracked for the duration of the study.

IMMUNOGENICITY EVALUATIONS

Blood will be drawn for assessments of immune responses at the time points indicated in the TIME AND EVENTS SCHEDULE. The site staff will perform sample collection and processing according to current versions of approved standard operating procedures.

Future scientific research may be conducted to further investigate Ebola vaccine- and disease-related questions and to study other infections of public health importance in Sierra Leone and neighbouring countries. This may include the development of new, or the improvement of, existing techniques to characterize EBOV-directed immune responses or diagnostic tests. No additional samples will be taken for these analyses, however, residual samples from the study tests may be retained for these purposes and analysed after the end of the study.

STATISTICAL METHODS

The primary analysis will be done when all subjects have completed their 28-day post-booster visit or discontinued earlier. This analysis will include all available data up to this point.

Sample Size Determination

The sample size is a convenience sample and is not based on formal hypothesis testing considerations.

SAFETY ANALYSES

No formal statistical testing of safety data is planned. Safety data will be analysed descriptively by age group, 1-3 years and 4-11 years (age the participant was when they received dose 1 vaccination in the EBOVAC-Salone trial).

IMMUNOGENICITY ANALYSES

No formal hypothesis on immunogenicity will be tested. Descriptive statistics (i.e. geometric mean and 95% confidence interval, as appropriate) will be calculated for continuous immunologic parameters at all available time points (i.e. Day 1, 8 and 22). Graphical representations of immunologic parameters will be made as applicable.

Frequency tabulations will be calculated for discrete (qualitative) immunologic parameters (i.e. responder rate), as applicable. Responders rate defined as >2.5-time increase over baseline value (or lower limit of quantitation [LLOQ]) pre-dose 1 vaccination in the EBOVAC-Salone trial, will be calculated depending on availability of sample results from the EBOVAC-Salone trial.

TIME AND EVENTS SCHEDULE

Study Procedures	Screening (≤28 days)[a]	Study Period				
		Day 1 Booster	Days 2-7	Day 8 (+ 3 days)	Day 22 (± 3 days)	Day 29[b] (± 3 days)
Test of Understanding (TOU)[c]	X					
Informed consent and assent (if applicable) [d]	X					
Medical history and demographics	X					
Inclusion/exclusion criteria[e]	X	X				
Urine pregnancy test[f]	X	X[g]				
Physical examination[h]	X	X[f]		X	X	X[b]
Vital signs [i]	X	X		X	X	X[b]
Vaccine Administration[d]		X				
30 minutes post-vaccination observation [j]		X				
Distribution of participant diary		X				
Completion of the diary at home[k]		X	X			
Completion and review of the diary by study site personnel				X		
Solicited adverse events recording [j]		X	X	X		
Unsolicited adverse events recording		From booster vaccination (Day 1) onwards until 28 days post booster				
Serious Adverse Events [l]	Continuous					
Concomitant medications [m]	X	X	X	X	X	X
Blood draw						
Haematology (i.e. full blood count)	X			X	X	
Immunogenicity (serum) [n]		X		X	X	
Approximate blood draw volumes						
Haematology: 2.0 mL per blood draw	2.0			2.0	2.0	
Immunogenicity: Participants aged<6 years, 2.5 mL per blood draw Particpcant aged 6 years and older, 5.0 mL per blood draw		2.5 5.0		2.5 5.0	2.5 5.0	
Total (Haematology + Immunogenicity) Participants aged **<6 years** Participant aged **6 years and older**	**2.0** **2.0**	**2.5** **5.0**		**4.5** **7.0**	**4.5** **7.0**	

NOTE: In case of early withdrawal due to an adverse event (AE), the investigator or clinical designee will collect all information relevant to the AE and safety of the participant, and will follow the participant until resolution of the AE or until reaching a clinically stable endpoint. Parents/guardians who withdraw consent, or participants who wish to withdraw assent, will be offered an optional visit for safety follow-up (before the formal withdrawal of consent). Participants and/or parents/guardians have the right to refuse such a visit for themselves/their child.

[a] Screening may be split into multiple days or visits. Retesting of values (eg, safety laboratory) that lead to exclusion is allowed once using an unscheduled visit during the screening period, provided there is an alternative explanation for the out of range value. The safety laboratory assessments at screening are to be performed within 28 days prior of the vaccination (including Day 1 before vaccination) and may be repeated if they fall outside this time window. If retesting is required, all screening procedures (except TOU) should be repeated

[b] The Day 29 visit can be conducted via phone call if the participant is unable, or does not wish, to attend the clinic for this visit. If Day 29 visit is conducted over the phone, no physical exam and no vital signs will be collected or recorded for this visit.

[c] The TOU should be administered to the parent or guardian who will provide consent, and will be administered after reading but before signing the informed consent form.

[d] Informed consent must be obtained before any study-related activities are performed.

[e] The investigators should ensure that all study enrollment criteria have been met at the end of the enrolment period and before the booster vaccination on Day 1. If a participant's clinical status changes (including available laboratory results or the receipt of additional medical records) after screening but before Day 1 such that the participant no longer meets all eligibility criteria, then the participant will be excluded from participating in the study.

[f] For all adolescent girls who are ≥12 years of age at the time of screening.

[g] Prior to study vaccine administration.

[h] A full physical examination including also body weight and height will be conducted at screening. At following vists, a brief physical examination will be symptom-directed. Physical examination findings (i.e. abnormalities) prior to vaccination are to be recorded as medical history, after vaccination as adverse event(s).

[i] Vital signs include blood pressure, pulse/heart rate (at rest), respiratory rate, and body temperature.

[j] After the vaccination, participants will remain under observation at the study site for at least 30 minutes for presence of any acute reactions, or longer if deemed necessary by the investigator. Solicited local (at the injection site) and systemic adverse events and unsolicited adverse events emerging during the observation period will be recorded in the CRF.

[k] Diaries will be completed at home by either a project field worker who will visit the participant during daily visits or by the parent/guardian, to document symptoms of solicited local (at the injection site) and systemic adverse events in the evening after the vaccination and then daily for the next 6 days at approximately the same time each day. Day 8 of the diary will be completed at the study clinic by a study doctor or nurse.

[l] Serious adverse events and/or special reporting situations that are related to study procedures will be reported from the time a signed and dated ICF is obtained onwards until the end of the study. All other serious adverse events and/or special reporting situations will be reported from the day of vaccination onwards until the end of the study.

[m]Concomitant therapies must be recorded from screening onwards until 28 days post-vaccination.

[n]Serum aliquots for FANG ELISA at all timepoints (D1, D8, D22). Serum aliquot for Ad26 VNA testing at D1.

1 INTRODUCTION

Ebola viruses belong to the Filoviridae family and cause Ebola virus disease (EVD), which can induce severe haemorrhagic fever in humans and nonhuman primates. Case fatality rates in EVD range from 25% to 90% (average: 50%) according to the World Health Organisation (WHO). [1]

Janssen Vaccines & Prevention B.V., in collaboration with Bavarian Nordic GmbH Denmark and in conjunction with an Innovative Medicines Initiative consortium led by the London School of Hygiene and Tropical Medicine (LSHTM), including the Institut National de la Santé et de la Recherche Médicale (INSERM), the University of Oxford and the Sierra Leone College of Medicine and Allied Health Sciences as partners, is investigating the potential of a prophylactic Ebola vaccine regimen (VAC52150) comprised of the following two candidate vaccines:

Ad26.ZEBOV is a non-replicating monovalent vaccine expressing the full-length Mayinga glycoprotein (GP) of the Ebola virus (formerly known as *Zaire ebolavirus*), and is produced in the human cell line PER.C6®.

MVA-mBN226B, further referred to as MVA-BN-Filo, is a non-replicating multivalent vaccine expressing the Sudan virus GP, the EBOV GP, the Marburg virus Musoke GP, and the Tai Forest virus (formerly known as *Côte d'Ivoire ebolavirus*) nucleoprotein, and is produced in chicken embryo fibroblast cells. The EBOV GP expressed by MVA-BN-Filo has 100% homology to the one expressed by Ad26.ZEBOV.

The GP of the Ebola virus (EBOV) responsible for the 2013-2016 epidemic in West Africa had 97% homology to the EBOV GP used in this vaccine regimen.

For the most up-to-date nonclinical and clinical information regarding Ad26.ZEBOV and MVA-BN-Filo, please refer to the latest versions of the Investigator's Brochures (IBs) and Addenda (if applicable) [2, 3]. A brief summary of the nonclinical and clinical information available at the time of the protocol writing is provided below.

1.1 BACKGROUND

The safety, reactogenicity, and immunogenicity of the Ad26.ZEBOV and MVA-BN-Filo vaccines have been evaluated in phase 1, 2 and 3 clinical trials. More than 100,000 participants, including children (1–17 years old) have received the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in a number of completed and ongoing clinical studies and in a large scale vaccination campaign in Rwanda. Data from these studies have shown that the two-dose heterologous Ad26.ZEBOV, MVA-BN-Filo vaccine regimen is generally well tolerated and able to induce humoral immune responses persisting for at least two years in adults and for at least one year in children.

Safety and immunogenicity of the Ad26.ZEBOV and MVA-BN-Filo vaccines in paediatric participants

In the VAC52150EBL2002 (Burkina Faso, Cote D'Ivoire, Uganda, Kenya) and VAC52150EBL3001 (Sierra Leone) studies, three age cohorts (12–17 years, 4–11 years and 1–3 years) were vaccinated with Ad26.ZEBOV or control followed by MVA-BN-Filo or control, 28 or 56 days later. The control used for Dose 1 and 2 in VAC52150EBL2002 was a placebo (i.e. saline injection). In VAC52150EBL3001, Dose 1 consisted of a meningococcal conjugate vaccine (MenACWY) and Dose 2 of placebo.

In these studies, the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen was well tolerated with no safety signals identified. Most solicited (local and systemic) AEs were mild to moderate. Overall, the frequency of grade 3 solicited AEs was low (<2%) in all children assigned to Ad26.ZEBOV, MVA-BN-Filo regimen. The most frequently reported solicited local AE was injection site pain; frequency was similar between the age cohorts, but higher in participants assigned to active vaccines than to placebo. The most frequently reported solicited systemic AEs was headache in the 12–17 years and 4–11 years age cohorts, and decreased appetite in the 1-3 years age cohort. The frequency of unsolicited AEs was higher in the 1-3 years age cohort compared to older children. The frequency of Grade 3 unsolicited AEs was similar across age cohorts. No serious adverse events (SAEs) were considered related to Ad26.ZEBOV or MVA-BN-Filo vaccines. The Ad26.ZEBOV, MVA-BN-

Filo vaccine regimen induced robust binding antibody responses, which persisted for at least up to 12 months. Younger children (1-3 years) had higher antibody responses compared to older children (4-11 years and 12-17 years age cohorts). [4, 5]

Safety and immunogenicity of an Ad26.ZEBOV booster dose in adult participants

An Ad26.ZEBOV booster vaccination was given to 29 adult participants, who were vaccinated approximately 2 years before with Ad26.ZEBOV followed by MVA-BN-Filo 56 days later in the VAC52150EBL3001 (EBOVAC-Salone) trial in Sierra Leone. The booster vaccination was safe and induced a strong anamnestic response in 96% of participants at seven days post-booster vaccination and in all 29 participants at 21 days post-booster vaccination. [6]

1.2 BENEFITS/RISKS OF PARTICIPATION

1.2.1 Potential Benefits

The 2-dose Ebola vaccine regimen (Ad26.ZEBOV followed by MVA-BN-Filo 56 days later) has received marketing authorisations for prophylactic use in adults and children ≥1 years old in the European Union.[7] The marketing authorisation also includes the possibility for an Ad26.ZEBOV booster dose to be given to subjects who received the 2-dose regimen more than 4 months earlier and are at imminent risk of infection with Ebola virus.[8] This vaccine regimen was previously shown to provide protection in vaccinated non-human primates against an EBOV challenge, which is fully lethal in unvaccinated control animals. Although clinical efficacy data are not available for this vaccine regimen, the marketing authorisation was granted on the basis of the potential clinical benefit induced by vaccination by correlating the magnitude of vaccine-elicited immune parameters in non-human primates with those observed in vaccinated humans in phase 1, 2 and 3 clinical studies.[9]

If the Ad26.ZEBOV booster dose is shown to be safe and immunogenic in children as in adults, the participants enrolled in this study may benefit from the potential protective benefit of the booster vaccination in the case of a future exposure to EBOV.

Participants will also benefit from clinical testing and physical examination; others may benefit from the knowledge that they may aid in the development of an Ebola vaccine.

1.2.2 Known Risks

Ad26.ZEBOV

The safety, reactogenicity, and immunogenicity of the Ad26.ZEBOV vaccine has been evaluated in a number of completed and ongoing clinical studies in adults and children. The vaccine was well tolerated, with no safety concerns identified. The vaccine mainly elicited some solicited local and systemic reactions, as expected with injectable vaccines, and there were no serious safety concerns in study participants. For details, see the safety data presented in Section 1.1. For the most up-to-date nonclinical and clinical information regarding Ad26.ZEBOV, refer to the latest versions of the IB and Addenda (if applicable) [2, 3].

1.2.3 Potential Risks

The following potential risks will be monitored during the study and are specified below:

Risks Related to Vaccination

In general, intramuscular (IM) injection may cause local itching, warmth, pain, tenderness, erythema, swelling, arm discomfort or bruising of the skin at vaccine injection sites.

Participants may exhibit general signs and symptoms associated with the administration of a vaccine, including fever, chills, rash, nausea/vomiting, general itching, headache, myalagia, arthralgia, and fatigue.

Young children may also experience loss of appetite, diarrhoea, decreased activity/lethargy, and irritability/crying. These side effects will be monitored, but are generally short-term and do not require treatment.

Participants may have an allergic reaction to the vaccine. An allergic reaction may cause a rash, hives or even anaphylaxis. Severe reactions are rare. Medications must be available in the clinic to treat serious allergic reactions.

Risks from Blood Draws

As with all clinical studies requiring blood sampling, there are risks associated with venipuncture and multiple blood sample collection. Blood drawing may cause pain, tenderness, bruising, bleeding, dizziness, vaso-vagal response, syncope, and, rarely, infection at the site where the blood is taken. The total blood volume to be collected is considered to be an acceptable amount of blood over this time period from the population in this study (see Section 15.1).

Concomitant Vaccination

Concomitant vaccination might have an influence on both the safety profile and immunogenicity of Ad26.ZEBOV. Likewise, the study intervention might have an influence on both the safety profile and immunogenicity of any concomitant vaccination. Therefore, a participant should not receive a live-attenuated vaccine from 30 days before the vaccination until 30 days after the vaccination unless a vaccine preventable disease such as measles emerges which would warrant administration of live-attenuated vaccines. Immunizations with inactivated vaccines should be administered at least 15 days before or after administration of any study intervention in order to avoid any potential interference with the efficacy of the routine immunizations or the interpretation of immune responses to study intervention, as well as to avoid potential confusion with regard to attribution of adverse reactions. However, if a vaccine is indicated in a post-exposure setting (e.g., rabies or tetanus), it must take priority over the study intervention. Otherwise, a participant will not postpone, forego or delay the receipt of any recommended vaccine according to local schedules (e.g., Expanded Program on Immunization [EPI] schedule according to the WHO regional office for West Africa).

Clinical Safety Experience with Ad26-based Vaccines

Thrombosis in combination with thrombocytopenia (thrombosis with thrombocytopenia syndrome [TTS]), in some cases accompanied by internal bleeding, has been observed very rarely following vaccination with the Janssen COVID-19 (Ad26.COV2.S) vaccine. Reports include severe cases of venous thrombosis at unusual sites such as cerebral venous sinus thrombosis (CVST), splanchnic vein thrombosis and arterial thrombosis, in combination with thrombocytopenia. The associated symptoms began approximately 1 to 2 weeks after vaccination, mostly in women under 60 years of age. Thrombosis in combination with thrombocytopenia can be fatal. The exact pathophysiology of TTS is unclear. This event has not been observed to date with any other Janssen Ad26-based vaccines. Participants should be instructed to seek immediate medical attention if they develop symptoms such as shortness of breath, chest pain, leg swelling, persistent abdominal pain, severe or persistent headaches, blurred vision, skin bruising or petechiae beyond the site of vaccination.

Vaccine Induced Seropositivity

The potential of a participant becoming polymerase chain reaction (PCR)-positive after vaccination was assessed in study VAC52150EBL1002. The risk for false positives is low and expected to decrease rapidly over time after administration of Ad26.ZEBOV.

Uninfected participants in Ebola vaccine studies may develop Ebola-specific antibodies as a result of an immune response to the candidate Ebola vaccine, referred to as vaccine induced seropositivity (VISP). These antibodies may be detected in Ebola serologic tests, causing the test to appear positive even in the absence of actual Ebola infection. VISP may become evident during the study, or after the study has been completed.

Unknown Risks

There may be other risks that are not known. If any significant new risks are identified, the Principal Investigator and participants (parents/guardians) will be informed.

1.2.3 Overall Benefit/Risk Assessment

Based on the available data and proposed safety measures, the overall benefit/risk assessment for this clinical study is considered acceptable for the following reasons:

- To date, safety data from the studies in the clinical development program revealed no significant safety issues (see Section 1.1). Further experience from Ad26.ZEBOV Filo will be gained from currently ongoing clinical studies.

- For all participants, there are pre-specified pausing rules that would result in pausing of further vaccination if predefined conditions occur, preventing exposure of new participants to study intervention until an independent medical reviewer evaluates all safety data (see Sections 3.1, 8.3, 10.7).

- Only participants who meet all inclusion criteria and none of the exclusion criteria (specified in Section 4) will be allowed to participate in this study. The selection criteria include adequate provisions to minimize the risk and protect the well-being of participants in the study.

- Several safety measures are included in this protocol to minimize the potential risk to participants, including the following:

  - Participants will remain at the site for at least 30 minutes after each vaccination to monitor the development of any acute reactions, or longer if deemed necessary by the investigator (e.g. in case of grade 3 AEs). Refer to Section 6 for more information on emergency care.

  - Safety evaluations (physical examinations and vital sign measurements) will be performed at scheduled visits during the study, as indicated in the TIME AND EVENTS SCHEDULE.

  - The investigator or clinical designee will document unsolicited AEs from the vaccination (Day 1) onwards until 28 days post-vaccination (Day 29). The investigator or clinical designee will document serious AEs and/or special reporting situations that are related to study procedures from the time a signed and dated informed consent form (ICF) is obtained onwards until the end of the study.

  - Any clinically significant abnormalities (including those persisting at the end of the study/early withdrawal) will be followed by the investigator until resolution or until a clinically stable endpoint is reached.

  - If acute illness (excluding minor illnesses such as diarrhoea or mild upper respiratory tract infection) or axillary temperature ≥38°C is present at the scheduled time for vaccination, the participant may be rescheduled for vaccination at a later time point within the window allowed for screening, or be withdrawn from vaccination at the discretion of the investigator and after consultation with the sponsor (see Section 6.1).

1.3 OVERALL RATIONALE FOR THE STUDY

Over 600 paediatric participants have received the Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen in the EBL2002 and EBL3001 clinical trials. [4, 5] The vaccine regimen was well-tolerated and highly immunogenic in children, however, the durability of the immune response is not known. In adults previously vaccinated with the Ad26.ZEBOV and MVA-BN-Filo regimen, an Ad26.ZEBOV booster vaccination was safe and induced a strong anamnestic response. [6] It is important to establish the safety and immunogenicity of a booster dose of Ad26.ZEBOV in children, as this can guide the clinical use of the vaccine regimen in this group. For example, it could support the strategy of boosting immunised children at the start of an Ebola outbreak.

2 OBJECTIVES, ENDPOINTS, AND HYPOTHESIS

2.1 OBJECTIVES AND ENDPOINTS

Primary Objectives

- To assess the safety and tolerability of a booster dose of Ad26.ZEBOV at a dose of $5x10^{10}$ viral particles (vp) in children previously vaccinated with the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen with a 56-day interval.

- To assess vaccine-induced humoral immune responses to the Ebola virus glycoprotein (EBOV GP), as measured by FANG ELISA, at 7 and 21 days following a booster dose of Ad26.ZEBOV at a dose of $5x10^{10}$ vp in children previously vaccinated with the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen with a 56-day interval.

Exploratory Objectives

- To assess neutralising antibody responses directed against the Ad26 vector before booster vaccination as measured by a virus neutralization assay (VNA).

2.2 HYPOTHESIS

As this study is designed to provide descriptive information regarding the safety and immunogenicity of a booster dose of Ad26.ZEBOV in children, no formal statistical hypothesis testing is planned.

3 STUDY DESIGN

3.1 OVERVIEW OF STUDY DESIGN

This is an open label phase 2 study evaluating the immune response to a booster dose of Ad26.ZEBOV administered to children who were previously vaccinated with Ad26.ZEBOV followed by MVA-BN-Filo 56 days later. Only subjects who received the Ad26.ZEBOV and MVA-BN-Filo regimen during their participation in the EBOVAC-Salone trial are eligible for enrolment in this study. Participants will be recruited in two age groups: children aged 4-11 years at the time of dose 1 vaccination and children aged 1-3 years at the time of dose 1 vaccination. Up to 25 subjects will be enrolled in each age group.

The study will consist of a screening period of up to 28 days, a booster vaccination (Day 1) and a post-booster vaccination follow-up period until 28 days post-vaccination (Day 29).

Parents/guardians will be asked to consent for the participation of their children in the study. Children aged 7 years and older at the time of enrolment in this study will be asked to give positive assent for their participation.

After informed consent and assent (if applicable) have been obtained, investigators should ensure that all study eligibility criteria have been met prior to the study vaccination on Day 1 (see list of inclusion and exclusion criteria in Section 4). Eligibility will be based on medical history, physical examinations (including body height and weight), vital sign measurements, a haematological (i.e. full blood count) assessment, and urine pregnancy test in all adolescent girls who are ≥12 years of age at the time of screening.

After vaccination, participants will remain under observation at the study site for at least 30 minutes for surveillance for any acute reactions, or longer if deemed necessary by the investigator. Following the vaccination, any unsolicited, solicited local or systemic AEs, and vital signs will be documented by study-site personnel at the end of this observation period.

The participant's parent/guardian will be given a thermometer, ruler and participant diary with instructions for the proper recording of events occurring after the booster vaccination. Diaries will be completed at home by either a project field worker who will visit the participant or by the parent/guardian to document solicited local (at the injection site) and systemic AEs and body temperature, beginning on the evening of the vaccination, and then daily for the next 6 days. Temperatures should be taken at approximately the same time each day, preferably in the evening and additionally whenever the child feels warm. Study-site personnel will collect, review, and complete the participant diary information at the 7-day post-vaccination visit (Day 8).

Unsolicited AEs will be recorded from the booster vaccination (Day 1) onwards until 28 days post-booster vaccination (Day 29). SAEs and/or special reporting situations that are related to study procedures will be reported from the time a signed and dated ICF is obtained onwards until the end of the study (Day 29).

Blood samples will be collected for immunogenicity assessments at Day 1 (i.e. the baseline sample before vaccination), 7 days post-vaccination (Day 8), and 21 days post-dose-vaccination (Day 22). At Day 8 and Day 22, a sample for haematology will also be collected.

Participants will exit the study after 28 days post-vaccination (Day 29). The study is considered completed at the site at final database lock, which will occur after the last participant has completed the last study visit or left the study.

4 PARTICIPANT POPULATION

The study will be open to healthy children (based on physical examination, medical history, a haematological assessment and clinical judgement) who received the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in the EBOVAC-Salone trial. The children must be enrolled in the long-term follow-up study to the EBOVAC-Salone trial, the VAC52150EBL3005 (EBOVAC-Salone Extension) study but not in the immunogenicity subset.

The inclusion and exclusion criteria for enrolling participants in this study are described in the following two subsections. If there is a question about the inclusion and exclusion criteria, the investigator must consult with the appropriate sponsor representative and resolve any issues before enrolling a participant in the study. Waivers are not allowed.

4.1 INCLUSION CRITERIA

Each potential participant must satisfy all of the following criteria to be enrolled in this current study:

1. Child must be enrolled in the VAC52150EBL3005 (EBOVAC-Salone Extension) study but not in the immunogenicity subset of EBOVAC-Salone Extension study.
2. Child must be a former participant in the VAC52150EBL3001 (EBOVAC-Salone) trial, and have received Ad26.ZEBOV (dose 1) vaccination followed by the MVA-BN-Filo (dose 2) vaccination within the EBOVAC-Salone trial window for dose 2 vaccination.
3. Child must have been aged 1 to 11 years old at the time of dose 1 vaccination in the EBOVAC-Salone trial.
4. The parent/guardian must consent for their child to participate in the VAC52150EBL2011 study by signing (or thumb printing, if illiterate) an ICF, indicating that he or she understands the purpose of, and procedures required for, the study, understands the potential risks and benefits of the study, and is willing to allow their child to participate in the study. If the parent/guardian cannot read or write, the procedures must be explained, and informed consent must be witnessed by a literate third party not involved in the conduct of the study. Children aged 7 years and older will be asked to give positive assent for their participation in the study and the assent procedure must be witnessed by an adult, literate parent/guardian/third party not involved in the conduct of the study, and documented.
5. The parent/guardian is willing/able to ensure that their child adheres to the prohibitions and restrictions specified in this protocol (see Section 4.3)

6. Child must be healthy in the investigator's clinical judgement (and the parent/guardian's judgement) on the basis of medical history, physical examination, vital signs, and a haematological assessment (i.e. full blood count) performed at screening. Subjects must meet the following haematology parameters within 28 days before Day 1:

    - Haemoglobin ≥8.0 g/dL for children aged 1 to <5 years, ≥9g/dL for children aged 5 or older
    - Platelet count ≥100 x 10^9/L
    - White blood cell count ≥5.0 x 10^9/L

    Note: The haematological assessment at screening is to be performed within 28 days prior to vaccination on Day 1 and may be repeated if it falls outside this time window.

    Note: If haematological screening assessment is out of range and deemed clinically significant, repeating screening test to assess eligibility is permitted once during the screening period, using an unscheduled visit.

7. Adolescent girls who have started their menstrual periods and/or are ≥12 years of age at the time of screening, must have a negative urine β-hCG pregnancy test at screening and immediately prior to the booster vaccination on Day 1.
8. The parent/guardian is available and willing to have their child participate for the duration of the study visits.
9. The parent/guardian must have the means to be contacted.
10. The parent/guardian must pass the Test of Understanding (TOU).

    Note: If the parent/guardian fails the TOU on the first attempt, he/she must be retrained on the purpose of the study and must take the test again (2 repeats are allowed). If he/she fails on the third attempt, he/she should not continue with enrolling or consenting procedures.

4.2 EXCLUSION CRITERIA

Any potential participant who meets any of the following criteria will be excluded from participating in the study:

1. Participants in the EBOVAC-Salone trial who were allocated to the control arm receiving the WHO-prequalified Meningococcal Group A, C, W135 and Y conjugate vaccine.
2. Participants in the EBOVAC-Salone trial who were age 12 years and older at the time of dose 1 vaccination.
3. Known allergy or history of anaphylaxis or other serious adverse reactions to vaccines or vaccine products (including any of the constituents of the study vaccine, e.g., polysorbate 80, ethylenediaminetetraacetic acid or L-histidine for Ad26.ZEBOV vaccine), including known allergy to chicken or egg proteins and aminoglycosides (gentamicin).
4. Presence of acute illness (this does not include minor illnesses such as mild diarrhoea or mild upper respiratory tract infection) or axillary temperature ≥38ºC on Day 1. Participants with such symptoms will be excluded from enrolment at that time but may be rescheduled for enrolment at a later date within the screening window.
5. Clinically significant history of skin disorder (e.g., psoriasis, contact dermatitis), allergy, symptomatic immunodeficiency, cardiovascular disease, respiratory disease, endocrine disorder, liver disease, renal disease, gastrointestinal disease, neurological illness as judged by the investigator or other delegated individual.
6. Adolescent girls who are known to be pregnant or breastfeeding at screening.

7. Received a blood transfusion or other blood products within 8 weeks of vaccination day.

8. Children who have been vaccinated with live-attenuated vaccines within 30 days before the study vaccination, and with inactivated vaccine within 15 days before the study vaccination.

9. Children who, in the opinion of the investigator, are unlikely to adhere to the requirements of the study or are unlikely to complete the vaccination and observation

10. Any other finding which in the opinion of the investigator or other delegated individual would increase the risk of an adverse outcome from participation in the study, e.g. history of thrombotic thrombocytopaenia syndrome (TTS) or heparin-induced thrombocytopaenia and thrombosis (HITT).

NOTE: Investigators should ensure that all study eligibility criteria have been met prior to the study vaccination on Day 1. If a subject's clinical status changes (including receipt of additional medical records or available laboratory results) after enrolment but before the vaccination (Day 1) so that he or she no longer meets all eligibility criteria, then the subject should be excluded from participation in the study.

4.3 PROHIBITIONS AND RESTRICTIONS

The parent/guardian must be willing and able to adhere to the following prohibitions and restrictions during the course of the study for their child to be eligible for participation:

1. In case of a new Ebola outbreak: participants and their parent/guardian must not travel to an area with an Ebola outbreak while the participant is enrolled in the study from the start of screening onwards until the last study visit. If applicable, any travelling to an area with an Ebola outbreak should be documented in the case report form (CRF). The date of travel and the destination should be clearly identified.

2. Ensure that their child does not receive any experimental medication (including experimental vaccines other than the study intervention) as described in Section 7.

5 INTERVENTION ALLOCATION AND BLINDING

5.1 BLINDING

As this is an open-label study, blinding procedures are not applicable.

6 DOSAGE AND ADMINISTRATION

All participants will receive the Ad26.ZEBOV vaccine, at a concentration of $5x10^{10}$vp, as a 0.5 millilitres (mL) IM injection into the deltoid muscle.

Study intervention will be prepared by a pharmacist or qualified staff member with primary responsibility for study intervention preparation and dispensing of the vaccine.

Ad26.ZEBOV will be administered as a 0.5 mL IM injections in the deltoid muscle, by a study intervention administrator. The injection site should be free from any injury, local skin conditions, or other issue that might interfere with the evaluation of local reactions. No local or topical anaesthetic will be used prior to the injection.

Participants will remain at the site for at least 30 minutes after the vaccination for the detection of any acute reactions, or longer if deemed necessary by the investigator (e.g. in case of a grade 3 AEs). As with any vaccine, allergic reactions following vaccination with the study intervention are possible. Therefore, appropriate drugs and medical equipment to treat acute anaphylactic reactions must be immediately available, and a medically qualified member of study-site personnel trained to recognize and treat

anaphylaxis must be present in the clinic during the entire vaccination procedure and post-vaccination monitoring period.

The investigator must provide emergency care as needed for any participant who experiences a life-threatening event. The site will have facilities, equipment and the ability to manage an anaphylactic reaction. If additional therapy is required, the investigator will arrange for transport to the closest appropriate facility for continuing care.

The Site Investigational Product Procedure Manual specifies the procedures for administration of the study intervention.

Ad26.ZEBOV will be manufactured by Janssen Vaccines & Prevention B.V. and provided under the responsibility of the sponsor, LSHTM. Please refer to the IB for a list of excipients.

6.1 CRITERIA FOR POSTPONEMENT OF VACCINATION

A participant will not be given any vaccination if he/she experiences any of the following events at the scheduled time for vaccination:

- An acute illness at the time of vaccination (this does not include minor illnesses such as diarrhoea or mild upper respiratory tract infection).
- An axillary temperature ≥38°C at the time of vaccination.

Participants experiencing any of these events may be rescheduled for vaccination at a later time point within the window allowed for screening, or be withdrawn from vaccination at the discretion of the investigator and after consultation with the sponsor.

7 CONCOMITANT THERAPY

Concomitant therapies that the participant is taking at the time of screening must be recorded in the CRF at screening.

Should immunisation with an inactivated vaccine be required, it should be administered at least 15 days before or after administration of the study intervention in order to avoid any potential interference in efficacy of the routine immunizations or the interpretation of immune responses to study intervention, as well as to avoid potential confusion with regard to attribution of adverse reactions. However, if a vaccine is indicated in a post-exposure setting (e.g., rabies or tetanus), it must take priority over the study intervention. A participant will not postpone, forego, or delay the receipt of any recommended vaccine according to local schedules (e.g. EPI schedule according to the WHO regional office for West Africa).

Analgesic/antipyretic medications and non-steroidal anti-inflammatory drugs may be used post-vaccination in case of medical need (e.g., fever or pain). Use of these medications as routine prophylaxis prior to study intervention administration is not recommended. The use of these medications must be documented.

Concomitant therapies must be recorded from screening onwards until 28 days post-vaccination.

Use of any experimental medication (including experimental vaccines other than the study intervention) during the study is not allowed.

The sponsor must be notified in advance (or as soon as possible thereafter) of any instances in which prohibited therapies are administered.

8 STUDY EVALUATIONS

Prior to any study-related activities being performed, participants, or participant's parent/guardian, must have signed a study ICF (see Section 15.2.3 Consent)

8.1 STUDY PROCEDURES

8.1.1 Overview

The TIME AND EVENTS SCHEDULE summarises the frequency and timing of all study procedures, which are provided in the following sections. Additional unscheduled study visits may be required if, in the investigator's opinion, further clinical or laboratory evaluation is needed.

Visit Windows

Visit windows are provided in the TIME AND EVENTS SCHEDULE. If a participant did not receive the study intervention on the planned day of vaccination, the timings of the next visits post-vaccination will be determined relative to the actual day of vaccination. The participant should be encouraged to come within these required windows.

Blood Sampling Volume

The approximate blood volumes collected in this study are indicated in the TIME AND EVENTS SCHEDULE. These study-related blood volumes obtained at each study visit (including any losses during phlebotomy) will not exceed 3% of the total blood volume. The total volume of blood is estimated at 80 to 90 mL/kg body weight; 3% is 2.4 mL blood per kg body weight. [10] The allowable blood volume calculations are based on the 3rd percentile for growth charts for 1 to 5-year-old [11] and 6- to 10-year-old children. [12]

Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

8.1.2 Screening Period

After signing and dating the ICF (see Section 16.2.3) and up to 28 days before Day 1 (day of vaccination), screening assessments will be performed as indicated in the TIME AND EVENTS SCHEDULE. Screening may be split into multiple days or visits.

Only participants complying with the criteria specified in Section 4 will be included in the study. The investigator will provide detailed information on the study to the participant and his/her parent/guardian will obtain written informed consent prior to study participation of the child.

After reading but before signing the ICF, the TOU will be administered to the parent/guardian. If the parent/guardian fails the TOU, they may repeat the test twice (and have to pass the third time for their child to be eligible) (for details, see Section 15.1).

The overall eligibility of the child to participate in the study will be assessed once all the screening results are available. Retesting of values (i.e. haematology) that lead to exclusion is allowed once using an unscheduled visit. If rescreening is required, all screening procedures (except TOU) should be repeated. Rescreening is allowed one time at the discretion of the study doctor. Study participants who qualify for inclusion will be contacted and scheduled for vaccination within 28 days.

A serum sample will be taken before vaccination at D1, to serve as a pre-vaccination baseline sample for immunogenicity assessments (see Section 8.4).

8.1.3 Vaccination Period

If eligible, the participant will be invited to come for the vaccination visit (Day 1). The investigator should ensure that all eligibility criteria have been met during the screening period. If a participant's clinical status changes (including available laboratory results or receipt of additional medical records) after screening but before the vaccination (Day 1) such that he/she no longer meets all eligibility criteria, then the participant should not be enrolled. Retesting of values and rescreening might be considered (see Section 8.1.2 for further information). Rescheduling of the vaccination (Day 1) visit, if the participant has an acute illness, is allowed within the screening window.

Before vaccination, a brief physical examination and measurement of vital signs will be performed.

Participants will be vaccinated as described in Section 6. After vaccination, participants will remain under observation at the study site for at least 30 minutes for surveillance for any acute reactions, or longer if deemed necessary by the investigator. Following the vaccination, any unsolicited, solicited local or systemic AEs, and vital signs will be documented by study-site personnel at the end of this observation period.

Upon discharge from the site, the parent/guardian will be provided with a thermometer (to measure body temperature), a ruler (to measure local injection site reactions), and a participant diary to record body temperature and solicited local (at the injection site) and systemic symptoms and will be trained on how to collect this information. Symptoms of solicited local and systemic AEs will be collected in the diary in the evening after the vaccination and then daily for the next 7 days at approximately the same time each day. Diaries will be completed at home by either a project field worker who will visit the participant during daily visits or by the parent/guardian and checked by a project field worker. The investigator or clinical designee will review information from the participant's diary.

Participants will come to the site 7 days after the vaccination (Day 8) as indicated in the TIME AND EVENTS SCHEDULE. The participant's diary will be reviewed by study-site personnel. The investigator will examine the injection site for occurrences of erythema, swelling, or tenderness at these visits in order to complete the relevant parts of the CRF.

Unsolicited AEs will be reported from the first day of vaccination until 28 days post-vaccination (Day 29).

SAEs and/or special reporting situations that are related to study procedures will be reported from the time a signed and dated ICF is obtained onwards until the end of the study (Day 29). All other SAEs and/or special reporting situations will be reported from the day of vaccination onwards until the end of the study (Day 29).

Participants will come to the site 7 and 21 days after the vaccination (Day 8 and Day 22) for safety and immunogenicity assessments. Please refer to 8.4 for details on the immunogenicity evaluations.

The parent/guardian will be instructed to contact the investigator anytime during the course of the study if their child experiences any AE or intercurrent illness that they perceive as relevant and/or can be possibly related to study intervention in their opinion.

When an enrolled participant completes or withdraws from the study, the investigator will complete an end-of-study form for the individual participant and provide a specific date for the end-of-study observation(s). When a participant withdraws before completing the study, the reason for withdrawal (if available) will be documented in the CRF and in the source documents.

Participants who appear to be lost to follow-up will be contacted, as per local practice, and the data recorded in the CRF. Participants lost to follow-up will have all data included for analysis.

8.2 PROCEDURES IN CASE OF A STUDY PAUSE

A study pause can affect participants that are awaiting the booster vaccination. After approval is granted to restart the study, participants who are awaiting the booster vaccination and whose screening period is longer than the protocol-defined 28 days as a result of a study pause, will be allowed to rescreen once (following the screening procedures described in Section 8.1.2, excluding TOU). Participants that are rescreened due to a pause must have new safety assessments (including full blood count, physical examination, and vital signs) within 28 days of the booster vaccination. The TOU does not need to be repeated. After screening, these participants will follow the same study procedures as those participants who were unaffected by a study pause (described in Section 8.1.3).

8.3 SAFETY EVALUATIONS

8.3.1 Safety Assessments

The Principal Investigator, together with the Chief Investigator and the sponsor's medical safety officer or delegate, will be responsible for the safety monitoring of the study, and will halt vaccination of further participants in case any of the pre-specified pausing rules described in Section 8.3.2 have been met. Further safety measures with regards to vaccination are described in Section 6.1.

An independent medical reviewer will be appointed by the sponsor before the start of the study to perform review of the safety data during the study. Details regarding this role are provided in Section 10.7.

Symptoms of solicited local and systemic AEs will be collected in the diary in the evening after the vaccination and then daily for the next 7 days. Unsolicited AEs will be collected from vaccination until 28 days post-vaccination (Day 29). Serious adverse events and/or special reporting situations that are related to study

procedures will be reported from the time a signed and dated ICF is obtained onwards until the end of the study (Day 29). All other SAEs and/or special reporting situations will be reported from the day of vaccination onwards until 28 days post-vaccination.

Any clinically relevant changes must be recorded on the AE section of the CRF.

Any clinically significant abnormalities persisting at the end of the study/early withdrawal will be followed by the investigator until resolution or until a clinically stable condition is reached.

All AEs will be coded for severity according to the criteria presented in Section 11.1.3.

The study will include the following evaluations of safety and reactogenicity according to the time points provided in the Time and Events Schedule:

Adverse Events

Adverse events will be reported as specified in Section 11.3.1.

Solicited Adverse Events

After vaccination, participants will remain under observation at the study site for at least 30 minutes for surveillance for any acute reactions, or longer if deemed necessary by the investigator. Symptoms of solicited local and systemic AEs will be collected in the diary in the evening after the vaccination and then daily for the next 7 days. Diaries will be completed at home by either a project field worker who will visit the participant during daily visits or by the parent/guardian and checked by a project field worker for 6 days. Day 8 of the diary will be completed by study site staff at the clinic. Diary information will be transcribed by the study personnel in the diary CRF pages. Once a solicited symptom from a diary is considered to be of severity Grade 1 or above, it will be referred to as a solicited AE.

Solicited Injection Site (Local) Adverse Events

Parents/guardians (or the project field worker) will be asked to note in the diary occurrences of tenderness, erythema and swelling at the study intervention injection site daily for 6 days post-vaccination (day of vaccination and the subsequent 6 days). The extent (largest diameter) of any erythema and swelling should be measured (using the ruler supplied) and recorded daily. Day 8 of the diary will be completed by study staff at the site clinic.

- **Injection Site Tenderness**

  Injection site tenderness is a painful sensation localized at the injection site upon palpation or movement of the limb. Due to the subjective nature of the reaction, the severity assessment of tenderness is self-reported (if a participant is unable to provide self-report, other reporters include parent/care giver or health care provider).

- **Injection Site Erythema**

  Injection site erythema is a redness of the skin caused by dilatation and congestion of the capillaries localized at the injection site. It can best be described by looking and measuring.

- **Injection Site Swelling**

  Injection site swelling is a visible enlargement of the site of injection. It may be either soft (typically) or firm (less typical).

  Note: Any other injection site events not meeting the above case definitions should be reported separately as unsolicited AEs.

Solicited Systemic Adverse Events

Parents/guardians will be instructed on how to record daily temperature using a thermometer provided for home use. The axillary temperature of the participant should be recorded in the diary in the evening of the day of vaccination, and then daily for the next 6 days at approximately the same time each day. The participant's temperature on Day 8 will be taken by study staff at the site clinic. If more than one measurement is made on any given day, the highest temperature of that day will be used in the CRF.

In this protocol, fever is defined as an endogenous elevation of body temperature ≥38° C, as recorded in at least one measurement.

Parents/guardians will also be instructed on how to note daily in the diary symptoms for 6 days post-vaccination (day of vaccination and the subsequent 6 days) any of the following events:

- body temperature
- fatigue/malaise
- chills
- headache
- nausea/vomiting
- muscle pain
- joint pain
- loss of appetite, in young children

Symptoms 7 days post vaccination (Day 8) will be assessed and recorded by study staff at the site clinic.

Physical Examination

A full physical examination will be performed at the screening visit. At subsequent study visits a brief, symptom-directed examination will be performed based on any clinically relevant issues, clinically relevant symptoms and medical history. The symptom-directed physical examination may be repeated if deemed necessary by the investigator. Physical examinations will be performed by the investigator or by a designated medically-trained clinician. Physical examination findings (i.e. abnormalities) prior to vaccination (Day 1) are to be recorded as medical history, but after vaccination as an AE.

Vital Signs

Axillary temperature, blood pressure, pulse/heart rate (beats per minute), and respiratory rate (breaths per minute) will be assessed.

Blood pressure and pulse/heart rate measurements will be assessed with a completely automated device. Manual techniques will be used only if an automated device is not available. Pulse/heart rate measurements should be preceded by at least 5 minutes of rest in a quiet setting without distractions.

Clinical Laboratory Tests

A haematological assessment (i.e., full blood count) will be performed by the local laboratory at the time points indicated in the TIME AND EVENTS SCHEDULE. The investigator must review the laboratory report, document this review, and record any clinically relevant changes on the AE page and/or the screening page of the CRF. Laboratory reports must be filed with the source documents.

A full blood count will include:

-haemoglobin
-haematocrit
-red blood cell (RBC) count
-white blood cell (WBC) count with differential
-platelet count

Note: a WBC evaluation may include any abnormal cells, which will then be reported by the laboratory. An RBC evaluation may include abnormalities in the RBC count, or RBC morphology, which will then be reported by the laboratory.

8.3.2 Study Pausing Rules

The investigators and the sponsor's medical safety officer or delegate will review the safety of enrolled participants on an ongoing basis and will halt vaccination of further participants in case any of the pre-specified pausing rules described in this section are met. The sponsor's medical safety officer or delegate will be involved in all discussions and decisions.

If any of the following events occur in any participant who received the study intervention, the site investigator will halt the vaccination of further participants in the study, and the sponsor's medical safety officer or delegate will be notified immediately:

1. Death of a participant considered related to the study intervention, or if the causal relationship to the study intervention cannot be excluded; *OR*

   Note: All cases of death will be sent to the independent medical reviewer for information. Upon their review, the independent medical reviewer may then also advise whether a study pause is required.

2. One or more participants experience a SAE (solicited or unsolicited) that is determined to be related to study intervention; *OR*

3. One or more participants experience anaphylaxis or generalized urticaria within 24 hours of vaccination, clearly not attributable to causes other than vaccination with the study intervention.

To enable prompt response to a situation that could trigger pausing rules, the investigator should notify the sponsor's medical safety officer or delegate immediately and no later than 24 h after becoming aware of any related AE of grade 3 or above AND update the CRF with relevant information on the same day the AE information is collected. A thorough analysis of all grade 3 cases will be carried out by the sponsor's medical safety officer or designee, irrespective of whether the criteria for pausing the study are met. Based on the pausing criteria, the sponsor's medical safety officer or delegate then decides whether a study pause is warranted. All investigator(s) will be notified immediately in case of a study pause. The sponsor's medical safety officer or delegate is responsible for the immediate notification of independent medical reviewer and coordination of a meeting with the independent medical reviewer in case of a study pause.

The sponsor's medical safety officer or delegate or the investigator (upon consultation with the sponsor's medical safety officer or delegate) may contact the independent medical reviewer in any case in which, in their professional opinion, the safety of the participants or the reliability of the data could be affected.

Vaccinations for the study may be suspended for safety concerns other than those described above, or before pausing rules are met, if, in the judgment of the independent medical reviewer, participant safety may be threatened.

Resumption of vaccinations will start only upon receipt of written recommendations by the independent medical reviewer. The clinical site(s) will be allowed to resume activities upon receipt of written notification from the sponsor. The communications from the independent medical reviewer will be forwarded by the investigator to the Independent Ethics Committee (IEC) and by the sponsor to the relevant health authorities, according to local standards and regulations.

8.4 IMMUNOGENICITY ASSESSMENTS

Venous blood samples for the determination of immune responses will be collected at the time points indicated in the TIME AND EVENTS SCHEDULE. Serum samples will be analysed to determine binding antibodies against EBOV GP using the FANG ELISA and neutralising antibodies against the Ad26 vector using the Ad26 VNA.

Sample collection and processing will be performed by the study-site personnel according to current versions of approved standard operating procedures. The Laboratory Manual contains further details regarding the collection, handling, labeling, and shipment of blood samples to the relevant laboratories.

8.4.1 Future scientific research

Future scientific research may be conducted to further investigate Ebola vaccine- and disease-related questions and to study other infections of public health importance in Sierra Leone and neighbouring countries. This may include the development of new, or the improvement of existing, techniques to characterise EBOV-directed immune responses or other diagnostic tests. No additional samples will be taken for these analyses, however, residual samples from the study tests may be retained for these purposes and analysed after the end of the study.

8.5 SAMPLE COLLECTION AND HANDLING

The actual dates and times of sample collection must be recorded in the CRF or laboratory requisition form.

Refer to the TIME AND EVENTS SCHEDULE for the timing and frequency of all sample collections.

Instructions for the collection, handling, storage, and shipment of samples are found in the laboratory manual that will be provided. Collection, handling, storage, and shipment of samples must be under the specified, and where applicable, controlled temperature conditions as indicated in the laboratory manual.

9 PARTICIPANT COMPLETION/WITHDRAWAL FROM THE STUDY

9.1 COMPLETION

A participant will be considered to have completed the study if he or she has completed all assessments at the 28-day post-vaccination visit (Day 29).

Participants who prematurely discontinue the study intervention for any reason before completion of the 28-day post-vaccination visit will not be considered to have completed the study

9.2 WITHDRAWAL FROM THE STUDY

A parent/guardian has the right to withdraw their child from the study at any time for any reason. The investigator should make an attempt to contact the parent/guardian of a child who did not return for scheduled visits. Although a participant and/or parent/guardian are not obliged to give reason(s) for withdrawing prematurely, the investigator should make a reasonable effort to ascertain the reason(s) while fully respecting the participant's rights.

A participant will be withdrawn from the study for any of the following reasons:

- Lost to follow-up
- Withdrawal of consent
- Death
- Repeated failure to comply with protocol requirements
- Decision by the sponsor or the investigator to stop or cancel the study
- Decision by local regulatory authorities or IEC to stop or cancel the study

If a participant is lost to follow-up, every reasonable effort must be made by the study-site personnel to contact the participant and/or the parent/guardian and determine the reason for discontinuation/withdrawal. The measures taken to follow up must be documented.

When a participant withdraws, or a parent/guardian withdraws a participant, before completing the study, the reason for withdrawal is to be documented in the CRF and in the source document.

A participant who wishes to withdraw consent, or a participant whose parent/guardian wishes to withdraw consent, will be offered an optional visit for safety follow-up (before the formal withdrawal of consent). The participant or participant's parent/guardian has the right to refuse this optional visit.

Withdrawal of Consent for the Use of Samples in Future Research

A participant, or a participant's parent/guardian, may withdraw consent for the use of samples for research (refer to Section 15.2.5). In such a case, every possible effort will be made to destroy samples after they are no longer needed for the study. Details of the sample retention for research are presented in the ICF.

10 STATISTICAL METHODS

Statistical analysis will be done by the sponsor or under the authority of the sponsor. A general description of the statistical methods to be used to analyze the safety and immunogenicity data is outlined below. Specific details will be provided in the Statistical Analysis Plan.

The final analysis will be performed at study completion, defined as the date of the final database lock, which will occur after all participants have completed the last study-related visit or left the study.

10.1 ANALYSIS SETS

Full Analysis Set: The full analysis set will include all participants with study intervention administration documented. This will be used for safety analysis.

Per-protocol Immunogenicity Population: The per-protocol immunogenicity population will include all vaccinated participants for whom immunogenicity data are available, excluding participants with major protocol deviations expecting to impact the immunogenicity outcomes.

10.2 SAMPLE SIZE DETERMINATION

Approximately 50 participants are expected to be enrolled into this trial (approximately 25 for each age group). The sample size is a convenience sample and is not based on formal hypothesis testing considerations.

10.3 PARTICIPANT INFORMATION

For all participants, demographic characteristics (e.g. age and sex), and other baseline characteristics (e.g. vital signs) will be tabulated and summarized with descriptive statistics.

10.4 SAFETY ANALYSES

No formal statistical testing of safety data is planned. Safety data will be analysed descriptively (including 95% CIs, if applicable).

Adverse Events (Including Reactogenicity)

The verbatim terms used in the CRF by investigators to identify AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). All reported AEs and events-related diary information (solicited local at injection site and systemic, and unsolicited) with onset within 28 days after the vaccination will be included in the analysis. For each AE, the number and percentage of participants who experience at least 1 occurrence of the given event will be summarized.

Summaries, listings, datasets, or participant narratives may be provided, as appropriate, for those participants who die, who discontinue study intervention due to an AE, or who experience a severe or a serious adverse event.

Physical Examination

Because only abbreviated, symptom-directed examinations are performed per the discretion of the investigator, physical examination findings (i.e., abnormalities) after vaccination are to be recorded as AEs, and will be analysed and presented as indicated above. When reported prior to vaccination, they will be recorded as medical history.

Vital Signs

Descriptive statistics of temperature, blood pressure, pulse/heart rate, and respiratory rate values will not be summarized at each scheduled time point. A listing of participants with clinically significant abnormal values will be provided.

Clinical Laboratory Tests

Laboratory abnormalities will be determined according to the United States National Institutes of Health, National Institute of Allegy and Infectious Disease, Division of Microbiology and Infectious Diseases (DMID)

paediatric toxicity table (November 2007) (see Appendix 1) and in accordance with the normal ranges of the clinical laboratory. The most severe laboratory abnormalities following vaccination will be listed.

10.5 IMMUNOGENICITY ANALYSIS

No formal hypothesis on immunogenicity will be tested. Descriptive statistics (e.g. geometric mean and 95% CI) will be calculated for continuous immunologic parameters at all available time points. Graphical representations of immunological parameters will be made as applicable. Frequency tabulations will be calculated for discrete (qualitative) immunologic parameters (i.e. responder rate), as applicable. Responders rate defined as >2.5-time increase over baseline value (or LLOQ) pre-dose 1 vaccination in the EBOVAC-Salone trial, will be calculated depending on availability of sample results from the EBOVAC-Salone trial.

10.6 INTERIM ANALYSIS

No interim analysis will be performed for this study.

10.7 INDEPENDENT DATA MONITORING COMMITTEE

An independent data monitoring committee (IDMC) will not be appointed for this study. The safety of the Ad26.ZEBOV vaccine has already been shown in children in previous studies. A booster dose of Ad26.ZEBOV was also shown to be safe in adults. There are no planned interim analyses. Thus the IDMC's role will be designated to an independent medical reviewer. The independent medical reviewer will be identified by the sponsor to review the accumulating safety data on an ongoing basis to ensure the continuing safety of the participants enrolled in this study.

The independent medical reviewer will be consulted periodically to review newly generated data. Ad hoc meetings may be requested via the sponsor if any of the pre-specified pausing rules for this study are met (see Section 8.2.) or in any situation that could affect the safety of the participants.

After the review, the independent medical reviewer will make recommendations regarding the continuation of the study. The independent medical reviewer responsibilities, authorities, frequency and timing of the evaluations and procedures will be documented in a role and responsibility description.

The independent medical reviewer will be external and independent of the sponsor. He or she will be a medical expert in the relevant field.

11 ADVERSE EVENT REPORTING

Timely, accurate, and complete reporting and analysis of safety information from clinical studies are crucial for the protection of participants, investigators, and the sponsor, and are mandated by regulatory agencies worldwide. The sponsor has established standard operating procedures in conformity with regulatory requirements worldwide to ensure appropriate reporting of safety information; all clinical studies conducted by the sponsor or its affiliates are conducted in accordance with those procedures.

Method of Detecting AEs and Serious Adverse Events

Care will be taken not to introduce bias when detecting AEs or SAEs. Open-ended and nonleading verbal questioning of the participant's parent(s)/guardian is the preferred method to inquire about AE occurrence.

Solicited Adverse Events

Solicited AEs are predefined local (at the injection site) and systemic events for which the participant's parent/guardian is specifically questioned, and which are noted in the participant's diary.

Unsolicited Adverse Events

Unsolicited AEs are all AEs for which the participant's parent/guardian is not specifically questioned in the participant diary.

11.1 DEFINITIONS

11.1.1 Adverse Event Definitions and Classifications

Adverse Event

An AE is any untoward medical occurrence in a clinical study subject administered a medicinal (investigational or non-investigational) product. An AE does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non-investigational) product, whether or not related to that medicinal (investigational or non-investigational) product. (Definition per International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use [ICH].)

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities.

Note: For the time period of AE collection, see Section 11.3.

Serious Adverse Event

A SAE, based on ICH and EU Guidelines on Pharmacovigilance for Medicinal Products for Human Use, is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (the participant was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.)
- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is a suspected transmission of any infectious agent via a medicinal product
- Is medically important*

*Medical and scientific judgment should be exercised in deciding whether expedited reporting is also appropriate in other situations (other than those listed above) , such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require intervention to prevent one of the other outcomes listed in the definition above. These should usually be considered serious.

If a serious and unexpected AE occurs for which there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis), the event must be reported as a suspected unexpected serious adverse reaction (SUSAR) (even after the study is over, if the sponsor, the independent medical reviewer, or the investigator becomes aware of it).

Unlisted (Unexpected) Adverse Event/Reference Safety Information

An AE is considered unlisted if the nature or severity is not consistent with the applicable product reference safety information. For Ad26.ZEBOV the expectedness of an AE will be determined by whether or not it is listed in the IB.

Adverse Event Associated With the Use of the Intervention

An AE is considered associated with the use of the intervention if the attribution is related by the definitions listed in Section 11.1.2.

An AE is considered not associated with the use of the intervention if the attribution is unrelated by the definitions listed in Section 11.1.2.

Adverse Events of Special Interest

AESIs (including potential AESIs) are significant AEs that are judged to be of special interest because of clinical importance, known or suspected effects of similar vaccines, or based on nonclinical signals. AESIs and potential AESIs will be carefully monitored during the study by the sponsor.

AESIs and potential AESIs must be reported to the sponsor within 24 hours of awareness, irrespective of seriousness (i.e., serious and nonserious AEs) or causality following the procedure described above for SAEs.

Specific requirements for the AESI are described below.

Thrombosis with Thrombocytopenia Syndrome (TTS)

TTS has been observed very rarely following vaccination with Janssen COVID vaccine and is considered to be an AESI in this study. TTS is a syndrome characterized by a combination of both a thrombotic event and thrombocytopenia. [13, 14]

Because this syndrome is rare and not completely understood, all cases of thrombosis and/or symptomatic thrombocytopenia will be considered a potential case of TTS and should be reported to the sponsor within 24 hours of awareness. Each potential AESI will be reviewed to identify a TTS case. A potential TTS case is defined as:

- Thrombotic events: suspected deep vessel venous or arterial thrombotic events as detailed in Appendix 2

and/or

- Symptomatic thrombocytopenia, defined as platelet count below LLN for the testing lab

Symptoms, signs, or conditions suggestive of a thrombotic event or symptomatic thrombocytopenia should be recorded and reported as a potential AESI even if the final or definitive diagnosis has not yet been determined, and alternative diagnoses have not yet been eliminated or shown to be less likely. Follow-up information and final diagnoses, if applicable, should be submitted to the sponsor as soon as they become available.

In the event of symptomatic thrombocytopenia, study site personnel should report the absolute value for the platelet count and the reference range for the laboratory test used.

For either a thrombotic event or symptomatic thrombocytopenia, it is recommended to test for anti-Platelet Factor 4 antibodies (anti-PF4) at the local laboratory or substitute local laboratory; repeat testing may be requested for confirmation upon sponsor discretion.

AESIs, including potential AESIs, will require enhanced data collection and evaluation. Every effort should be made to report as much information as possible about the event to the sponsor in a reasonable timeframe.

Treatment and Follow-up Recommendation

The medical management of thrombotic events with thrombocytopenia is different from the management of isolated thromboembolic diseases. Study site personnel and/or treating physicians should follow available guidelines for treatment of thrombotic thrombocytopenia (e.g., American Society of Hematology 2021; British Society of Haematology 2021; CDC 2021). The use of heparin may be harmful and alternative treatments may be needed. Consultation with a hematologist is strongly recommended.

11.1.2 Attribution Definitions

Every effort should be made by the investigator to explain any AE and to assess its potential causal relationship, i.e., to administration of the study intervention or to alternative causes (e.g., natural history of an underlying diseases, concomitant therapies). This applies to all AEs, whether serious or non-serious.

Causality of AEs should be assessed by the investigator based on the following:

Related

There is suspicion that there is a relationship between study intervention and AE (without determining the extent of that probability); there is a reasonable possibility that the study intervention contributed to the AE.

All AEs assessed as possibly, probably or definitely related to the study intervention will be considered related to the study intervention.

Unrelated

There is no suspicion that there is a relationship between the study intervention and the AE; there are other more likely causes and administration of the study intervention is not suspected to have contributed to the AE.

All AE assessed as unrelated or doubtfully related to the study intervention will be considered unrelated to the study intervention.

By definition, all solicited AEs at the injection site (local) will be considered related to the study intervention administration.

11.1.3 Severity Criteria

All AEs, except for solicited AEs, will be coded for severity using a modified version of the November 2007 DMID paediatric toxicity table (see Appendix 1).

For AEs not identified in the table, the following guidelines will apply:

Mild	Grade 1	Symptoms causing no or minimal interference with usual social and functional activities.
Moderate	Grade 2	Symptoms causing greater than minimal interference with usual social and functional activities.
Severe	Grade 3	Symptoms causing inability to perform usual social and functional activities.
Potentially Life-threatening	Grade 4	Any grade 3 symptom that requires hospitalization/in-patient medical intervention.

Note: Only clinically significant abnormalities in laboratory data occurring from signing of the ICF onwards will be reported as adverse events and graded using the table above.

The investigator should use clinical judgment in assessing the severity of events not directly experienced by the participant (e.g. laboratory abnormalities).

11.2 SPECIAL REPORTING SITUATIONS

Safety events of interest on a study intervention that may require expedited reporting or safety evaluation include, but are not limited to:

- Overdose of a study intervention
- Suspected abuse/misuse of a study intervention
- Medication error involving a product (with or without participant exposure to the study intervention, e.g., name confusion)

Special reporting situations should be recorded in the CRF. Any special reporting situation that meets the criteria of a SAE should be recorded on the serious adverse event page of the CRF.

11.3 PROCEDURES

Depending on the nature of the event, the reporting procedures below will be followed. Any questions concerning AE reporting will be directed to the sponsor. Further details on AE reporting can be found in the AE reporting flowchart.

11.3.1 All Adverse Events

Symptoms of solicited local and systemic AEs will be collected in the diary in the evening after the vaccination and then daily for the next 7 days. Unsolicited AEs will be reported from vaccination until 28 days post-vaccination.

Serious adverse events and/or special reporting situations that are related to study procedures will be reported from the time a signed and dated ICF is obtained onwards until the end of the study. All other SAEs and/or special reporting situations will be reported from the day of vaccination onwards until 28 days post-vaccination. The sponsor will evaluate any safety information that is spontaneously reported by an investigator beyond the time frame specified in the protocol.

The investigator will monitor and analyse the study data including all AE and clinical laboratory data as they become available and will make determinations regarding the severity of the adverse experiences and their relation to study intervention. All AEs will be deemed related to study intervention or not related to study intervention, according to Section 11.1.

The investigator or clinical designee must review both post-injection reactogenicity and other AE CRFs to insure the prompt and complete identification of all events that require expedited reporting as SAEs, invoke pausing rules, or are other serious and unexpected events.

All AEs, regardless of seriousness, severity, or presumed relationship to study intervention, must be recorded using medical terminology in the source document and the CRF. Whenever possible, diagnoses should be given when signs and symptoms are due to a common aetiology (e.g., cough, runny nose, sneezing, sore throat, and head congestion should be reported as "upper respiratory infection"). Investigators must record in the CRF their opinion concerning the relationship of the AE to study therapy. All measures required for AE management must be recorded in the source document and reported according to sponsor instructions.

The LSHTM assumes responsibility for appropriate reporting of AEs to the regulatory authorities. The LSHTM will also report to the vaccine manufacturer and investigator (and the head of the investigational institute, as required) all SUSARs. The investigator (or sponsor, as required) must report SUSARs to the appropriate IEC that approved the protocol unless otherwise required and documented by the IEC.

Janssen Vaccines & Prevention B.V., as the vaccine manufacturer, will report any SUSAR to all the investigators of studies using the experimental vaccine.

The parent/guardian will be provided with a "wallet (study) card" and instructed to carry this card with their child for the duration of the study indicating the following:

- Study number
- Statement, in the local language(s), that the child is participating in a clinical study.
- Investigator's name and 24-hour contact telephone number
- Local sponsor's name and 24-hour contact telephone number (for medical staff only)
- Participant number

11.3.2 Serious Adverse Events

All SAEs occurring during the study must be reported to the appropriate person nominated by the sponsor by study-site personnel within 24 hours of their knowledge of the event and to IEC and local regulatory authority, as required.

Information regarding SAEs will be transmitted to the sponsor and to IEC and local regulatory authority, as required, using the Serious Adverse Event Form, which must be completed and signed by a physician from the study site, and transmitted to the sponsor, IEC and local regulatory authority, within 24 hours. The initial and follow-up reports of a SAE should scanned and sent by email.

All SAEs that have not resolved by the end of the study, or that have not resolved upon discontinuation of participation in the study, must be followed until any of the following occurs:

- The event resolves
- The event stabilizes
- The event returns to baseline, if a baseline value/status is available
- The event can be attributed to agents other than the study intervention or to factors unrelated to study conduct
- It becomes unlikely that any additional information can be obtained (participant or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts)

Suspected transmission of an infectious agent by a medicinal product will be reported as a SAE. Any event requiring hospitalisation (or prolongation of hospitalisation) that occurs during the course of a subject's participation in a study must be reported as a SAE, except hospitalisations for the following:

- Hospitalisations not intended to treat an acute illness or AE (e.g., social reasons such as pending placement in a long-term care facility)
- Surgery or procedure planned before entry into the study (this must be documented in the CRF).

  Note: Hospitalisations that were planned before the signing of the ICF, and where the underlying condition, for which the hospitalisation was planned, has not worsened, will not be considered SAEs. Any AE that results in a prolongation of the originally planned hospitalisation is to be reported as a new SAE.

During the entire study, the cause of death of a participant in a study, whether or not the event is expected or associated with the study intervention, is considered a SAE.

11.4 CONTACTING SPONSOR REGARDING SAFETY

The names (and corresponding telephone numbers) of the individuals who should be contacted regarding safety issues or questions regarding the study are listed in the Contact Information page(s), which will be provided as a separate document.

12 PRODUCT QUALITY COMPLAINT HANDLING

A product quality complaint (PQC) is defined as any suspicion of a product defect related to manufacturing, labeling, or packaging, i.e., any dissatisfaction relative to the identity, quality, durability, or reliability of a product, including its labeling or package integrity. A PQC may have an impact on the safety and efficacy of the product. Timely, accurate, and complete reporting and analysis of PQC information from studies are crucial for the protection of participants, investigators, and the sponsor, and are mandated by regulatory agencies worldwide. Janssen Vaccines & Prevention B.V. and the sponsor have established procedures in conformity with regulatory requirements worldwide to ensure appropriate reporting of PQC information; all studies conducted by the sponsor or its affiliates will be conducted in accordance with those procedures.

12.1 PROCEDURES

All initial PQCs must be reported to the sponsor by the study-site personnel within 24 hours after being made aware of the event.

If the defect is combined with a SAE, the study-site personnel must report the PQC to the sponsor according to the SAE reporting timelines (refer to Section 11.3). A sample of the suspected product should be maintained for further investigation if requested by the sponsor and Janssen Vaccines & Prevention B.V.

12.2 CONTACTING SPONSOR REGARDING PRODUCT QUALITY

The names (and corresponding telephone numbers) of the individuals who should be contacted regarding product quality issues are listed in the Contact Information page(s), which will be provided as a separate document.

13 STUDY INTERVENTION INFORMATION

Ad26.ZEBOV

Ad26.ZEBOV is a monovalent, replication-incompetent adenovirus serotype 26-based vector that expresses the full-length EBOV Mayinga GP and is produced in the human cell line PER.C6®.

The Ad26.ZEBOV vaccine will be supplied at a concentration of $1x10^{11}$ vp/mL in 2-mL single-use glass vials as a frozen liquid to be thawed before use. Each vial contains an extractable volume of 0.5 mL. Refer to the IB for a list of excipients.

The Ad26.ZEBOV vaccine is manufactured by IDT Biologika GmbH for Janssen Vaccines & Prevention B.V., The Netherlands.

13.1 PACKAGING

All study intervention will be manufactured and packaged in accordance with Good Manufacturing Practice. All study intervention will be packaged and labeled under the responsibility of the sponsor. No study intervention can be repacked or re-labeled without prior approval from the sponsor.

Further details for study intervention packaging and labeling can be found in the Site Investigational Product Procedures Manual.

13.2 LABELLING

Study intervention labels will contain information to meet the applicable regulatory requirements.

13.3 PREPARATION, HANDLING, STORAGE

All study intervention must be stored at controlled temperatures. Guidance on storage temperature is provided in the Site Investigational Product Procedures Manual.

Vials must be stored in a secured location with no access for unauthorized personnel. All equipment for storage of the study intervention (including refrigerators, freezers) must be equipped with a continuous temperature monitor and alarm, and with back-up power systems. In the event that the study intervention is exposed to temperatures outside the specified temperature ranges, all relevant data will be sent to the sponsor to determine if the affected study intervention can be used or will be replaced. The affected study intervention must be quarantined and not used until further instruction from the sponsor is received.

A pharmacist/qualified staff member will prepare all doses for vaccine administration and provide it for dispensing.

Full details on the preparation, the holding time, and storage conditions from the time of preparation to delivery of Ad26.ZEBOV are provided in the Site Investigational Product Procedures Manual.

13.4 INTERVENTION ACCOUNTABILITY

The investigator is responsible for ensuring that all study intervention received at the site is inventoried and accounted for throughout the study. The study intervention administered to the participant must be documented on the intervention accountability form. All study intervention will be stored and disposed of according to the sponsor's instructions. Study-site personnel must not combine contents of the study intervention containers.

Study intervention must be handled in strict accordance with the protocol and the container label, and must be stored at the study site in a limited-access area or in a locked cabinet under appropriate environmental

conditions. Unused study intervention must be available for verification by the sponsor's study site monitor or delegate during on-site monitoring visits. The return to the sponsor of unused study intervention will be documented on the Investigational Product Destruction Form. When the study site is an authorized destruction unit and study intervention supplies are destroyed on-site, this must also be documented on the Investigational Product Destruction Form.

Potentially hazardous materials such as used ampules, needles, syringes, and vials containing hazardous liquids, should be disposed of immediately in a safe manner and therefore will not be retained for intervention accountability purposes.

Study intervention should be dispensed under the supervision of the investigator or a qualified member of the study-site personnel, or by a hospital/clinic pharmacist. Study intervention will be supplied only to study participants. The investigator agrees neither to dispense the study intervention from, nor store it at, any site other than the study site agreed upon with the sponsor.

14 STUDY -SPECIFIC MATERIALS

The investigator will be provided with the following supplies:

- IB and Addendum (if applicable) for Ad26.ZEBOV
- Site Investigational Product Procedures Manual
- Laboratory manual
- Electronic Data Capture (eDC) Manual/electronic CRF Completion Guidelines
- Sample ICF
- Participant diaries
- TOU
- Rulers, thermometers
- Participant wallet card

15 ETHICAL ASPECTS

15.1 STUDY-SPECIFIC DESIGN CONSIDERATIONS

Potential participants, and/or their parent/guardian, will be fully informed of the risks and requirements of the study. During the study, participants and/or their parent/guardian will be given any new information that may affect their decision to continue participation. They will be told that their consent to participate in the study is voluntary and may be withdrawn at any time with no reason given and without penalty or loss of benefits to which they would otherwise be entitled. Only parents/guardians of participants who are fully able to understand the risks, benefits, and potential AEs of the study, and provide their consent voluntarily will be allowed to enrol their child.

The primary ethical concern is the safety of the enrolled children.

When referring to the signing of the ICF, the term guardian refers to the traditionally or legally appointed guardian of the child with authority to authorise participation in research. For each participant, his or her parent or guardian, must give permission and written consent (according to local requirements) after the nature of the study has been fully explained and before any study-related activities are performed. Only parents/guardians who are fully able to understand the risks and benefits, and who provide their consent voluntarily, can enrol their child into the study. Assent must be obtained from children capable of understanding the nature of the study, typically potential participants 7 years of age and older. For the purposes of this study, all references to participants who have provided consent (and assent as applicable) refer to the participant and his or her parent/guardian who has provided consent according to this process. Children who assent to a study and later withdraw that assent will not be maintained in the study against their will, even if their parent/guardian still want them to participate.

Test of Understanding

The TOU is a short assessment of the parent/guardian of the potential participant's understanding of key aspects of the study. The test will help the study staff to determine how well the parent/guardian understands the study and their requirements for participation of their child.

The parent/guardian must pass the TOU, indicating that he or she understands the purpose of, and procedures required for the study, after reading the informed consent and after the investigator or designee has provided detailed information on the study and has answered the questions of the parent/guardian. The parent/guardian must subsequently sign the ICF, indicating that he or she is willing to allow their child to participate in the study.

If a parent/guardian fails to achieve the passing score on an attempt, further information and counselling will be provided to the parent/guardian by a study team member. The parent/guardian is allowed to retake the test twice to achieve the passing score (≥90%) required for participation of their child in the study. If the parent/guardian fails to achieve the passing score on the third attempt they will not be able to re-take the test again, and their child will not be allowed to participate in the study.

Any parent/guardian of a potential participant not capable of understanding the key aspects of the study, and their requirements for participation, should not be allowed to enrol their child.

15.2 REGULATORY ETHICS COMPLIANCE

All references to the IEC refer to the LSHTM Ethics Committee and the Sierra Leone Ethics and Scientific Review Committee.

15.2.1 Investigator Responsibilities

The investigator will be responsible for ensuring that the study is performed in accordance with the protocol, current ICH guidelines on Good Clinical Practice (GCP), and applicable regulatory and country-specific requirements.

GCP is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and well-being of study subjects are protected, consistent with the principles that originated in the Declaration of Helsinki, and that the study data are credible.

15.2.2 Independent Ethics Committee

Before the start of the study, the investigator (or sponsor where required) will provide the IEC with current and complete copies of the following documents (as required by local regulations):

- Final protocol and, if applicable, amendments
- Sponsor-approved ICF (and any other written materials to be provided to the study subjects, e.g. diary)
- IB (or equivalent information) and amendments
- Sponsor-approved subject recruiting materials
- Information on compensation for study-related injuries or payment to study participants for participation in the study, if applicable
- Investigator's curriculum vitae or equivalent information (unless not required, as documented by the IEC)
- Information regarding funding, name of the sponsor, institutional affiliations, other potential conflicts of interest, and incentives for study participants
- Any other documents that the IEC requests to fulfil its obligation

This study will be undertaken only after the IEC has given full approval of the final protocol, amendments (if any, excluding the ones that are purely administrative, with no consequences for participants, data or study

conduct, unless required locally), the ICF, applicable recruiting materials, and subject compensation programmes, and after the sponsor has received a copy of this approval. This approval letter must be dated and must clearly identify the IEC and the documents being approved.

During the study the investigator (or sponsor where required) will send the following documents and updates to the IEC for their review and approval, where appropriate:

- Protocol amendments (excluding the ones that are purely administrative, with no consequences for participants, data, or study conduct)
- Revision(s) to ICF and any other written materials to be provided to participants
- New or revised participant recruiting materials approved by the sponsor (when applicable)
- Revisions to compensation for study-related injuries or payment to participants for participation in the study, if applicable
- New edition(s) of the IB and amendments/addenda
- Summaries of the status of the study at intervals stipulated in guidelines of the IEC (at least annually)
- Reports of AEs that are serious, unlisted/unexpected, and associated with the study vaccine
- New information that may adversely affect the safety of the participants or the conduct of the study
- Deviations from or changes to the protocol to eliminate immediate hazards to the participants
- Report of deaths of participants under the investigator's care
- Notification if a new investigator is responsible for the study at the centre
- Development Safety Update Report and Line Listings, where applicable
- Any other requirements of the IEC

For all protocol amendments (excluding the ones that are purely administrative, with no consequences for participants, data, or study conduct), the amendment and applicable ICF revisions will be submitted promptly to the IEC for review and approval before implementation of the change(s).

At least once a year, the IEC will be asked to review and reapprove this study, where required.

At the end of the study, the investigator (or sponsor where required) will notify the IEC about the study completion.

15.2.3 Informed Consent

In this community-based study, informed consent will take place at several levels ranging from approval from the government authorities, followed by community level consent, and finally, individual consent.

Approval from Respective Health Authorities

Approval for this project will be obtained from the respective health authorities in the country where the study is conducted.

Additionally, during the planning process of the study, approval will be sought from other authorities such as district or local councillors, political leaders, and traditional leaders.

Consent at the Community Level

Documented community-level consent by a community leader must be available.

Consent at the Individual Level

The parent/guardian of a participant (in this section referred to as the legally acceptable representative) must give written consent according to local requirements after the nature of the study has been fully explained. The ICF must be signed before performance of any study-related activity. The ICF(s) that is/are used must be

approved by both the sponsor and by the reviewing IEC and be in a language that the legally acceptable representative and can read and understand. The informed consent should be in accordance with principles that originated in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and sponsor policy.

Before enrolment in the study, the investigator or an authorized member of the study-site personnel must explain to the subject and/or the subject's legally acceptable representative the aims, methods, reasonably anticipated benefits, and potential hazards of the study, and any discomfort participation in the study may entail. The subject and/or the legally acceptable representative will be informed that their participation/the participation of their child is voluntary and that they may withdraw consent to participate at any time. They will be told that the investigator will maintain a participant identification register if needed and that their records may be accessed by health authorities and authorized sponsor personnel without violating the confidentiality of the participant, to the extent permitted by the applicable law(s) or regulations. By signing the ICF the legally acceptable representative is authorizing such access, which includes permission to obtain information about their child's health status. It also denotes that the legally acceptable representative agrees to allow his or her child's study physician to recontact the them for the purpose of obtaining consent for additional safety evaluations.

The legally acceptable representative will be given sufficient time to read the ICF and the opportunity to ask questions. After this explanation and before their child's entry into the study, consent should be appropriately recorded by means of the legally acceptable representative's personally dated signature. After having obtained the consent, a copy of the ICF must be given to the legally acceptable representative.

If the legally acceptable representative is unable to read or write, an impartial witness should be present for the entire informed consent process (which includes reading and explaining all written information) and the impartial witness should personally date and sign the ICF after the consent of the legally acceptable representative is obtained.

Children will be enrolled only after obtaining consent of a parent/guardian. Assent will be obtained from children capable of understanding the nature of the study, typically subjects 7 years of age and older. Written assent will be obtained from subjects who are able to write. Children who assent to a study and later withdraw that assent will not continue in the study against their will, even if their parent/guardian still want them to participate.

Children who turn 7 years of age will be asked to provide assent, if it is deemed they are able to comprehend the information provided, for continuation in the study at the first available opportunity.

15.2.4 Privacy of Personal Data

The collection and processing of personal data from participants enrolled in this study will be limited to those data that are necessary to fulfill the objectives of the study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data agree to keep the identity of participants confidential.

The informed consent obtained from the legally acceptable representative includes explicit consent for the processing of personal data of the participant and for the investigator/institution to allow direct access to his or her original medical records (source data/documents) for study-related monitoring, audit, IEC review, and regulatory inspection. This consent also addresses the transfer of the data to other entities and to other countries.

The legally acceptable representative has the right to request through the investigator access to the personal data of their child and the right to request rectification of any data that are not correct or complete. Reasonable steps will be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.

Exploratory research is not conducted under standards appropriate for the return of data to subjects. In addition, the sponsor cannot make decisions as to the significance of any findings resulting from exploratory research. Therefore, exploratory research data will not be returned to subjects or investigators, unless required by law or local regulations. Privacy and confidentiality of data generated in the future on stored samples will be protected by the same standards applicable to all other clinical data.

15.2.5 Long-Term Retention of Samples for Additional Future Research

Samples collected in this study may be stored for up to 15 years (or according to local regulations) for additional research. Future scientific research may be conducted to further investigate Ebola vaccine- and disease-related questions and to study other infections of public health importance in Sierra Leone and neighbouring countries. This may include the development of new or the improvement of existing techniques to characterize EBOV-directed immune responses or diagnostic tests. No additional samples will be taken for these analyses, however, residual samples from other tests may be retained for these purposes and analysed after the end of the study.

Parents/guardians will be asked to consent voluntarily for their child's blood samples to be stored for other research studies that may be done after this study is completed. Participants for whom such consent is not given, can participate in the study without having their blood samples stored for future testing (see also Section 9.2). In such case, their blood samples will be destroyed after all the immunogenicity tests have been concluded (as agreed by the sponsor).

All samples, for which consent has been obtained and for which additional material is available after study-specified testing is complete, will be stored for future testing.

Stored samples will be coded throughout the sample storage and analysis process and will not be labeled with personal identifiers. Parents/guardians may withdraw consent for their child's samples to be stored for research at any time during the study.

16 ADMINISTRATIVE REQUIREMENTS

16.1 PROTOCOL AMENDMENTS

Neither the investigator nor the sponsor will modify this protocol without a formal amendment by the sponsor. All protocol amendments will be issued by the sponsor, and signed and dated by the relevant investigator. Protocol amendments will not be implemented without prior IEC approval, or when the relevant competent authority has raised any grounds for non-acceptance, except when necessary to eliminate immediate hazards to the participants, in which case the amendment will be promptly submitted to the IEC and relevant competent authority. Documentation of amendment approval by the investigator and IEC will be provided to the sponsor. When the change(s) involves only logistic or administrative aspects of the study, the IEC (where required) will be notified.

During the course of the study, in situations where a departure from the protocol is unavoidable, the investigator or other physician in attendance will contact the appropriate sponsor representative (listed in the Contact Information page(s), which will be provided as a separate document). Except in emergency situations, this contact will be made before implementing any departure from the protocol. In all cases, contact with the sponsor will be made as soon as possible to discuss the situation and agree on an appropriate course of action. The data recorded in the CRF and source documents will reflect any departure from the protocol, and the source documents will describe this departure and the circumstances requiring it.

16.2 REGULATORY DOCUMENTATION

16.2.1 Regulatory Approval and Notification

This protocol and any amendment(s) must be submitted to the appropriate regulatory authority in the country where the study is being conducted. A study may not be initiated until all local regulatory requirements are met. All references to the local Regulatory Authority refer to the Pharmacy Board of Sierra Leone.

16.2.2 Required Pre-study Documentation

The following documents will be provided to the sponsor before shipment of study intervention to the study site:

- Protocol and amendment(s), if any, signed and dated by the Principal Investigator
- A copy of the dated and signed (or sealed, where appropriate per local regulations), written IEC approval of the protocol, amendments, ICF, any recruiting materials, and if applicable, participant compensation programs. This approval must clearly identify the specific protocol by title and number and must be signed (or sealed, where appropriate per local regulations) by the chairman or authorised designee.
- Name and address of the IEC, including a current list of the IEC members and their function, with a statement about how it is organised and operates according to GCP and the applicable laws and regulations. If accompanied by a letter of explanation, or equivalent, from the IEC, a general statement may be substituted for this list. If an investigator or a member of the study-site personnel is a member of the IEC, documentation must be obtained to state that this person did not participate in the deliberations or in the vote/opinion of the study.
- Regulatory authority approval or notification
- Signed and dated statement of investigator (e.g. Form FDA 1572), If applicable
- Documentation of investigator qualifications (e.g. curriculum vitae)
- Completed investigator financial disclosure form from the Principal Investigator, where required
- Signed and dated clinical trial agreement, which includes financial agreement
- Any other documentation required by local regulations

The following documents must be provided to the sponsor before enrolment of the first participant:

- Completed investigator financial disclosure forms from all sub investigators
- Documentation of sub-investigator qualifications (e.g., curriculum vitae)
- Name and address of any local laboratory conducting tests for the study, and a dated copy of current laboratory normal ranges for these tests, if applicable

16.3 SUBJECT IDENTIFICATION AND ENROLMENT LOGS

The investigator agrees to complete a subject identification and enrolment log to permit easy identification of each subject during and after the study. This document will be reviewed by the sponsor study-site representative for completeness.

The participant identification and enrolment log will be treated as confidential and will be filed by the investigator in the study file. To ensure participant confidentiality, no copy will be made. All reports and communications relating to the study will identify subjects by participant identification and age at initial informed consent.

The investigator must also complete a participant screening log, which reports on all participants who were seen to determine eligibility for inclusion in the study.

16.4 SOURCE DOCUMENTATION

At a minimum, source documents consistent in the type and level of detail with that commonly recorded at the study site as a basis for standard medical care must be available for the following: subject identification, eligibility, and study identification; study discussion and date of signed informed consent; dates of visits; results of safety and efficacy parameters as required by the protocol; record of all AEs and follow-up of AEs; concomitant medication; intervention receipt/dispensing/return records; study intervention administration

information; and date of study completion and reason for early discontinuation of study intervention or withdrawal from the study, if applicable.

The author of an entry in the source documents should be identifiable.

Specific details required as source data for the study and source data collection methods will be reviewed with the investigator before the study and will be described in the monitoring guidelines (or other equivalent document).

The TOU and participant's diary used to collect information regarding solicited symptoms after vaccination will be considered source data.

Inclusion and exclusion criteria not requiring documented medical history must be verified at a minimum by participant interview or other protocol required assessment (e.g., physical examination, laboratory assessment) and documented in the source documents.

16.5 CASE REPORT FORM COMPLETION

Case report forms are prepared and provided by the sponsor for each subject in electronic format. All CRF entries, corrections, and alterations must be made by the investigator or authorized study-site personnel. The investigator must verify that all data entries in the CRF are accurate and correct.

The study data will be transcribed by study-site personnel from the source documents onto an electronic case report form (eCRF). Study-specific data will be transmitted in a secure manner to the sponsor within the timeframe agreed upon between the sponsor and the study site.

Worksheets may be used for the capture of some data to facilitate completion of the eCRF. Any such worksheets will become part of the participant's source documentation. Data must be entered into eCRFs in English. Study site personnel must complete the eCRF promptly after a participant visit, and the forms should be available for review at the next scheduled monitoring visit.

If necessary, queries will be generated in the eDC tool. If corrections to a CRF are needed after the initial entry into the eCRF, this can be done in either of the following ways:

- Investigator and study site personnel can make corrections in the eDC tool at their own initiative or as a response to an auto query (generated by the eDC tool).
- Sponsor or sponsor delegate can generate a query for resolution by the investigator and study site personnel.

16.6 DATA QUALITY ASSURANCE/QUALITY CONTROL

Steps to be taken to ensure the accuracy and reliability of data include the selection of qualified investigators and appropriate study sites, review of protocol procedures with the investigator and study-site personnel before the study, and periodic monitoring visits by the sponsor and/or remote monitoring by the sponsor. Written instructions will be provided for collection, handling, storage, and shipment of samples.

Guidelines for CRF completion will be provided and reviewed with study-site personnel before the start of the study. The sponsor will review CRFs for accuracy and completeness during onsite monitoring visits and after transmission to the sponsor; any discrepancies will be resolved with the investigator or designee, as appropriate. The data will be entered into the study database and verified for accuracy and consistency with the data sources.

Representatives of the sponsor may visit the participating site at any time during or after completion of the study to conduct an audit of the study in compliance with regulatory guidelines and/or LSHTM policy. Similar procedures may also be conducted by a regulatory authority. Further details of on-site audit policies are presented in Section 16.10.

16.7 RECORDS RETENTION

In compliance with ICH/GCP guidelines, the investigator/institution will maintain all CRFs and all source documents that support the data collected from each participant, as well as all study documents as specified

in ICH/GCP Section 8, Essential Documents for the Conduct of a Clinical Trial, and all study documents as specified by the applicable regulatory requirement(s). The investigator/institution will take measures to prevent accidental or premature destruction of these documents.

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, as per LSHTM SOP (LSHTM-SOP-049 Site Close out).

If it becomes necessary for the sponsor or the appropriate regulatory authority to review any documentation relating to this study, the investigator/institution must permit access to such reports.

16.8 MONITORING

The sponsor, or their delegate, will perform study site visits to monitor this study.

The sponsor, or their delegate will perform on-site monitoring visits as frequently as necessary. The monitor will record dates of the visits in a study site visit log that will be kept at the study site. The first post-initiation visit will be made as soon as possible after enrolment has begun. At these visits, the monitor will compare the data entered into the CRF with the vaccination unit and/or clinic records (source documents) (e.g., hospital / clinic / physician's office medical records). The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the CRF are known to the sponsor and study-site personnel and are accessible for verification by the sponsor study-site contact. If electronic records are maintained at the study site, the method of verification must be discussed with the study-site personnel.

Direct access to source documents (medical records) must be allowed for the purpose of verifying that the recorded data are consistent with the original source data. Findings from this review will be discussed with the study-site personnel. The sponsor expects that, during monitoring visits, the relevant study-site personnel will be available, the source documents will be accessible, and a suitable environment will be provided for review of study-related documents. The monitor will meet with the investigator on a regular basis during the study to provide feedback on the study conduct.

In addition to on-site monitoring visits, remote contacts can occur. It is expected that during these remote contacts, study-site personnel will be available to provide an update on the progress of the study at the site.

16.9 STUDY COMPLETION/TERMINATION

16.12.1 Study Completion/End of Study

The study is considered completed at final database lock, which will occur after the last participant in the study has completed their last study-related visit or left the study. The final data from the study site will be sent to the sponsor (or designee) after completion of the final participant visit at that study site, in the time frame specified in the Clinical Trial Agreement.

16.12.2 Study Termination

The sponsor reserves the right to close the study for data collection or terminate the study at any time for any reason at the sole discretion of the sponsor. The study will be closed upon study completion. The study is considered closed when all the required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of the study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirement of the IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of participants by the investigator

- Discontinuation of further study intervention development

16.10 ON-SITE AUDITS

Representatives of the sponsor's clinical quality assurance department may visit the study site at any time during or after completion of the study to conduct an audit of the study in compliance with regulatory guidelines and company policy. These audits will require access to all study records, including source documents, for inspection. Subject privacy must, however, be respected. The investigator and study-site personnel are responsible for being present and available for consultation during routinely scheduled study-site audit visits conducted by the sponsor or its designees.

Similar auditing procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The investigator should immediately notify the sponsor if he or she has been contacted by a regulatory agency concerning an upcoming inspection.

16.11 USE OF INFORMATION AND PUBLICATION

All information, including but not limited to information regarding the Ad26.ZEBOV vaccine or the sponsor's operations (e.g., manufacturing processes, basic scientific data, prior clinical data, formulation information) supplied by the sponsor to the investigator and not previously published, and any data, including exploratory research data, generated as a result of this study, are considered confidential. The investigator agrees to maintain this information in confidence and use this information only to accomplish this study, and will not use it for other purposes without the sponsor's prior written consent.

The investigator understands that the information developed in the study will be used by the sponsor or the vaccine manufacturer, Janssen Vaccines & Prevention B.V., in connection with the continued development of the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen, and thus may be disclosed as required to other clinical investigators or regulatory agencies. To permit the information derived from the clinical studies to be used, the investigator is obligated to provide the sponsor with all data obtained in the study.

Consistent with Good Publication Practices and International Committee of Medical Journal Editors guidelines, the sponsor shall have the right to publish such primary data and information without approval from the investigator. Authorship of publications resulting from this study will be based on the guidelines on authorship, such as those described in the International Committee of Medical Journal Editors (ICMJE), Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals, which state that the named authors must have made a significant contribution to the conception or design of the work; or the acquisition, analysis, or interpretation of the data for the work; and drafted the work or revised it critically for important intellectual content; and given final approval of the version to be published; and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Registration of Clinical Studies and Disclosure of Results

The sponsor will register and disclose the existence of, and the results of, clinical studies as required by law.

APPENDIX 1

Toxicity Grading Scale for Healthy Paediatric Participants (older than 3 months) Enrolled in Preventive Vaccine Clinical Trials

Adapted from: DMID° Pediatric Toxicity Tables (November 2007, draft). For adverse events not included in the tables below, refer to the severity criteria guidelines in section 11.1.3 Severity Criteria. Local lab references take preference over the DMD table and the different grades.

The abbreviations used in the following tables are: LLN: lower limit of normal; IV: intravenous; ULN: upper limit of normal.

LOCAL REACTIONS				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Tenderness	Mild discomfort to touch; minimal to no limitation of use of limb	Notable discomfort to touch; Greater than minimal limitation of use of limb	Significant discomfort at rest; Severe limitation of use of limb	Hospitalization or ER visit for treatment
Erythema	<10 mm	10-25 mm	26-50 mm	>50 mm or any grade 3 with hospitalization or ER visit for treatment
Swelling	<10 mm	10-25 mm	26-50 mm	>50 mm or any grade 3 with hospitalization or ER visit for treatment
HEMATOLOGY				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Hemoglobin for children greater than 3 months and less than 2 years of age	**9.0-9.9 gm/dL**	**7.0-8.9 gm/dL**	**<7.0 gm/dL**	**Cardiac Failure secondary to Anemia**
Hemoglobin for children greater than 2 years of age	10.0-10.9 gm/dL	7.0-9.9 gm/dL	<7.0 gm/dL	Cardiac Failure secondary to Anemia
Absolute Neutrophil Count	**750-1200/mm³**	**400-749/mm³**	**250-399/mm³**	**<250/mm³**
Platelets	75,000-99,999/mm³	50,000-74,999/mm³	25,000-49,999/mm³	<25,000/mm³
Prothrombin Time (PT)	1.1-1.2 x ULN	1.3-1.5 x ULN	1.6-3.0 x ULN	>3.0 x ULN
Partial Thromboplastin Time (PTT)	1.1-1.6 x ULN	1.7-2.3 x ULN	2.4-3.0 x ULN	>3.0 x ULN

GASTROINTESTINAL				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Bilirubin (Fractionated bilirubin test must be performed when total bilirubin is elevated)				
Bilirubin for children greater than 3 months of age (when accompanied by any increase in other liver function test)	**1.1- <1.25 x ULN**	**1.25- <1.5 x ULN**	**1.5-1.75 x ULN**	**>1.75 x ULN**
Bilirubin for children greater than 3 months of age (when other liver functions are in the normal range)	1.1- <1.5 x ULN	1.5- <2.0 x ULN	2.0-3.0 x ULN	>3.0 x ULN
AST (SGOT)	1.1- <2.0 x ULN	2.0- <3.0 x ULN	3.0-8.0 x ULN	>8.0 x ULN
ALT (SGPT)	1.1- <2.0 x ULN	2.0- <3.0 x ULN	3.0-8.0 x ULN	>8.0 x ULN
GGT	1.1- <2.0 x ULN	2.0- <3.0 x ULN	3.0-8.0 x ULN	>8.0 x ULN
Pancreatic Amylase	1.1- 1.4 x ULN	1.5- 1.9 x ULN	2.0-3.0 x ULN	>3.0 x ULN
Uric Acid	7.5-9.9 mg/dL	10.0-12.4 mg/dL	12.5-15.0 mg/dL	>15.0 mg/dL
Loss of Appetite	Feeding minimally reduced	Feeding reduced by more than 50% of normal for the child	Refusing all feeds	No solid or liquid taken orally for in the last 24 hours; requires intravenous fluids
Diarrhea	Change in consistency of stools OR increase of 1-3 stools over baseline per 24-hour period	liquid/watery stools OR increase of 4 to 6 stools over baseline per 24-hour period	Increase of ≥7 stools over baseline per 24-hour period	Requires IV fluid resuscitation and electrolytes repletion OR hypotensive shock
Constipation	Slight change in consistency and/or frequency of stools	Hard, dry stools with a change in frequency	Intestinal obstruction accompanied with abdominal pain	Hospitalization; Severe abdominal distention and vomiting accompanied with severe abdominal pain
Vomiting	1 episode/ day (24h)	2-3 episodes per day (24h)	4-6 episodes per day (24h)	Greater than 6 episodes per day (24h) OR intractable vomiting

ELECTROLYTES				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Creatinine				
3 Months – 2 Years of age	0.6-0.8 x ULN	0.9-1.1 x ULN	1.2-1.5 x ULN	> 1.5 x ULN
2 Years – 3 Years of age	0.7-1.0 x ULN	1.1-1.6 x ULN	1.7-2.0 x ULN	>2.0 x ULN
Hypernatremia	----	145-149 mEq/L	150-155 mEq/L	>155 mEq/L or abnormal sodium AND mental status changes
Hyponatremia	----	130-135 mEq/L	129-124 mEq/L	<124 mEq/L or abnormal sodium AND mental status changes
Hyperkalemia	5.0-5.9 mEq/L	6.0-6.4 mEq/L	6.5-7.0 mEq/L	>7.0 mEq/L or abnormal potassium AND cardiac arrhythmia
Hypokalemia	3.0-3.5 mEq/L	2.5-2.9 mEq/L	2.0-2.4 mEq/L	<2.0 mEq/L or abnormal potassium AND cardiac arrhythmia
Hypercalcemia	10.5-11.2 mg/dL	11.3-11.9 mg/dL	12.0-12.9 mg/dL	>13.0 mg/dL
Hypocalcemia	7.8-8.4 mg/dL	7.0-7.7 mg/dL	6.0-6.9 mg/dL	>6.0 mg/dL
Hypomagnesemia	1.2-1.4 mEq/L	0.9-1.1 mEq/L	0.6-0.8 mEq/L	<0.6 mEq/L or abnormal magnesium AND cardiac arrhythmia
hypoglycemia	55-65 mg/dL	40-54 mg/dL	30-39 mg/dL	<30 mg/dL or adnormal glucose AND mental status changes
Hyperglycemia	116-159 mg/dL	160-249 mg/dL	250-400 mg/dL	>400 mg/dL or ketoacidosis
Proteinuria	Tr-1+ or <150 mg/day	2+ or 150-499 mg/day	3+ or 500-1000 mg/day	4+ or Nephrotic syndrome >1000 mg/day
Hematuria	Microscopic <25 cells/hpf	Microscopic >25 cells/hpf	Gross hematuria	Hospitalization; Life-threatening consequences

Neurologic				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Irritability	Easily consolable; minimal or no interference with activity. Episodes of continuous crying <60 min.	Difficult to console. Episodes of continuous crying >60 min <120 min	Inconsolable, prevents daily activity. Episodes of continuous crying >120 min	Hospitalization or ER visit for treatment
Decreased Activity	Minimal decrease in alertness, minimal or no interference with activity	Some interference with activity, slightly subdued	unable to achieve normal level of alertness, lethargic	ER visit or hospitalization for treatment or life-threatening consequences
Neuropathy/ Lower Motor Neuropathy	----	Mild transient Paresthesia only	Persistent or progressive paresthesia, burning sensation in feet, or mild dysesthesia; no weakness; mild to moderate deep tendon reflex changes; no sensory loss	Onset of significant weakness, decrease or loss of DTRs, sensory loss in "stocking glove" distribution, radicular sensory loss, multiple cranial nerve involvement; bladder or bowel dysfunction, fasciculations, respiratory embarrassment from chest wall weakness.
Myopathy or Neuromuscular Junction Impairment	Normal or mild (<2 x ULN) CPK elevation	Mild proximal weakness and/or atrophy not affecting gross motor function. Mild myalgias, +/- mild CPK elevation (<2 x ULN)	Proximal muscle weakness and/or atrophy affecting motor function +/- CPK elevation; or severe myalgias with CPK >2 x ULN;	Onset of myasthenia-like symptoms (fatigable weakness with external, variable ophthalmoplegia and/or ptosis), or neuromuscular junction blockade (acute paralysis) symptoms
OTHER				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Fever/pyrexia	38.0 - 38.4 °C or 100.4 - 101.1 °F	38.5 - 38.9 °C or 101.2 - 102.0 °F	39.0 - 40.0 °C or 102.1 - 104.0 °F	Greater than 40 °C or 104.0 °F
Acute allergic reaction	Pruritus without Rash	Pruritic Rash	Mild Urticaria	Severe Urticaria Anaphylaxis, Angioedema
Stomatitis	Mild discomfort	Painful, difficulty swallowing, but able to eat and drink	Painful: unable to swallow solids	Painful: unable to swallow liquids; requires IV fluids
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	Hospitalization

APPENDIX 2:

THROMBOTIC EVENTS TO BE REPORTED AS POTENTIAL ADVERSE EVENTS OF SPECIAL INTEREST

The list of thrombotic events to be reported to the sponsor as potential AESIs is provided below. Further guidance may become available on thrombotic events of interest.

• MedDRA PTs for large vessel thrombosis and embolism
Aortic embolus, aortic thrombosis, aseptic cavernous sinus thrombosis, brain stem embolism, brain stem thrombosis, carotid arterial embolus, carotid artery thrombosis, cavernous sinus thrombosis, cerebral artery thrombosis, cerebral venous sinus thrombosis, cerebral venous thrombosis, superior sagittal sinus thrombosis, transverse sinus thrombosis, mesenteric artery embolism, mesenteric artery thrombosis, mesenteric vein thrombosis, splenic artery thrombosis, splenic embolism, splenic thrombosis, thrombosis mesenteric vessel, visceral venous thrombosis, hepatic artery embolism, hepatic artery thrombosis, hepatic vein embolism, hepatic vein thrombosis, portal vein embolism, portal vein thrombosis, portosplenomesenteric venous thrombosis, splenic vein thrombosis, spontaneous heparin-induced thrombocytopenia syndrome, femoral artery embolism, iliac artery embolism, jugular vein embolism, jugular vein thrombosis, subclavian artery embolism, subclavian vein thrombosis, obstetrical pulmonary embolism, pulmonary artery thrombosis, pulmonary thrombosis, pulmonary venous thrombosis, renal artery thrombosis, renal embolism, renal vein embolism, renal vein thrombosis, brachiocephalic vein thrombosis, vena cava embolism, vena cava thrombosis, truncus coeliacus thrombosis

• MedDRA PTs for more common thrombotic events
Axillary vein thrombosis, deep vein thrombosis, pulmonary embolism, MedDRA PTs for acute myocardial infarction*, MedDRA PTs for stroke*

Source: Shimabukuro T. CDC COVID-19 Vaccine Task Force. Thrombosis with thrombocytopenia syndrome (TTS) following Janssen COVID-19 vaccine. Advisory Committee on Immunization Practices (ACIP). April 23, 2021. https://www.cdc.gov/vaccines/acip/meetings/slides-2021-04-23.html.

*Vaccine Adverse Event Reporting System (VAERS)Standard Operating Procedures for COVID-19 (as of 29 January 2021) https://www.cdc.gov/vaccinesafety/pdf/VAERS-v2-SOP.pdf

REFERENCES

1. WHO. Ebola virus disease - key facts. 2021; Available from: http://www.who.int/news-room/fact-sheets/detail/ebola-virus-disease (Accessed 27 May 2021).
2. Investigator's Brochure: VAC52150 (Ad26.ZEBOV, MVA-BN-Filo [MVA-mBN226B]) Edition 1. Janssen Vaccines & Prevention B.V. (18 December 2020).
3. Addendum 2 to Investigator's Brochure VAC52150 (Ad26.ZEBOV, MVA-BN-Filo [MVA-mBN226B]) Edition 1. Janssen Vaccines & Prevention (06 May 2021).
4. Afolabi M.O., Mutua G., Barry H. et al. Safety and Immunogenicity of a 2-Dose Heterologous Vaccine Regimen Against Ebola in African Children and Adolescents. American Society of Tropical Medicine and Hygiene 68th Annual Meeting, National Harbor, Maryland USA November 20-24, 2019.
5. Afolabi M.O., Ishola D., Manno D. at al. Safety and immunogenicity of a two-dose heterologous vaccine regimen against Ebola in children in Sierra Leone: a randomised, double-blind, placebo-controlled trial. Lancet Infect Dis 2021; (accepted).
6. Ishola D., Manno D., Afolabi M.O., et al. Safety and long-term immunogenicity of a two-dose Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen in adults: a randomised trial initiated during the Ebola outbreak in Sierra Leone. Lancet Infect Dis 2021; (accepted).
7. European Commission. Vaccine against Ebola: Commission grants new market authorisations. Accessible at: https://ec.europa.eu/commission/presscorner/detail/en/ip_20_1248 (Accessed 27 May 2021).
8. European Medicines Agency. Zabdeno. Accessible at: https://www.ema.europa.eu/en/medicines/human/EPAR/zabdeno (Accessed 27 May 2021).
9. Bockstal V., Roozendaal R., Van Effelterre T. et al. Immunobridging approach to assess clinical benefit as the basis for licensure of the monovalent Ebola vaccine. Accessible at: https://www.imi.europa.eu/sites/default/files/events/2018/ScientificSymposium/37-%20Viki%20Bockstal.pdf (Accessed 27 May 2021).
10. Ethical Considerations for Clinical Trials on Medicinal Products Conducted With the Pediatric Population: Recommendations of the Ad Hoc Group for the Development of Implementing Guidelines for Directive 2001/20/EC Relating to Good Clinical Practice in the Conduct of Clinical Trials on Medicinal Products for Human Use. 2008.
11. WHO. Weight-for-age: Birth to 5 years in girls. Available from: http://www.who.int/childgrowth/standards/cht_wfa_girls_p_0_5.pdf?ua=1 (Accessed 27 May 2021).
12. WHO. Weight-for-age (5-10 years) percentiles in girls. Available from: http://www.who.int/growthref/cht_wfa_girls_perc_5_10years.pdf?ua=1 (Accessed 27 May 2021).
13. American Society of Hematology. COVID-19 resources. Thrombosis with Thrombocytopenia Syndrome (also termed Vaccine-induced Thrombotic Thrombocytopenia). Updated 6 May 2021. https://www.hematology.org/covid-19/vaccine-induced-immune-thrombotic-thrombocytopenia (Accessed 27 May 2021).
14. Brighton Collaboration. Interim Case Definition of Thrombosis with Thrombocytopenia Syndrome (TTS). 21 April 2021. https://brightoncollaboration.us/thrombosis-with-thrombocytopenia-syndrome-interim-case-definition/ (Accessed 29 April 2021)

Paper 4: 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

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	150330	**Title**	Dr
First Name(s)	Daniela		
Surname/Family Name	Manno		
Thesis Title	Testing a prophylactic vaccine regimen against Ebola virus disease in Sierra Leone: vaccine safety, immunogenicity and factors affecting immunogenicity		
Primary Supervisor	Professor Deborah Watson Jones		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	Vaccines		
When was the work published?	August 2023		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	This work was published after the registration for my research degree. I consulted the Head of the Doctoral College and I was allowed to include this paper in my PhD portfolio.		
Have you retained the copyright for the work?*	**Yes**	Was the work subject to academic peer review?	**Yes**

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

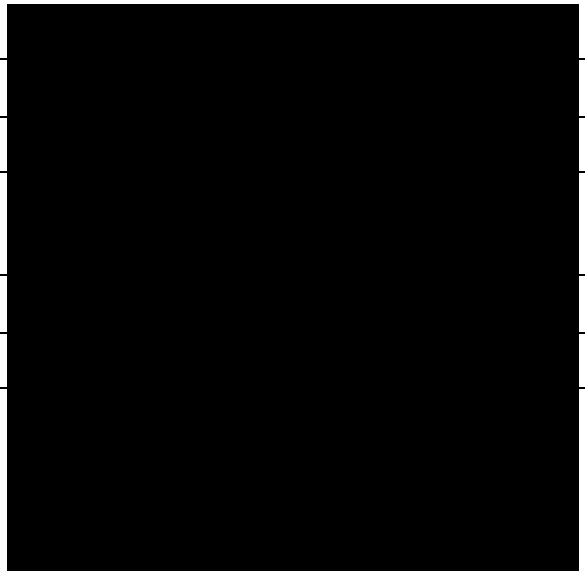
Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	

Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I was one of the coordinators of the study. I drafted the statistical analysis plan and conducted the statistical analysis in collaboration with the study statistician, Philip Ayieko. I am the first and corresponding author of the paper. I drafted the manuscript and I was responsible for communication with the journal during the manuscript submission, peer-review and publication process.

SECTION E



Student Signature	
Date	

Supervisor Signature	
Date	

RETENTION OF COPYRIGHT EVIDENCE

Paper 3. The Effect of Previous Exposure to Malaria Infection and Clinical Malaria Episodes on the Immune Response to the Two-Dose Ad26.ZEBOV, MVA-BN-Filo Ebola Vaccine Regimen

Vaccines MDPI, Basel, Switzerland

vaccines

MDPI

Article

The Effect of Previous Exposure to Malaria Infection and Clinical Malaria Episodes on the Immune Response to the Two-Dose Ad26.ZEBOV, MVA-BN-Filo Ebola Vaccine Regimen

Daniela Manno [1,*], Catriona Patterson [1], Abdoulie Drammeh [1,2], Kevin Tetteh [1], Mattu Tehtor Kroma [2,3], Godfrey Tuda Otieno [1,2], Bolarinde Joseph Lawal [1,2], Seyi Soremekun [1], Philip Ayieko [1,4], Auguste Gaddah [5], Abu Bakarr Kamara [2,3], Frank Baiden [1,2], Muhammed Olanrewaju Afolabi [1,2], Daniel Tindanbil [1,2], Kwabena Owusu-Kyei [1,2], David Ishola [1,2], Gibrilla Fadlu Deen [3], Babajide Keshinro [6], Yusupha Njie [1,2], Mohamed Samai [3], Brett Lowe [1,7], Cynthia Robinson [6], Bailah Leigh [3], Chris Drakeley [1], Brian Greenwood [1] and Deborah Watson-Jones [1,4]

1 London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK
2 EBOVAC Project Office, Kukuna Road, Kambia, Sierra Leone
3 College of Medicine and Allied Health Sciences, University of Sierra Leone, New England Ville, Freetown, Sierra Leone
4 Mwanza Intervention Trials Unit, National Institute for Medical Research, Mwanza P.O. Box 11936, Tanzania
5 Janssen Research and Development, 2340 Beerse, Belgium
6 Janssen Vaccines and Prevention, 2333 CB Leiden, The Netherlands
7 KEMRI-Wellcome Trust Research Programme, Kilifi P.O. Box 230, Kenya
* Correspondence: daniela.manno@lshtm.ac.uk

Abstract: We assessed whether the immunogenicity of the two-dose Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen with a 56-day interval between doses was affected by exposure to malaria before dose 1 vaccination and by clinical episodes of malaria in the period immediately after dose 1 and after dose 2 vaccinations. Previous malaria exposure in participants in an Ebola vaccine trial in Sierra Leone (ClinicalTrials.gov: NCT02509494) was classified as low, intermediate, and high according to their antibody responses to a panel of *Plasmodium falciparum* antigens detected using a Luminex MAGPIX platform. Clinical malaria episodes after vaccinations were recorded as part of the trial safety monitoring. Binding antibody responses against the Ebola virus (EBOV) glycoprotein (GP) were measured 57 days post dose 1 and 21 days post dose 2 by ELISA and summarized as Geometric Mean Concentrations (GMCs). Geometric Mean Ratios (GMRs) were used to compare groups with different levels of exposure to malaria. Overall, 587 participants, comprising 188 (32%) adults (aged $\geq$ 18 years) and 399 (68%) children (aged 1–3, 4–11, and 12–17 years), were included in the analysis. There was no evidence that the anti-EBOV-GP antibody GMCs post dose 1 and post dose 2 differed between categories of previous malaria exposure. There was weak evidence that the GMC at 57 days post dose 1 was lower in participants who had had at least one episode of clinical malaria post dose 1 compared to participants with no diagnosed clinical malaria in the same period (GMR = 0.82, 95% CI: 0.69–0.98, p-value = 0.02). However, GMC post dose 2 was not reduced in participants who experienced clinical malaria post-dose 1 and/or post-dose 2 vaccinations. In conclusion, the Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen is immunogenic in individuals with previous exposure to malaria and in those who experience clinical malaria after vaccination. This vaccine regimen is suitable for prophylaxis against Ebola virus disease in malaria-endemic regions.

Keywords: malaria; Ebola; vaccine; immunogenicity; Ad26.ZEBOV; MVA-BN-Filo

Citation: Manno, D.; Patterson, C.; Drammeh, A.; Tetteh, K.; Kroma, M.T.; Otieno, G.T.; Lawal, B.J.; Soremekun, S.; Ayieko, P.; Gaddah, A.; et al. The Effect of Previous Exposure to Malaria Infection and Clinical Malaria Episodes on the Immune Response to the Two-Dose Ad26.ZEBOV, MVA-BN-Filo Ebola Vaccine Regimen. *Vaccines* **2023**, *11*, 1317. https://doi.org/10.3390/vaccines11081317

Academic Editor: Junki Maruyama

Received: 1 July 2023
Revised: 26 July 2023
Accepted: 28 July 2023
Published: 2 August 2023

1. Introduction

Ebola disease indicates a group of severe, often fatal, infections caused by viruses of the genus *Orthoebolavirus* [1,2]. In 2018, the World Health Organization (WHO) included

Ebola disease among the infections that pose the greatest public health risk due to their epidemic potential and for which vaccine development is urgently needed [3]. Since then, two vaccine regimens against Ebola virus (EBOV), species *Zaire ebolavirus*, which causes Ebola virus disease (EVD) [4], have reached a more advanced stage of clinical development. A single-dose recombinant vesicular stomatitis virus-vectored vaccine expressing the EBOV glycoprotein (rVSV-ZEBOV-GP, Ervebo) has been licensed for use in adults in the EU, USA, and various African countries [5–7] and has received WHO pre-qualification [8]. Similarly, a heterologous two-dose regimen, consisting of an adenovirus type 26 (Ad26) vector-based vaccine encoding the EBOV glycoprotein (Ad26.ZEBOV, Zabdeno) and the modified vaccinia Ankara (MVA) vector-based vaccine, encoding EBOV, Sudan virus and Marburg virus glycoproteins, and the Taï Forest virus nucleoprotein (MVA-BN-Filo, Mvabea), has obtained conditional marketing authorization in the EU and various African countries and WHO pre-qualification for prophylactic use in adults and children aged 1 year or older [9–11].

All EVD outbreaks have occurred in sub-Saharan Africa [4] and vaccination against EVD will likely be implemented most frequently in countries in this region. Sub-Saharan Africa also has the highest burden of malaria in the world. In 2021, the WHO estimated that 95% of all malaria cases and 96% of all malaria deaths occurred in this region [12]. It is therefore important to evaluate whether malaria can affect the immune responses after vaccination against EVD.

The effect of malaria on reducing the immune response to some vaccines is well recognized. Impaired humoral responses have been observed in children with symptomatic malaria following vaccination with tetanus toxoid and typhoid [13], *Hemophilus influenzae* type b conjugate vaccine [14], and meningococcal polysaccharide vaccine [15]. In a study in Nigeria, children receiving malaria chemoprophylaxis showed higher antibody responses to a meningococcal polysaccharide vaccine than children of the same age who did not receive malaria chemoprophylaxis [15]. However, malaria infection did not impair the immune responses to the prophylactic HPV-16/18 virus-like particle, AS04-adjuvanted vaccine in adolescents and adults in East Africa [16]. A study in adults who received the rVSV-ZEBOV-GP Ebola vaccine found no evidence of an impaired immune response in participants with asymptomatic malaria infection at vaccination [17]. Another study in adults and children who received the Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen found that anti-EBOV-GP antibody concentrations after dose 1 and before dose 2 were lower in 1–3-year-old children with asymptomatic malaria infection at vaccination compared with malaria-negative children of similar age [18]. However, antibody concentrations after dose 2, a measure of the overall immunogenicity of the vaccine regimen, were not significantly different between the two groups [18]. This study also found no consistent effect of asymptomatic malaria infection on vaccine-induced immune responses across other age groups.

In this analysis of participants from the same study [18], we evaluated whether the immunogenicity of the two-dose Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen was affected by exposure to malaria before dose 1 vaccination and by clinical episodes of malaria in the period immediately after dose 1 or dose 2 vaccinations. To assess previous exposure to malaria, we measured antibodies to a panel of six *Plasmodium falciparum* (*P. falciparum*) recombinant antigens with a Luminex MAGPIX quantitative suspension array technology (qSAT) platform [19]. This method allows the evaluation of both long-term and recent malaria exposure and has been previously validated in children with malaria infection [20].

2. Materials and Methods

2.1. Study Design and Participants

A nested cohort study on malaria was implemented in the EBOVAC-Salone trial, which investigated the safety and immunogenicity of the two-dose regimen with the Ad26.ZEBOV and MVA-BN-Filo Ebola vaccines in adults and children in Sierra Leone. This is the same

cohort of participants in which we previously examined the effect of asymptomatic malaria parasitemia at the time of vaccination on the vaccine-induced immune responses [18].

The EBOVAC-Salone trial occurred between September 2015 and July 2018 in Kambia District, an area in the North of Sierra Leone affected by intense malaria transmission [21]. Information on the design of the clinical trial can be found on the trial registration page in ClinicalTrials.gov (NCT02509494) and has been described previously [22]. The trial was conducted in two stages: Stage 1 in which a small number of adults were all vaccinated with Ad26.ZEBOV followed by MVA-BN-Filo after 56 days, and Stage 2 in which adults and children were randomized to receive either the same Ebola vaccine regimen as in Stage 1 or an active control vaccine [22]. All participants in Stage 2 of the EBOVAC-Salone trial, which included 400 adults (≥18 years of age) and 576 children in three age cohorts (1–3 years, 4–11 years, and 12–17 years), were offered the opportunity to take part in the malaria cohort study on the day of dose 1 vaccination. A separate informed consent process from the one used for the main trial was implemented to obtain informed consent (including assent in children aged 7–17 years) for the malaria study. Only individuals who received the Ebola vaccine regimen in accordance with the trial protocol and gave their consent to the malaria study were included in the analysis after the trial was over and the data were unblinded. Additional information on the design of the malaria cohort study has been presented in a previous publication [18].

2.2. Assessment of Exposure to Malaria Infection

Exposure to malaria infection was categorized in three ways: (1) exposure to malaria before vaccination, (2) exposure to malaria at vaccination (described in a previous publication [18]) and (3) exposure to malaria after vaccination but before assessment of vaccine immunogenicity (Figure 1).

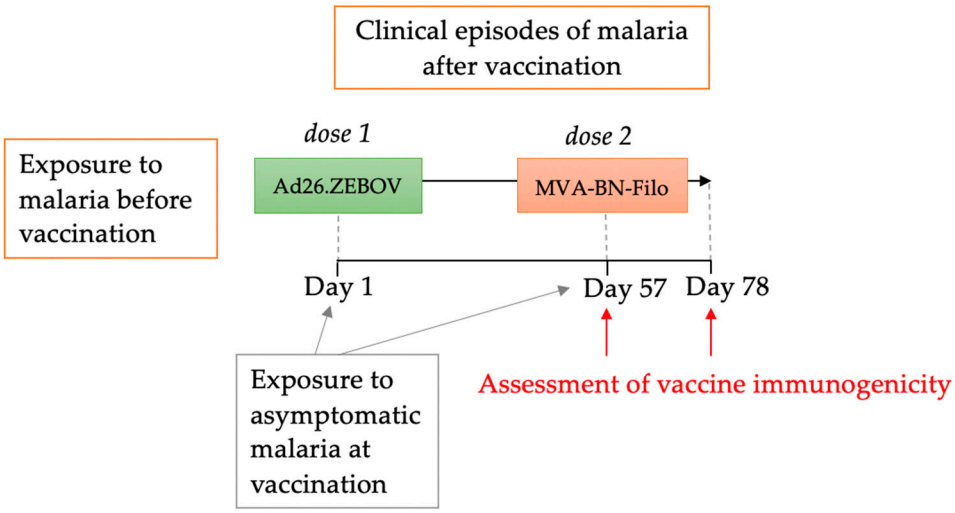


Figure 1. Malaria study conceptual framework. Note: the potential effect of malaria infection on vaccine immunogenicity was considered in three ways: (1) exposure to malaria before vaccination, (2) exposure to malaria at the time of vaccination, described previously [18], and (3) exposure to malaria after vaccination. Assessment of vaccine immunogenicity post dose 1 was evaluated on Day 57. Assessment of vaccine immunogenicity post dose 2 (a measure of the overall immunogenicity of the vaccine regimen), was evaluated on Day 78 (21 days after dose 2 vaccination).

To assess prior exposure to malaria, we categorized participants based on their antibody responses to a panel of *P. falciparum* antigens, using serum samples collected for an EBOV seroprevalence study [23] at the screening visit of the EBOVAC-Salone trial, which occurred within 28 days before the administration of dose 1 vaccination. Since the sample identification numbers for the seroprevalence study were unlinked from the EBOVAC-Salone trial and the malaria cohort study identification numbers, a matching

algorithm based on participants' date of birth, age, sex, date of screening, and study clinic number was used to link the seroprevalence study samples to the participants included in the malaria cohort study. Only participants with an exclusive one-to-one matching, which allowed the identification of an available serum sample for the laboratory analysis, were included in this analysis. The data linkage and the use of samples were approved by both the Sierra Leone Ethics Committee and the London School of Hygiene and Tropical Medicine Ethics Committee.

Samples were analyzed using the Luminex MAGPIX qSAT platform as described in previous publications [19]. A brief description of the technique is also provided in the Supplementary Material (see Luminex xMAP technique in Supplementary methods). In a previous study by Achan et al. [19], which employed the same technique, antibody responses to the following six *P. falciparum* antigens were considered the most appropriate to determine exposure to malaria infection: apical membrane antigen 1 (AMA-1), merozoite surface protein 1.19 (MSP-1.19), and glutamate-rich protein (GLURP.R2) reflecting long-term exposure to malaria; reticulocyte-binding protein homologue (Rh2.2030), gametocyte exported protein (GEXP18), and early transcribed membrane protein (Etramp5.Ag1) reflecting recent exposure to malaria (i.e., infection in the past ~9 months). Antibody responses to each antigen are expressed as Median Fluorescent Intensity (MFI) [19]. Participants were ranked into quartiles of their MFI to each antigen within their age group and were given a score from 1 to 4 according to the quartile to which they belonged (1 for the lowest quartile, 4 for the highest quartile). The scores obtained for each antigen were added to obtain an age-adjusted cumulative quartile score. Participants were then categorized according to the quartiles of this score. The highest (4th quartile) and lowest (1st quartile) scores were assigned to high-exposure and low-exposure groups, respectively [19]. Participants with intermediate scores (2nd and 3rd quartiles) were assigned to the intermediate-exposure group [19]. We considered participants in the high-exposure group to have the highest exposure to malaria infection, while participants in the low-exposure group had the lowest exposure to malaria infection. Participants in the intermediate-exposure group were considered to have intermediate exposure to malaria infection. Since long-term and recent exposure to malaria might have a different effect on vaccine-induced immune responses, we conducted a secondary analysis of the data, ranking participants separately for their responses to the long-term exposure antigens (AMA-1, MSP-1.19, GLURP.R2) and antigens indicative of recent malaria infection (Rh2.2030, GEXP18, Etramp5.Ag1).

To assess the potential impact of malaria after vaccination, we considered episodes of symptomatic malaria recorded after vaccination and before the assessment of immunogenicity. As part of the safety monitoring in the EBOVAC-Salone clinical trial, adverse events (AEs) were collected for 28 days after each vaccine dose, while serious adverse events (SAEs) were collected throughout the study. Malaria was the most frequent unsolicited AE after each vaccination in all age groups and the most frequent SAE in 1–3-year-old children [22]. Diagnosis of malaria was based on clinical symptoms and positivity to the First Response Malaria Ag. pLDH HRP2 Combo Rapid Diagnostic Test (Premier Medical Corporation Private Limited, Mumbai). Participants with clinical malaria received a complete course of age-appropriate antimalarial drugs according to national malaria treatment guidelines [24].

2.3. Assessment of Vaccine-Induced Immune Responses

Serum samples were obtained at baseline on Day 1 (immediately before dose 1 vaccination), on Day 57 (immediately before dose 2), and on Day 78 (21 days post dose 2) for the assessment of IgG antibodies to EBOV glycoprotein (GP), using the Filovirus Animal Non-Clinical Group (FANG) ELISA at Q2 Solutions Vaccine Testing Laboratory in the USA (https://www.q2labsolutions.com, accessed on 31 July 2023). The FANG ELISA was validated by the USA Food and Drug Administration (FDA) in February 2017 (Q2 Solutions, pers. comm., 2017).

2.4. Sample Size Calculation and Statistical Analysis

We performed a lognormal power calculation and determined that 460 participants with an available serum sample at screening, divided into quartiles for previous exposure to malaria, would allow a comparison of approximately 115 high-exposure participants with 115 low-exposure participants giving 90% power to detect a geometric mean ratio (GMR) of 0.81 and 80% power to detect a GMR of 0.83 for EBOV GP binding antibodies in high-exposure participants compared with low-exposure participants, assuming a coefficient of variation of 0.5 within each of the two groups, and an alpha (Type I) error of 0.05. For the assessment of the effect of clinical malaria episodes after vaccination on vaccine immunogenicity, we assumed that at least 117 (20%) of the 587 participants would have had at least one episode of malaria post-dose 1 vaccination, based on data from the EBOVAC-Salone trial [22]. This gave us 90% power to detect a GMR of 0.81 and 80% power to detect a GMR of 0.83 for EBOV GP binding antibodies in participants with no malaria episodes compared to those with at least one episode, using the same lognormal power calculation and assumptions as before.

Demographic characteristics were summarized using descriptive statistics. An age-adjusted cumulative quartile score and categories of previous exposure to malaria were obtained from the MFI for the six *P. falciparum* antigens as described previously. Malaria symptomatic infections after vaccination were analyzed as a categorical variable (i.e., at least one episode of malaria) and as a continuous variable (i.e., number of episodes). The measurements of EBOV GP binding antibody concentration were transformed on the logarithmic scale and summarized as geometric mean concentration (GMC) with 95% confidence interval (CI). Mean log-transformed EBOV GP antibody concentrations were compared between exposure categories, overall, and in each age group, using linear regression. The regression coefficients and 95% CI were back-transformed to obtain GMR, i.e., ratios of GMCs between exposure categories, with 95% CI. Statistical evidence of a difference in antibody GMC between different categories was assessed using a log likelihood-ratio test. We considered age as an a priori confounder. For this reason, we used age-adjusted categories to assess the effect of previous exposure to malaria on vaccine immunogenicity, while the effect of clinical episodes of malaria after vaccination was adjusted for age by including age group as an independent variable in the linear regression model. The analysis of the effect of previous exposure to malaria on vaccine immunogenicity was also adjusted for baseline EBOV GP-specific antibody concentrations pre-dose 1 vaccination because this variable was considered a potential confounder. This was achieved by including the baseline EBOV GP-specific antibody concentrations as an independent variable in the linear regression model.

3. Results

3.1. Study Participants

Among 976 participants enrolled in Stage 2 of the EBOVAC-Salone trial, 730 were randomized to the Ebola vaccine group and received the first dose of this regimen (Figure 2).

Among these participants, 140 were not eligible for inclusion in the malaria ancillary study, consisting of 55 who did not receive dose 2, 72 who received a delayed dose 2 beyond the protocol visit window due to a temporary halt of the trial, three who did not consent to the malaria study, and 10 who were not eligible for other reasons (Figure 2). For three participants, the vaccine immunogenicity data were not available. The remaining 587 participants were included in the malaria study. Of those, 188 (32.0%) were adults aged $\geq$ 18 years, and 399 (68.0%) were children (125 were aged 1–3 years, 133 aged 4–11 years, and 141 aged 12–17 years). The demographic characteristics of the study participants have been published previously [18] and are presented in the Supplementary Material (Supplementary Table S1).

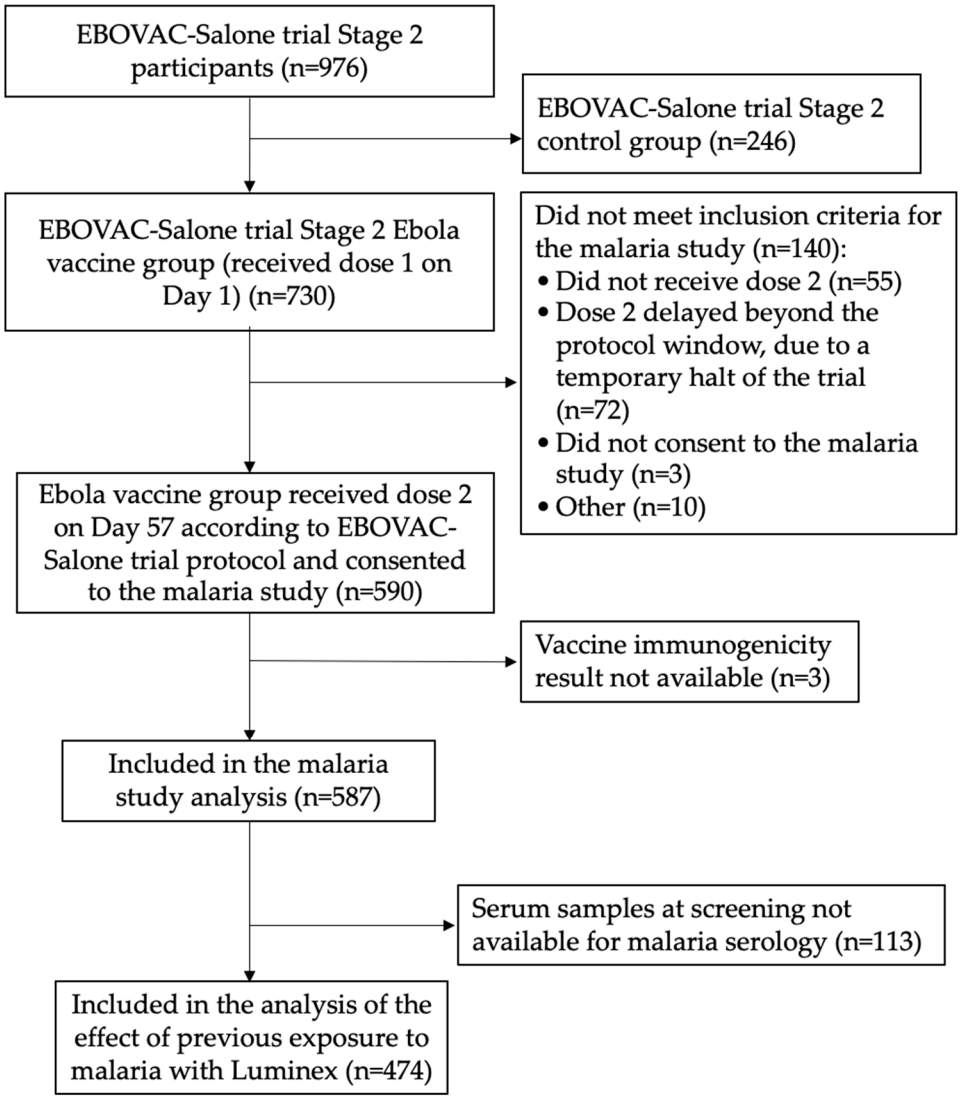


Figure 2. Study flow diagram showing the recruitment process and sample availability for laboratory analysis.

3.2. The Effect of Previous Exposure to Malaria on Vaccine Immunogenicity

Overall, 474 (80.7%) of 587 study participants had an available serum sample collected at screening, which allowed the assessment of previous exposure to malaria before vaccination (Figure 2). When the EBOV GP–specific antibody GMC after each vaccine dose was compared between categories of previous exposure to malaria at screening in all participants and then stratified by age cohort (Tables 1 and 2), there was no evidence that the EBOV GP binding antibody GMC post dose 1 (Day 57) and post dose 2 (Day 78) differed between different categories of previous exposure to malaria. The analysis in which participants were ranked separately for their responses to the long-term exposure antigens and antigens indicative of recent malaria infection showed similar results (Supplementary Tables S2–S5).

Table 1. Ebola Virus (EBOV) Glycoprotein (GP)-specific binding antibody geometric mean concentrations (GMCs) post dose 1 (measured on Day 57) by categories of previous exposure to malaria, based on participants' serologic response to a panel of *Plasmodium falciparum* (*P. falciparum)* antigens, indicative of long-term and recent exposure to malaria at the screening visit, overall, and by age cohort.

Long-Term and Recent Exposure to Malaria at Screening	N (%)	Post-Dose 1 EBOV GP-Specific Binding Antibody GMC, EU/mL	GMR [1] (95% CI)	*p*
All participants [2]	N = 474			
Low	144 (30.4)	361 (306–426)	1	0.39
Intermediate	213 (44.9)	324 (283–371)	0.88 (0.72–1.08)	
High	117 (24.7)	402 (337–481)	0.99 (0.78–1.25)	
By age group				
1–3 years	N = 96			
Low	37 (38.5)	783 (612–1002)	1	0.75
Intermediate	28 (29.2)	777 (575–1050)	0.94 (0.65–1.37)	
High	31 (32.3)	694 (491–981)	0.86 (0.55–1.35)	
4–11 years	N = 116			
Low	32 (27.6)	288 (208–400)	1	0.06
Intermediate	56 (48.3)	453 (367–560)	1.35 (0.92–1.98)	
High	28 (24.1)	358 (247–520)	0.89 (0.58–1.37)	
12–17 years	N = 115			
Low	33 (28.7)	365 (263–506)	1	0.83
Intermediate	54 (47.0)	308 (241–393)	0.91 (0.62–1.33)	
High	28 (24.3)	323 (233–447)	0.90 (0.59–1.38)	
≥18 years	N = 147			
Low	42 (28.6)	214 (163–283)	1	0.28
Intermediate	75 (51.0)	189 (152–235)	0.92 (0.67–1.25)	
High	30 (20.4)	314 (230–428)	1.19 (0.85–1.67)	

[1] Adjusted for baseline EBOV GP-specific antibody concentrations. [2] Categories of previous exposure to malaria are age-adjusted. GMR = geometric mean ratio.

Table 2. EBOV GP-specific binding antibody GMCs post-dose 2 (measured on Day 78) by categories of previous exposure to malaria, based on participants' serologic response to a panel of *P. falciparum* antigens, indicative of long-term and recent exposure to malaria at the screening visit, overall and by age cohort.

Long-Term and Recent Exposure to Malaria at Screening	N (%)	Post-Dose 2 EBOV GP-Specific Binding Antibody GMC, EU/mL	GMR [1] (95% CI)	*p*
All participants [2]	N = 466			
Low	143 (30.7)	8717 (7102–10,699)	1	0.70
Intermediate	206 (44.2)	7927 (6629–9479)	0.94 (0.72–1.23)	
High	117 (25.1)	9331 (7392–11,778)	1.12 (0.82–1.51)	
By age group				
1–3 years	N = 96			
Low	37 (38.5)	23,263 (17,681–30,607)	1	0.90
Intermediate	28 (29.2)	24,544 (17,102–35,225)	1.00 (0.60–1.65)	
High	31 (32.3)	19,313 (10,757–34,676)	0.89 (0.50–1.59)	
4–11 years	N = 115			
Low	32 (27.8)	11,046 (7571–16,116)	1	0.45
Intermediate	55 (47.8)	11,069 (8284–14,791)	1.06 (0.65–1.71)	
High	28 (24.4)	7472 (4794–11,644)	0.76 (0.41–1.42)	

Table 2. *Cont.*

Long-Term and Recent Exposure to Malaria at Screening	N (%)	Post-Dose 2 EBOV GP-Specific Binding Antibody GMC, EU/mL	GMR [1] (95% CI)	*p*
12–17 years	N = 112			
Low	33 (29.5)	8038 (4998–12,926)	1	0.31
Intermediate	51 (45.5)	11,561 (8392–15,927)	1.48 (0.82–2.65)	
High	28 (25.0)	9803 (7338–13,096)	1.21 (0.69–2.12)	
≥18 years	N = 143			
Low	41 (28.7)	3190 (2576–3950)	1	0.06
Intermediate	72 (50.3)	3029 (2372–3869)	0.94 (0.69–1.29)	
High	30 (21.0)	5170 (3676–7271)	1.50 (1.01–2.24)	

[1] Adjusted for baseline EBOV GP-specific antibody concentrations. [2] Categories of previous exposure to malaria are age-adjusted.

3.3. The Effect of Clinical Episodes of Malaria after Vaccination on Vaccine Immunogenicity

Among the 587 participants in the malaria study, 175 (29.8%) had at least one episode of clinical malaria recorded between Day 1 and Day 57, when the immunogenicity of dose 1 was assessed (Table 3). When considering participants overall and after adjusting for age group, there was weak evidence that participants who had at least one episode of clinical malaria post dose 1 had a lower GMC at Day 57 compared to participants who had no recorded episodes of clinical malaria (GMR = 0.82, 95% CI: 0.69–0.98, *p*-value = 0.02), (Table 3). After stratifying for age group, there was also weak evidence that adults who had at least one episode of clinical malaria had a lower GMC at Day 57 than adults with no recorded episodes of malaria (age group–specific GMR = 0.76, 95% CI: 0.57–1.00, *p*-value = 0.05), (Table 3). In the other age groups, there was no evidence of a difference, although the GMCs tended to be lower in participants with at least one episode of clinical malaria in the 1–3 and 4–11 year old groups (Table 3).

Table 3. EBOV GP-specific binding antibody GMCs post dose 1 (measured on Day 57) and clinical malaria episodes, which occurred in between Day 1 and Day 57 [1], overall and by age cohort.

Clinical Malaria Post-Dose 1 Vaccination	N (%)	Post-Dose 1 EBOV GP-Specific Binding Antibody GMC, EU/mL	GMR (95% CI)	*p*
All participants	N = 587			
None	412 (70.2)	371 (338–407)	1	0.02
At least one episode	175 (29.8)	323 (275–379)	0.82 (0.69–0.98) [2]	
By age group				
1–3 years	N = 125			
None	74 (59.2)	750 (630–892)	1	0.23
At least one episode	51 (40.8)	618 (460–830)	0.82 (0.58–1.16)	
4–11 years	N = 133			
None	99 (74.4)	413 (342–498)	1	0.22
At least one episode	34 (25.6)	331 (254–431)	0.80 (0.58–1.11)	
12–17 years	N = 141			
None	120 (85.1)	312 (264–368)	1	0.83
At least one episode	21 (14.9)	327 (209–510)	1.05 (0.65–1.69)	
≥18 years	N = 188			
None	119 (63.3)	260 (220–308)	1	0.05
At least one episode	69 (36.7)	197 (156–249)	0.76 (0.57–1.00)	

[1] All malaria episodes between Day 1 and Day 29 were recorded (28 days after dose 1 vaccination); between Day 30 and Day 57 only malaria episodes considered serious were recorded. [2] Adjusted for age group.

Among 579 participants who received dose 2 and had immunogenicity results available 21 days post dose 2 (Day 78), 229 (39.5%) had at least one episode of malaria recorded since dose 1 vaccination, in between Day 1 and Day 78 (Table 4). When considering participants overall and after adjusting for age group, there was no evidence of a difference in EBOV GP-specific binding antibody GMC between participants who had at least one episode of clinical malaria compared to participants who had no clinical malaria (Table 4). The result was similar when participants were stratified by age group (Table 4) or when only post-dose 2 malaria episodes, recorded between Day 57 and Day 78, were considered (Table 5).

Table 4. EBOV GP-specific binding antibody concentrations post dose 2 (measured on Day 78) and clinical malaria episodes, which occurred in between Day 1 and Day 78 [1], overall and by age cohort.

Clinical Malaria Post-Dose 1 and 2 Vaccinations	N (%)	Post-Dose 2 EBOV GP-Specific Binding Antibody GMC, EU/mL	GMR (95% CI)	*p*
All participants	N = 579			
None	350 (60.5)	8489 (7498–9610)	1	0.69
At least one episode	229 (39.5)	9133 (7678–10,863)	1.04 (0.87–1.24) [2]	
By age group				
1–3 years	N = 125			
None	54 (43.2)	22,601 (18,039–28,317)	1	0.88
At least one episode	71 (56.8)	21,909 (16,020–29,963)	0.97 (0.66–1.43)	
4–11 years	N = 132			
None	92 (69.7)	9470 (7576–11,839)	1	0.24
At least one episode	40 (30.3)	12,062 (8469–17,178)	1.27 (0.84–1.94)	
12–17 years	N = 138			
None	106 (76.8)	9428 (7549–11,775)	1	0.74
At least one episode	32 (23.2)	10,186 (6864–15,117)	1.08 (0.69–1.70)	
≥18 years	N = 184			
None	98 (53.3)	3987 (3275–4852)	1	0.65
At least one episode	86 (46.7)	3741 (3104–4509)	0.94 (0.72–1.23)	

[1] All malaria episodes between Day 1 and Day 29 (28 days after dose 1 vaccination) and between Day 57 and Day 78 (21 days after dose 2 vaccination) were recorded, and only malaria episodes considered serious were recorded between Day 30 and dose 2 administration (Day 57). [2] Adjusted for age group at dose 1 vaccination.

Table 5. Ebola virus glycoprotein binding antibody GMCs post dose 2 (measured on Day 78) and clinical malaria episodes, which occurred after dose 2 in between Day 57 and Day 78, overall and by age cohort.

Clinical Malaria Post-Dose 2 Vaccination	N (%)	Post-Dose 2 EBOV GP-Specific Binding Antibody GMC, EU/mL	GMR (95% CI)	*p*
All participants	N = 579			
None	481 (83.1)	8372 (7511–9333)	1	0.83 [1]
At least one episode	98 (16.9)	10,775 (8189–14,178)	1.03 (0.79–1.33) [1]	
By age group				
1–3 years	N = 125			
None	82 (65.6)	24,019 (19,917–28,966)	1	0.29
At least one episode	43 (34.4)	19,117 (11,996–30,467)	0.80 (0.48–1.32)	
4–11 years	N = 132			
None	123 (93.2)	10,051 (8273–12,212)	1	0.59
At least one episode	9 (6.8)	12,299 (5419–27,914)	1.22 (0.53–2.85)	

Table 5. *Cont.*

Clinical Malaria Post-Dose 2 Vaccination	N (%)	Post-Dose 2 EBOV GP-Specific Binding Antibody GMC, EU/mL	GMR (95% CI)	*p*
12–17 years	N = 138			
None	122 (88.4)	9474 (7678–11,689)	1	0.71
At least one episode	16 (11.6)	10,607 (6589–17,076)	1.12 (0.66–1.89)	
≥18 years	N = 184			
None	154 (83.7)	3743 (3217–4356)	1	0.27
At least one episode	30 (16.3)	4591 (3397–6205)	1.23 (0.87–1.72)	

[1] Adjusted for age group at dose 1 vaccination.

Among the 229 who had at least one episode of malaria recorded between Day 1 and Day 78, 177 (77.3%) had only one episode, 49 (21.4%) had two episodes, and 3 (1.3%) had three episodes. There was no evidence of an association between the number of episodes of malaria and the binding antibody concentration at 21 days post dose 2 (GMR = 0.97, 95%CI = 0.85–1.11, *p*-value = 0.65).

4. Discussion

Countries in Sub-Saharan Africa that are considered at risk of future EVD outbreaks also have a high burden of malaria [4,12,25]. This means that vaccination against EVD will be implemented most frequently in areas with a high malaria prevalence. Since malaria is recognized as having an effect on the immune responses of some vaccines [13–15], we conducted a study to assess whether malaria impaired the immunogenicity of the two-dose Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen in adults and children from Kambia district in Sierra Leone, an area where malaria is highly prevalent [18,21].

The results presented in this paper show no evidence of a difference in EBOV GP-specific binding antibody concentrations between categories of previous exposure to malaria infection based on antibody responses to six *P. falciparum* antigens indicative of long-term exposure (AMA-1, MSP-1.19 and GLURP.R2) and recent exposure, i.e., infection in the past ~9 months, (Rh2.2030, GEXP18 and Etramp5.Ag1) to malaria. Participants who had at least one episode of symptomatic malaria after dose 1 had lower antibody concentrations 57 days after dose 1 compared with participants with no malaria. However, when we assessed the cumulative effect of malaria episodes post dose 1 and post dose 2 on the antibody concentrations 21 days after dose 2, we did not observe any evidence of a difference.

These results could be explained by transient suppression of heterologous antibody production during clinical malaria episodes. Malaria is known to dysregulate B-cell functions, which could affect the production of antibodies [13]. These results are consistent with previous results from the same study [18], which showed that young children (1–3 years old) who had asymptomatic malaria infection at dose 1 (Ad26.ZEBOV) vaccination had lower antibody concentration post dose 1 compared with malaria-negative children (age group–specific GMR = 0.56; 95% CI: 0.39–0.81), but this was not observed in older age groups post dose 1 or across all age groups post dose 2.

The fact that we did not observe any effect of previous exposure to malaria on vaccine-induced immune responses, while we observed some effect of asymptomatic malaria infection at vaccination and of clinical malaria after vaccination, could be due to the different time intervals between these infections and the evaluation of vaccine immunogenicity. A recent malaria infection is probably more likely to have a detectable effect on vaccine immunogenicity than an infection that occurred in the past. However, this result could also be due to the way we assessed these exposures. Previous exposure to malaria at screening was based on the antibody response to malaria antigens, and it is possible that participants with higher levels of antibodies to malaria might also be better at producing an antibody response after vaccination against EVD. Asymptomatic malaria infection assessed through

microscopy [18] or clinical episodes of malaria recorded in the clinical trial and confirmed with a positive RDT might have been a better way to capture exposure to malaria infection.

However, the current and the previous analysis [18] show that the effect of malaria infection on vaccine immunogenicity was only detected post dose 1 at Day 57, while no effect was observed at 21 days post dose 2 when the immunogenicity of the two-dose Ebola vaccine regimen was primarily evaluated in the EBOVAC-Salone trial [22]. Thus, even if participants with asymptomatic malaria infection at the time of vaccination or clinical malaria after vaccination have lower antibody responses post dose 1, they respond after the administration of dose 2 and produce antibody concentrations that are similar to those observed in participants not affected by malaria [18].

These findings are also consistent with those from another study conducted in Sierra Leone [17], which showed robust immune responses to another Ebola vaccine (rVSVΔG-ZEBOV-GP) in asymptomatic adults with malaria parasitemia at vaccination, and this is reassuring because it confirms that both vaccines are immunogenic in malaria-endemic areas. rVSVΔG-ZEBOV-GP was also shown to be effective in preventing EVD in Guinea, a malaria-endemic country [26,27].

Our analysis has some limitations. Serum samples were no longer available from the EBOVAC-Salone trial and, therefore, serum samples from an ancillary EBOV seroprevalence study of the EBOVAC-Salone trial were used for the Luminex analysis [23]. Since the IDs of this study were not linked to the malaria study, we had to use a matching algorithm and could only identify a matching serum sample in about 81% of our study participants. Another limitation involved the assessment of clinical episodes of malaria. In Stage 2 of the EBOVAC-Salone trial, adverse events were collected up to 28 days after each vaccine dose, while only serious adverse events were collected throughout the study. This means that episodes of non-serious clinical malaria were not recorded from Day 30 to Day 56, which could have caused misclassification of participants who had a non-serious malaria infection in that time interval. If present, this misclassification likely occurred randomly and independently from the assessment of vaccine immunogenicity. This could have resulted in a dilution of the effect of clinical malaria episodes on vaccine immunogenicity if present [28]. Another limitation of this study is that the diagnosis of clinical episodes of malaria was based on a positive RDT in a population with a high background level of infection. RDT positivity may persist for several weeks after recovery from malaria infection, and this could lead to an overestimation of episodes of clinical malaria. However, the presence of a positive RDT suggests that participants had some exposure to malaria parasites recently even if this was not the cause of their illness. Finally, since the presence of fever was a contraindication to vaccination in the EBOVAC-Salone trial, we were not able to evaluate if the Ebola vaccine regimen is equally immunogenic in subjects with symptomatic malaria infection at vaccination, a situation that could happen outside a clinical trial, especially during mass vaccination in response to an ongoing EVD outbreak.

The strength of this study is the evaluation of the effect of malaria in different age groups because malaria is known to affect children more than older individuals. Moreover, by assessing the effect of previous exposure to malaria at screening and episodes of symptomatic malaria after vaccination, our results complement the results presented previously [18], providing a full picture of the effect of malaria on the immune response to the Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen.

5. Conclusions

Overall, the results of this analysis and the results presented in a previous article [18] confirm that there is no indication that malaria substantially affects the immunogenicity of the two-dose Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen, and that this vaccine regimen is suitable for EVD prophylaxis in areas where malaria is highly endemic and where the vaccine may be most needed in the future. However, as the clinical trial could not assess the safety and immunogenicity of the Ebola vaccine regimen in participants with clinical

malaria at the time of vaccination, the feasibility of delaying vaccination until recovery in people who have clinical malaria should be considered outside outbreak conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/vaccines11081317/s1, Supplementary material including Supplementary methods and Supplementary tables.

Author Contributions: D.M., K.T., S.S., P.A., A.G., B.K., C.R., C.D., B.G. and D.W.-J. were involved in the conceptualization of the study and data analysis plan. D.M. drafted the manuscript and conducted the statistical analysis. C.P., A.D., K.T., M.T.K., G.T.O., B.J.L. and B.L. (Brett Lowe) were responsible for the laboratory sample analysis with Luminex, sample management, and laboratory results interpretation. A.B.K., F.B., M.O.A., D.T., K.O.-K., D.I., G.F.D., M.S. and B.L. (Bailah Leigh) were involved in the malaria study conduct and clinical care of participants. Y.N. was the senior data manager and performed the data linkage for the retrieval of serum samples for the Luminex analysis. D.W.-J. was the lead scientist for the program (EBOVAC1) at the London School of Hygiene & Tropical Medicine. C.R. was the lead scientist for the program at Janssen Vaccines and Prevention. B.L. (Bailah Leigh) was the study principal investigator in Sierra Leone. All authors have read and agreed to the published version of the manuscript.

Funding: This project has received funding from the Innovative Medicines Initiative (IMI) 2 Joint Undertaking under grant agreement No 115854. This Joint Undertaking received support from the European Union's Horizon 2020 research and innovation program and the European Federation of Pharmaceutical Industries and Association.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Sierra Leone Ethics and Scientific Review Committee and the London School of Hygiene and Tropical Medicine Ethics Committee (reference number: 10538).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Potential participants and their parent/guardian (if the participant's age < 18 years) were given all the details regarding the malaria study, including information that they could withdraw their consent at any moment without affecting their participation in the main EBOVAC-Salone trial.

Data Availability Statement: Following publication, individual deidentified participants' data and a data dictionary will be made available upon request via the London School of Hygiene & Tropical Medicine research data repository, LSHTM Data Compass at http://datacompass.lshtm.ac.uk (accessed on 31 July 2023). Requests with a defined analysis plan can be sent via LSHTM Data Compass.

Acknowledgments: The authors thank the staff and management of Kambia Government Hospital, Kambia District Health Management Team, the College of Medicine and Allied Health Sciences (COMAHS) and the London School of Hygiene and Tropical Medicine (LSHTM) EBOVAC1 project management and administrative teams, the social science team, the community engagement team, and colleagues from World Vision, GOAL, and the Ebola Vaccine Deployment, Acceptance and Compliance (EBODAC) project. The authors thank all study participants and their families.

Conflicts of Interest: A.G., B.K. (Babajide Keshinro) and C.R. are full-time employees of Janssen, Pharmaceutical Companies of Johnson & Johnson. A.G. and C.R. report ownership of shares in Janssen, Pharmaceutical Companies of Johnson & Johnson. All other authors declare funding from the IMI 2 Joint Undertaking. The funders (IMI) had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Centers for Disease Control and Prevention (CDC). What Is Ebola Disease? Available online: https://www.cdc.gov/vhf/ebola/about.html (accessed on 31 July 2023).
2. International Committee on Taxonomy of Viruses. Genus: Ebolavirus. Available online: https://ictv.global/report/chapter/filoviridae/filoviridae/orthoebolavirus (accessed on 25 July 2023).
3. Mehand, M.S.; Al-Shorbaji, F.; Millett, P.; Murgue, B. The WHO R&D Blueprint: 2018 review of emerging infectious diseases requiring urgent research and development efforts. *Antivir. Res.* **2018**, *159*, 63–67. [CrossRef]
4. Centers for Disease Control and Prevention (CDC). Ebola Virus Disease Distribution Map: Cases of Ebola Virus Disease in Africa Since 1976. Available online: https://www.cdc.gov/vhf/ebola/history/distribution-map.html (accessed on 25 July 2023).

5. US Food and Drug Administration. First FDA-Approved Vaccine for the Prevention of Ebola Virus Disease, Marking a Critical Milestone in Public Health Preparedness and Response. Available online: https://www.fda.gov/news-events/press-announcements/first-fda-approved-vaccine-prevention-ebola-virus-disease-marking-critical-milestone-public-health (accessed on 25 July 2023).
6. European Commission. Vaccine against Ebola: Commission Grants First-Ever Market Authorisation. Available online: https://ec.europa.eu/commission/presscorner/detail/en/ip_19_6246 (accessed on 25 July 2023).
7. Merck. ERVEBO® (Ebola Zaire Vaccine, Live) Now Registered in Four African Countries, within 90 Days of Reference Country Approval and WHO Prequalification. Available online: https://www.merck.com/news/ervebo-ebola-zaire-vaccine-live-now-registered-in-four-african-countries-within-90-days-of-reference-country-approval-and-who-prequalification/ (accessed on 25 July 2023).
8. Burki, T. Ebola virus vaccine receives prequalification. *Lancet* **2019**, *394*, 1893. [CrossRef]
9. Johnson & Johnson. Johnson & Johnson Joins World Health Organization in Efforts to Prevent Spread of Ebola in West Africa. 13 May 2021. Available online: https://www.jnj.com/johnson-johnson-joins-world-health-organization-in-efforts-to-prevent-spread-of-ebola-in-west-africa (accessed on 25 July 2023).
10. European Commission. Vaccine against Ebola: Commission Grants New Market Authorisations. Available online: https://ec.europa.eu/commission/presscorner/detail/%20en/ip_20_1248 (accessed on 25 July 2023).
11. Johnson & Johnson. Ebola. Available online: https://healthforhumanityreport.jnj.com/2022/global-health-equity/pandemics-epidemics/ebola.html (accessed on 25 July 2023).
12. World Health Organization. World Malaria Report 2022. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO. Available online: https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2022 (accessed on 25 July 2023).
13. Portugal, S.; Tipton, C.M.; Sohn, H.; Kone, Y.; Wang, J.; Li, S.; Skinner, J.; Virtaneva, K.; Sturdevant, D.E.; Porcella, S.F.; et al. Malaria-associated atypical memory B cells exhibit markedly reduced B cell receptor signaling and effector function. *Elife* **2015**, *4*, e07218. [CrossRef] [PubMed]
14. Usen, S.; Milligan, P.; Ethevenaux, C.; Greenwood, B.; Mulholland, K. Effect of fever on the serum antibody response of Gambian children to Haemophilus influenzae type b conjugate vaccine. *Pediatr. Infect Dis. J.* **2000**, *19*, 444–449. [CrossRef] [PubMed]
15. Bradley-Moore, A.M.; Greenwood, B.M.; Bradley, A.K.; Bartlett, A.; Bidwell, D.E.; Voller, A.; Craske, J.; Kirkwood, B.R.; Gilles, H.M. Malaria chemoprophylaxis with chloroquine in young Nigerian children. II. Effect on the immune response to vaccination. *Ann. Trop Med. Parasitol.* **1985**, *79*, 563–573. [CrossRef] [PubMed]
16. Nakalembe, M.; Banura, C.; Namujju, P.B.; Mirembe, F.M. Immunogenicity to the bivalent HPV-16/18 vaccine among adolescent African students exposed to helminths and malaria. *J. Infect. Dev. Ctries* **2015**, *9*, 197–205. [CrossRef] [PubMed]
17. Mahon, B.E.; Simon, J.; Widdowson, M.A.; Samai, M.; Rogier, E.; Legardy-Williams, J.; Liu, K.; Schiffer, J.; Lange, J.; DeByle, C.; et al. Baseline Asymptomatic Malaria Infection and Immunogenicity of Recombinant Vesicular Stomatitis Virus-Zaire Ebola Virus Envelope Glycoprotein. *J. Infect. Dis.* **2021**, *224*, 1907–1915. [CrossRef] [PubMed]
18. Ishola, D.; The EBOVAC-Salone Malaria Infection Sub-Study Team. Asymptomatic Malaria Infection and the Immune Response to the 2-Dose Ad26.ZEBOV, MVA-BN-Filo Ebola Vaccine Regimen in Adults and Children. *Clin. Infect. Dis.* **2022**, *75*, 1585–1593. [CrossRef] [PubMed]
19. Achan, J.; Reuling, I.J.; Yap, X.Z.; Dabira, E.; Ahmad, A.; Cox, M.; Nwakanma, D.; Tetteh, K.; Wu, L.; Bastiaens, G.J.H.; et al. Serologic Markers of Previous Malaria Exposure and Functional Antibodies Inhibiting Parasite Growth Are Associated with Parasite Kinetics Following a *Plasmodium falciparum* Controlled Human Infection. *Clin. Infect. Dis.* **2020**, *70*, 2544–2552. [CrossRef] [PubMed]
20. Helb, D.A.; Tetteh, K.K.; Felgner, P.L.; Skinner, J.; Hubbard, A.; Arinaitwe, E.; Mayanja-Kizza, H.; Ssewanyana, I.; Kamya, M.R.; Beeson, J.G.; et al. Novel serologic biomarkers provide accurate estimates of recent *Plasmodium falciparum* exposure for individuals and communities. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, E4438–E4447. [CrossRef] [PubMed]
21. Baiden, F.; Fleck, S.; Leigh, B.; Ayieko, P.; Tindanbil, D.; Otieno, T.; Lawal, B.; Tehtor, M.; Rogers, M.; Odeny, L.; et al. Prevalence of malaria and helminth infections in rural communities in northern Sierra Leone, a baseline study to inform Ebola vaccine study protocols. *PLoS ONE* **2022**, *17*, e0270968. [CrossRef]
22. Ishola, D.; Manno, D.; Afolabi, M.O.; Keshinro, B.; Bockstal, V.; Rogers, B.; Owusu-Kyei, K.; Serry-Bangura, A.; Swaray, I.; Lowe, B.; et al. Safety and long-term immunogenicity of the two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in adults in Sierra Leone: A combined open-label, non-randomised stage 1, and a randomised, double-blind, controlled stage 2 trial. *Lancet Infect. Dis.* **2022**, *22*, 97–109. [CrossRef] [PubMed]
23. Manno, D.; Ayieko, P.; Ishola, D.; Afolabi, M.; Rogers, B.; Baiden, F.; Serry-Bangura, A.; Bah, O.; Kohn, B.; Swaray, I.; et al. Ebola Virus Glycoprotein IgG Seroprevalence in Community Previously Affected by Ebola, Sierra Leone. *Emerg Infect. Dis.* **2022**, *28*, 734–738. [CrossRef] [PubMed]
24. Government of Sierra Leone, Ministry of Health and Sanitation, National Malaria Control Programme. Guidelines for Case Management of Malaria. Fourth Edition. 2015. Available online: https://www.afro.who.int/publications/guidelines-case-management-malaria-2015 (accessed on 25 July 2023).

25. Redding, D.W.; Atkinson, P.M.; Cunningham, A.A.; Lo Iacono, G.; Moses, L.M.; Wood, J.L.N.; Jones, K.E. Impacts of environmental and socio-economic factors on emergence and epidemic potential of Ebola in Africa. *Nat. Commun.* **2019**, *10*, 4531. [CrossRef] [PubMed]
26. Henao-Restrepo, A.M.; Longini, I.M.; Egger, M.; Dean, N.E.; Edmunds, W.J.; Camacho, A.; Carroll, M.W.; Doumbia, M.; Draguez, B.; Duraffour, S.; et al. Efficacy and effectiveness of an rVSV-vectored vaccine expressing Ebola surface glycoprotein: Interim results from the Guinea ring vaccination cluster-randomised trial. *Lancet* **2015**, *386*, 857–866. [CrossRef] [PubMed]
27. Henao-Restrepo, A.M.; Camacho, A.; Longini, I.M.; Watson, C.H.; Edmunds, W.J.; Egger, M.; Carroll, M.W.; Dean, N.E.; Diatta, I.; Doumbia, 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 Ca Suffit!). *Lancet* **2017**, *389*, 505–518. [CrossRef]
28. Hennekens, C.; Buring, J.; Mayrent, S.L. *Epidemiology in Medicine*; Lippincott Williams & Wilkins: Philadelphia, PA, USA, 1987; Volume 7, p. 169.

The Effect of Previous Exposure to Malaria Infection and Clinical Malaria Episodes on the Immune Response to the Two-Dose Ad26.ZEBOV, MVA-BN-Filo Ebola Vaccine Regimen

Supplementary Material

Table of Contents

1. SUPPLEMENTARY METHODS....2

1.1. Luminex xMAP Technique....2

1.2. Data Validation of the Categorical Variable of Previous Exposure to Malaria....2

2. SUPPLEMENTARY TABLES....3

Supplementary Table S1....3

Supplementary Table S2....4

Supplementary Table S3....5

Supplementary Table S4....6

Supplementary Table S5....7

Supplementary Table S6....8

Reference....8

1. SUPPLEMENTARY METHODS

1.1. Luminex xMAP Technique

The Luminex xMAP technique (Luminex Corp, Austin TX) offers the advantage of simultaneously detecting and quantifying antibodies to multiple antigens. Colour-coded beads are coated with malaria antigens (each bead colour is coated with a different antigen) before the sample (serum, plasma, etc.) is added. If the sample contains a specific antibody against a malaria antigen coating the beads, the antibody binds to the antigen and forms an antigen-antibody complex. A secondary fluorescent antibody is then added, which binds the fragment crystallizable region (Fc region) of the sample antibody in each antigen-antibody complex. A MAGPIX analyser aspirates and transports the beads into its imaging chamber, exposing them to LED lights. These excite the fluorescent molecule attached to the antibody-antigen complexes leading to the emission of fluorescent light whose intensity is directly proportionate to the amount of antibody-antigen binding. The machine detects the fluorescence and sorts the data for each antigen according to the colour code of the bead. The analyser counts every bead for each antigen per sample and takes a median value. The final reading is the Median Fluorescent Intensity (MFI).

1.2. Data Validation of the Categorical Variable of Previous Exposure to Malaria

In a previous study by Achan et al. [1], which employed the Luminex MAGPIX platform, antibody responses to the following six *Plasmodium falciparum* (*P. falciparum*) antigens were considered the most appropriate to determine exposure to malaria infection: apical membrane antigen 1 (AMA-1), merozoite surface protein 1.19 (MSP-1.19), and glutamate-rich protein (GLURP.R2) reflecting long-term exposure to malaria; reticulocyte-binding protein homologue (Rh2.2030), gametocyte exported protein (GEXP18), and early transcribed membrane protein (Etramp5.Ag1) reflecting recent exposure to malaria (i.e., infection in the past ~9 months). Antibody responses to each antigen are expressed as MFI (see above 1.1 Luminex xMAP technique).

To check if the categories of previous exposure to malaria, which were obtained by combining the MFI to the six *P. falciparum* antigens, adequately summarised the MFI of each antigen, we evaluated how the MFI for each antigen varied according to the categories of previous exposure to malaria.

The mean and the median MFI for each *P. falciparum* antigen increased consistently with increasing age-adjusted categories of previous exposure to malaria, being lower in the low-exposure group, intermediate in the intermediate-exposure group and higher in the high-exposure group (Supplementary Table S6). This showed that the previous exposure to malaria categorisation was able to adequately summarise the immune response to each of the six *P. falciparum* antigens, as expected.

2. SUPPLEMENTARY TABLES

Supplementary Table S1. Sociodemographic characteristics of the malaria study participants.

Characteristic		n (%) N=587
Age cohort	1-3 years	125 (21)
	4-11 years	133 (23)
	12-17 years	141 (24)
	≥ 18 years	188 (32)
Sex	Male	368 (63)
	Female	219 (37)
Ethnicity	Themne	405 (69)
	Limba	71 (12)
	Soso	50 (9)
	Mende	24 (4)
	Fula	14 (2)
	Other ethnicities	23 (4)
Religion	Muslim	484 (82)
	Christian	100 (17)
	None or not stated	3 (1)
Level of Education	No formal education	160 (27)
	Primary (1-6 grades)	200 (34)
	Secondary/High School	213 (36)
	Tertiary level	14 (2)

Supplementary Table S2. Ebola Virus (EBOV) Glycoprotein (GP)-specific binding antibody geometric mean concentrations (GMCs) post dose 1 (measured on Day 57) by categories of previous exposure to malaria, based on participants' serologic response to a panel of *Plasmodium falciparum (P. falciparum)* long-term exposure antigens [1] at the screening visit, overall and by age cohort.

Long-term Exposure to Malaria at Screening	**N (%)**	**Post-Dose 1 EBOV GP-Specific Binding Antibody GMC, EU/mL**	**GMR [2] (95% CI)**	***p***
All participants [3]	N=474			
Low	177 (37.3)	344 (295-401)	1	0.39
Intermediate	180 (38.0)	325 (283-373)	0.93 (0.77-1.14)	
High	117 (24.7)	418 (347-504)	1.09 (0.86-1.37)	
By age group				
1-3 years	N=96			
Low	37 (38.5)	852 (646-1123)	1	0.51
Intermediate	28 (29.2)	655 (518-828)	0.79 (0.55-1.14)	
High	31 (32.3)	733 (516-1041)	0.87 (0.55-1.37)	
4-11 years	N=116			
Low	45 (38.8)	300 (228-395)	1	0.18
Intermediate	42 (36.2)	428 (334-547)	1.39 (0.96-2.00)	
High	29 (25.0)	452 (320-637)	1.13 (0.76-1.67)	
12-17 years	N=115			
Low	43 (37.4)	367 (279-482)	1	0.67
Intermediate	44 (38.3)	297 (226-390)	0.87 (0.60-1.25)	
High	28 (24.3)	318 (226-448)	0.89 (0.61-1.29)	
≥ 18 years	N=147			
Low	52 (35.4)	192 (149-249)	1	0.83
Intermediate	66 (44.9)	215 (172-269)	0.96 (0.72-1.27)	
High	29 (19.7)	276 (192-397)	1.06 (0.76-1.48)	

[1]Apical membrane antigen 1 (AMA-1), merozoite surface protein 1.19 (MSP-1.19) and glutamate-rich protein (GLURP.R2). [2]Adjusted for baseline EBOV GP-specific antibody concentrations. [3]Categories of previous exposure to malaria are age-adjusted. GMR=geometric mean ratio.

Supplementary Table S3. EBOV GP-specific binding antibody GMCs post-dose 2 (measured on Day 78) by categories of previous exposure to malaria, based on participants' serologic response to a panel of *P. falciparum* long-term exposure antigens [1] at the screening visit, overall and by age cohort.

Long-Term Exposure to Malaria at Screening	**N (%)**	**Post-Dose 2 EBOV GP-Specific Binding Antibody GMC, EU/mL**	**GMR [2] (95% CI)**	***p***
All participants [3]	N=466			
Low	176 (37.8)	7908 (6571- 9516)	1	0.50
Intermediate	174 (37.3)	8673 (7176-10482)	1.15 (0.88-1.49)	
High	116 (24.9)	9212 (7206-11777)	1.20 (0.88-1.63)	
By age group				
1-3 years	N=96			
Low	37 (38.5)	22596 (17121-29823)	1	0.98
Intermediate	28 (29.2)	22762 (16540-31324)	1.06 (0.66-1.70)	
High	31 (32.3)	21404 (11677-39233)	1.00 (0.56-1.77)	
4-11 years	N=115			
Low	45 (39.1)	9000 (6463-12532)	1	0.06
Intermediate	41 (35.7)	13835 (9935-19266)	1.64 (1.03-2.62)	
High	29 (25.2)	7601 (5100-11329)	0.95 (0.54-1.65)	
12-17 years	N=112			
Low	43 (38.4)	8848 (5960-13137)	1	0.49
Intermediate	42 (37.5)	11725 (8329-16506)	1.32 (0.78-2.23)	
High	27 (24.1)	9359 (6624-13223)	1.04 (0.62-1.74)	
≥ 18 years	N=143			
Low	51 (35.7)	2996 (2413-3720)	1	0.40
Intermediate	63 (44.0)	3409 (2623-4431)	1.15 (0.81-1.64)	
High	29 (20.3)	4468 (3105-6428)	1.34 (0.89-2.02)	

[1]Apical membrane antigen 1 (AMA-1), merozoite surface protein 1.19 (MSP-1.19) and glutamate-rich protein (GLURP.R2). [2]Adjusted for baseline EBOV GP-specific antibody concentrations. [3]Categories of previous exposure to malaria are age-adjusted.

Supplementary Table S4. EBOV GP-specific binding antibody GMCs post dose 1 (measured on Day 57) by categories of previous exposure to malaria, based on participants' serologic response to a panel of *P. falciparum* recent exposure antigens [1] at the screening visit, overall and by age cohort.

Recent Exposure to Malaria at Screening	**N (%)**	**Post-dose 1 EBOV GP-Specific Binding Antibody GMC, EU/mL**	**GMR [2] (95% CI)**	***p***
All participants [3]	N=474			
Low	170 (35.9)	364 (313-424)	1	0.41
Intermediate	187 (39.4)	335 (290-388)	0.88 (0.72-1.07)	
High	117 (24.7)	367 (307-438)	0.97 (0.78-1.22)	
By age group				
1-3 years	N=96			
Low	42 (43.8)	741 (584-940)	1	0.96
Intermediate	24 (25.0)	771 (573-1038)	0.96 (0.65-1.42)	
High	30 (31.2)	750 (521-1081)	0.95 (0.61-1.49)	
4-11 years	N=116			
Low	34 (29.3)	344 (243-487)	1	0.66
Intermediate	53 (45.7)	411 (331-511)	0.99 (0.66-1.48)	
High	29 (25.0)	361 (255-511)	0.84 (0.53-1.34)	
12-17 years	N=115			
Low	43 (37.4)	340 (255-452)	1	0.88
Intermediate	43 (37.4)	304 (227-408)	0.92 (0.64-1.32)	
High	29 (25.2)	344 (261-452)	0.94 (0.66-1.36)	
≥ 18 years	N=147			
Low	51 (34.7)	224 (176-286)	1	0.71
Intermediate	67 (45.6)	225 (173-293)	1.05 (0.78-1.42)	
High	29 (19.7)	190 (151-238)	0.92 (0.68-1.24)	

[1]Reticulocyte-binding protein homologue (Rh2.2030), gametocyte exported protein (GEXP18) and early transcribed membrane protein (Etramp5.Ag1). [2]Adjusted for baseline EBOV GP-specific antibody concentrations. [3] Categories of previous exposure to malaria are age-adjusted.

Supplementary Table S5. EBOV GP-specific binding antibody GMCs post-dose 2 (measured on Day 78) by categories of previous exposure to malaria, based on participants' serologic response to a panel of *P. falciparum* recent exposure antigens [1] at the screening visit, overall and by age cohort.

Recent Exposure to Malaria at Screening	**N (%)**	**Post-dose 2 EBOV GP-Specific Binding Antibody GMC, EU/mL**	**GMR [2] (95% CI)**	***p***
All participants [3]	N=466			
Low	169 (36.3)	9003 (7345- 11037)	1	0.91
Intermediate	181 (38.8)	8174 (6836-9774)	0.94 (0.71-1.23)	
High	116 (24.9)	8318 (6587-10502)	0.98 (0.73-1.33)	
By age group				
1-3 years	N=96			
Low	42 (43.8)	23257 (17613-30709)	1	0.86
Intermediate	24 (25.0)	25239 (17305-36810)	1.04 (0.62-1.76)	
High	30 (31.2)	18911 (10462-34184)	0.89 (0.50-1.58)	
4-11 years	N=115			
Low	34 (29.6)	12534 (8378-18750)	1	0.28
Intermediate	52 (45.2)	10321 (7740-13763)	0.87 (0.53-1.43)	
High	29 (25.2)	7405 (4932-11118)	0.64 (0.36-1.14)	
12-17 years	N=112			
Low	43 (38.4)	9216 (5804-14636)	1	0.50
Intermediate	40 (35.7)	11757 (9006-15348)	1.28 (0.74-2.19)	
High	29 (25.9)	8914 (6619-12005)	0.95 (0.55-1.64)	
≥ 18 years	N=143			
Low	50 (35.0)	3175 (2543-3964)	1	0.66
Intermediate	65 (45.4)	3577 (2710-4721)	1.12 (0.79-1.59)	
High	28 (19.6)	3622 (2705-4850)	1.20 (0.83-1.74)	

[1]Reticulocyte-binding protein homologue (Rh2.2030), gametocyte exported protein (GEXP18) and early transcribed membrane protein (Etramp5.Ag1). [2]Adjusted for baseline EBOV GP-specific antibody concentrations. [3] Categories of previous exposure to malaria are age-adjusted.

Supplementary Table S6. Serologic responses (Median Fluorescent Intensity) to *Plasmodium falciparum* antigens indicative of long-term and recent exposure to malaria infection, by category of previous exposure to malaria.

***Plasmodium falciparum* Antigens** [1]		**Serologic Responses (Median Fluorescent Intensity** [2]**)**		
		Low-Exposure to Malaria N=144	**Intermediate-Exposure to Malaria** N=213	**High-Exposure to Malaria** N=117
Long-term exposure				
AMA-1	mean (SD)	15654.6 (11143.0)	24064.1 (8768.3)	26863.4 (7682.4)
	median (IQR)	16314.5 (2845.5-25930.5)	27130.0 (22271.0- 29568.0)	29028.0 (25181.0- 31246.0)
MSP1.19	mean (SD)	6712.3 (7101.6)	17905.1 (9567.8)	23848.1 (8592.5)
	median (IQR)	4397.0 (1280.5-9883.3)	18811.0 (8932.5-26674.0)	26976.5 (18627.0-30274.0)
GLURP.R2	mean (SD)	13402.2 (14660.0)	27037.2 (15529.3)	30453.1 (14693.6)
	median (IQR)	5888.0 (452.3-27124.5)	33506.5 (10232.0-39559.5)	36470.0 (19701.0-41219.0)
Recent exposure				
Rh2.2030	mean (SD)	7327.7 (7693.1)	15210.4 (9646.5)	19892.2 (10622.2)
	median (IQR)	4249.0 (966.0-12496.8)	15339.0 (6800.0-23962.0)	22729.0 (10209.0-29284.0)
GEXP18	mean (SD)	1401.2 (1050.3)	3343.5 (2327.0)	5204.7 (3291.0)
	median (IQR)	1100.5 (614.0-1863.8)	2624.0 (1695.0-4490.0)	4895.5 (2708.0-6875.5)
Etramp5.Ag1	mean (SD)	2995.2 (5489.7)	9624.7 (9362.1)	15985.4 (11034.0)
	median (IQR)	1475.3 (745.5-2957.0)	6192.0 (2536.0.5-13036.5)	13187.0 (7101.0-23062.0)

[1]Long-term exposure antigens: apical membrane antigen 1 (AMA-1), merozoite surface protein 1.19 (MSP-1.19) and glutamate-rich protein (GLURP.R2). Recent exposure (malaria infection in the past ~9 months): reticulocyte-binding protein homologue (Rh2.2030), gametocyte exported protein (GEXP18) and early transcribed membrane protein (Etramp5.Ag1). [2]Median Fluorescent Intensity is the final reading of the Luminex analysis (see 1.1. Luminex xMAP technique in Supplementary methods). SD = standard deviation. IQR = interquartile range.

Reference

1. Achan, J.; Reuling, I.J.; Yap, X.Z.; Dabira, E.; Ahmad, A.; Cox, M.; Nwakanma, D.; Tetteh, K.; Wu, L.; Bastiaens, G.J.H.; et al. Serologic Markers of Previous Malaria Exposure and Functional Antibodies Inhibiting Parasite Growth Are Associated With Parasite Kinetics Following a Plasmodium falciparum Controlled Human Infection. Clin. Infect. Dis. 2020, 70, 2544–2552. https://doi.org/10.1093/cid/ciz740.

APPENDIX 1 – Studies reporting immunogenicity and safety data of vaccines against Ebola disease, reported by vaccine and study type

a. Vaccines licensed in more than one country and WHO-prequalified

rVSV-ZEBOV, single dose or 2-dose homologous regimen						
Phase 1-2 clinical trials						
Study	**Registration**	**Design**	**Comparisons / vaccine dose**	**Participants**	**Sites**	**Key results**
Huttner et al. 2015[33]	NCT02287480	Randomised, double-blind, placebo-controlled trial	Randomisation to a vaccine injection at dose of 10 or 50 million plaque-forming units (PFU) vs placebo and 300,000 PFU (low dose) vs placebo	**142** adults (aged 18-65 years)	Geneva (Switzerland)	No SAEs reported. Dose reduction from 10-50 million to 300,000 PFU decreased occurrence and magnitude of viraemia, monocyte activation, and early reactogenicity but lowered antibody responses and did not prevent vaccine-induced arthritis, dermatitis, or vasculitis.
Agnandji et al. 2016[34]	NCT02283099 NCT02287480 NCT02296983 PACTR201411000919191	3 non-randomised, open-label, dose-escalation trials 1 randomised, double-blind, placebo-controlled trial	Dose-escalation trials: one vaccine injection at doses from 300,000 to 20 million PFU. RCT: one vaccine injection at dose of 10 or 50 million PFU compared to placebo	**158** adults (aged 18-55 years)	Hamburg (Germany), Geneva (Switzerland), Kilifi (Kenya), Lambaréné (Gabon)	No vaccine-related SAEs. Mild-to-moderate, early-onset reactogenicity of short duration. Fever observed in up to 30% of vaccinated participants. Two weeks post vaccination: arthritis observed in 22% of participants in Geneva and 3% in Hamburg and Kilifi. EBOV-GP-specific antibody responses detected in all the participants, with similar GP-binding antibody titres but significantly higher neutralising antibody titres at higher doses. GP-binding antibodies sustained for 180 days in all participants.
Agnandji et al. 2017[35]	PACTR201411000919191	Randomised, open-label, dose-escalation trial	Participants randomised to one injection of vaccine at doses, from 3000 to 20 million PFU in adults and 20 million in children	**115** adults (aged 18-50 years) **40** children (aged 6-17 years)	Lambaréné (Gabon)	Acceptable safety and immunogenicity profile of the 20 million PFU dose in adults. Higher vaccine replication in children, leading to shedding of the vaccine in saliva and urine. Authors stated that lower vaccine doses may be needed in paediatric populations.
ElSherif et al. 2017[36]	NCT02374385	Randomised, observer-blind, placebo-controlled, dose-ranging trial	Participants randomised to one injection of vaccine at either 100,000 or 500,000 or 3 million PFU vs placebo	**40** adults (aged 18-65 years)	Halifax (Canada)	Solicited AEs mostly mild to moderate and self-limited. No vaccine-related SAEs. Viremia following vaccination no longer detectable after day 3, with no virus shedding in saliva or urine. The vaccine elicited GP-binding antibodies in recipients of all 3 doses. Antibody titres persisted up to 180 days.
Heppner et al. 2017[37]	NCT02314923	Randomised, double-blind, placebo-controlled, dose-response trial	Cohort 1, randomisation to one injection of vaccine at doses of either 3000, 30,000, 300,000 or 3 million PFU vs placebo. Cohort 2, randomisation to doses of 3 million, 9 million, 20 million or 100 million PFU vs placebo.	**513** adults (aged 18-61 years)	USA (8 sites)	Most AEs were early-onset, mild to moderate, of short duration, and more frequent at high vaccine doses (≥9 million PFU). At 20 million PFU, the most common AEs were local pain and tenderness, headache, fatigue, myalgia, shivering or chills, fever and joint aches and pain. Self-limited, post-vaccination arthritis occurred in 5% of vaccinees. Antibody responses observed in most participants by day 14. On day 28, at the 20 million PFU dose, binding and neutralising antibody seroconversion in 95.7% of participants. Immunological responses sustained for 1 year.

rVSV-ZEBOV, single dose or 2-dose homologous regimen						
Phase 1-2 clinical trials (continued)						
Study	**Registration**	**Design**	**Comparisons / vaccine dose**	**Participants**	**Sites**	**Key results**
Regules et al. 2017[38]	NCT02269423 (single dose regimen) NCT02280408 (2-dose regimen)	Randomised, double-blind, placebo-controlled, dose-escalation trials	Participants randomised to one injection of vaccine at doses of 3 million, 20 million, or 100 million PFU or placebo. A second identical dose administered 28 days after the first, in one site.	**78** adults	Washington, USA (2 sites)	Most common AEs: injection-site pain, fatigue, myalgia, and headache. Transient viremia noted in all the vaccine recipients after dose 1. Number of AEs and viremia were lower after dose 2 than after dose 1. By day 28, all vaccine recipients had seroconversion with titres of binding and neutralising antibodies higher in the groups that received 20 or 100 million PFU than in the group that received 3 million PFU. Dose 2 significantly increased antibody titres at day 56, but the effect was diminished at 6 months.
Huttner et al. 2018[39]	NCT02287480 NCT02933931 NCT02296983 PACTR201411000919191	Cohort of adults vaccinated in phase 1 trials	Participants vaccinated with one dose of rVSV-ZEBOV at doses of 300,000 PFU (low dose) or 10-50 million PFU (high dose)	**217** adults	Geneva (Switzerland), Kilifi (Kenya), Lambaréné (Gabon)	In the Geneva group, the percentage of responders at 2 years was higher in high dose compared to low dose recipients (100% vs 89%, p=0.04). In high dose recipients, EBOV-GP IgG GMC decreased from peak (at 1-3 months) to 6 months in Geneva (p<0.01) and Lambaréné (p=0.03) but not in Kilifi (p=0.58) and remained stable apart from the Geneva group, where GMC in high dose recipients increased from 6 months to 1 year (p=0.03). In the Geneva group, binding antibodies decreased between 1 and 2 years (p<0.01). High-dose was associated with higher GMCs after 6 months (Geneva p=0.01; Lambaréné p=0.01). nAbs seropositivity decreased from 64-71% at 28 days to 27-31% at 6 months in the Geneva group.
Ehrhardt et al. 2019[40]	NCT02283099	Cohort of participants vaccinated in a phase 1 trial	Participants vaccinated with one dose of rVSV-ZEBOV at doses of 300,000, 3 million or 20 million PFU.	**7** adults	Cologne (Germany)	Polyclonal B cell responses observed in 4 vaccinated participants. EBOV-targeting antibodies cross-reacted with other ebolaviruses and had overlapping target epitopes with antibodies from EVD survivors. In all vaccinees, potent EBOV-neutralising antibodies.
Huttner et al. 2023[97]	NCT02287480 NCT02296983 PACTR201411000919191	Cohort of adults vaccinated in phase 1 trials	Participants vaccinated with one dose of rVSV-ZEBOV at doses of 300,000 PFU or 10-50 million PFU	**168** adults	Geneva (Switzerland), Lambaréné (Gabon)	EBOV-GP antibodies plateaued from 1 year to 5 years in Geneva participants receiving a high dose of vaccine, with similar avidity of EVD convalescents. nAbs decreased from 56% at year 1 to 42% at year 5 in Geneva volunteers, irrespective of vaccine dose.
Phase 3 clinical trials						
Henao-Restrepo et al. 2015[41]	PACTR201503001057193 ('Ebola ca suffit')	Open-label, cluster-randomised ring vaccination trial	Clusters randomisation to immediate vaccination with rVSV-ZEBOV (one dose of 20 million PFU) or delayed vaccination (21 days after randomisation).	**7651** adults	Guinea	In the immediate vaccination group, no cases of EVD with symptom onset at least 10 days after randomisation, vs 16 cases of EVD in the delayed vaccination group (VE 100%, 95% CI 74.7-100.0; p=0.004). No new cases of EVD diagnosed in vaccinees from the immediate or delayed groups from 6 days post vaccination. 43 SAEs reported with one considered causally related to vaccination (a febrile episode with no sequelae).

rVSV-ZEBOV, single dose or 2-dose homologous regimen						
Phase 3 studies (continued)						
Study	**Registration**	**Design**	**Comparisons / vaccine dose**	**Participants**	**Sites**	**Key results**
Henao-Restrepo et al. 2017[42]	PACTR201503001057193 ('Ebola ca suffit')	Open-label, cluster-randomised ring vaccination trial	Same as Henao-Restrepo et al. 2015. After an independent data and safety monitoring board advice, randomisation was stopped and immediate vaccination was offered to all adults and children (age 6-17 years) to all identified rings.	**11,841** adults and children (aged 6-17 years)	Guinea and Sierra Leone	No EVD cases ≥10 days after randomisation among randomly assigned contacts and contacts of contacts vaccinated in immediate clusters vs 16 cases among all eligible individuals in delayed clusters. VE=100% (95% CI 68.9-100.0, p=0.005). Results from all clusters (randomised and non-randomised): no EVD cases ≥10 days after randomisation among all immediately vaccinated vs 23 cases among all eligible contacts and contacts of contacts in delayed clusters plus all eligible contacts and contacts of contacts never vaccinated in immediate clusters. VE in all clusters = 100% (95% CI 79.3-100.0, p=0.003).
Halperin et al. 2017[43]	NCT02503202	Randomised, double-blind, placebo-controlled, lot-to-lot consistency trial	Participants randomised to one injection of vaccine of 1 of 3 lots at a dose of 20 million PFU (combined-lots group), a single high-dose lot (100 million PFU (high-dose group), or placebo.	**1197** adults (aged 18-65 years)	USA (40 sites) Spain (1 site) Canada (1 site)	Vaccine well tolerated. Fever (≥38.0°C) observed in 20.2% (3.2% with ≥39.0°C) in the lot consistency groups, 32.2% in the high-dose (4.3% with ≥39.0°C), and 0.8% in the placebo group (0.8% with ≥39.0°C). Arthralgia reported in 17.1% combined lots, 20.4% high-dose, 3.0% placebo. Arthritis in 5.1% combined lots, 4.2% high-dose, 0.0% placebo. Rash reported in 3.8% combined lots, 3.8% high-dose and 1.5% placebo. No vaccine-related SAEs.
Kennedy et al. 2017[51]	NCT02344407 (PREVAIL I)	Randomised, double-blind, placebo-controlled trial	Participants randomised to one injection of rVSV-ZEBOV (1 ml, 20 million PFU/ml), or 1 ml of placebo, or ChAd3-EBO-Z (2 ml, 100 billion pu/ml) or 2 ml of placebo.	**1500** adults	Liberia	AEs more frequent after active vaccines than placebo at 7 days: injection-site reactions (30.9% in rVSV-ZEBOV group, 28.5% in ChAd3-EBO-Z group vs 6.8% in placebo group), headache (31.9% and 25.1% vs 16.9%), muscle pain (26.9%, 22.3% vs 13.3%), feverishness (30.5%, 23.9% vs 9.0%), and fatigue (15.4%, 14.0% vs 8.8%) (p<0.001 for all comparisons). No evidence of a difference in frequency of SAEs at 30 days between vaccines vs placebo. At 1 month, antibody responses in 83.7% in rVSV-ZEBOV group, 70.8% in ChAd3-EBO-Z group, vs 2.8% in placebo group (p<0.001 for both comparisons). At 12 months, antibody responses in 79.5% in rVSV-ZEBOV group, 63.5% in ChAd3-EBO-Z group, vs 6.8% in placebo group (p<0.001 for both comparisons).
Samai et al. 2018[44]	NCT02378753 PACTR201502001037220 (STRIVE)	Randomised, open-label, controlled trial with phased vaccine introduction	Randomised to immediate (within 7 days of enrolment) or deferred (18-24 weeks after enrolment) vaccination with one injection of vaccine (20 million PFU)	**8651** adults (HCWs and FLWs)	Sierra Leone (7 sites)	AEs at 7 days more frequent in vaccinated than unvaccinated participants: fever >38°C (20.5% vs 3.9%), headache (71.2% vs 22.1%), fatigue (50.7% vs 10.4%), and joint pain (31.7% vs 6.5%). Vaccinated participants more commonly reported joint pain (17.0% vs 4.8%) and rash (7.8% vs 1.7%) from 5 to 28 days; skin vesicles (2.0% vs 0%) and mouth ulcers (2.0% vs 0%) from 8 to 14 days. 1.5% of participants reported SAEs, none considered vaccine-related.
Bolay et al. 2019[45]	NCT02344407 (PREVAIL I)	Non-randomised, open label, ring vaccination trial	All rings immediately vaccinated with one dose of rVSV-ZEBOV (20 million PFU)	**210** adults and children (aged ≥6 years)	Liberia	No SAEs reported. Among participants without an elevated antibody level at baseline, 77.3% had an antibody response at 1 month.

rVSV-ZEBOV, single dose or 2-dose homologous regimen						
Phase 3 studies (continued)						
Study	**Registration**	**Design**	**Comparisons / vaccine dose**	**Participants**	**Sites**	**Key results**
Halperin et al. 2019[46]	NCT02503202	Randomised, double-blind, placebo-controlled, lot-to-lot consistency trial	Participants randomised to one injection of vaccine of 1 of 3 lots at a dose of 20 million PFU (combined-lots group), a single high-dose lot (100 million PFU (high-dose group), or placebo.	**1197** adults (aged 18-65 years)	USA (40 sites), Spain (1 site), Canada (1 site)	EBOV-GP binding antibody GMTs increased in all rVSV-ZEBOV groups by 28 days (>58-fold) and persisted through 24 months. The 3 lots had equivalent immunogenicity. nAbs GMTs increased by 28 days in all rVSV-ZEBOV groups, peaking at 18 months with no decrease through 24 months. At 28 days, ≥94% of vaccine recipients sero-responded (EBOV-GP ELISA, ≥2-fold increase and titre ≥200 Eu/mL), with responses persisting at 24 months in ≥91%.
Juan-Giner et al. 2019[47]	PACTR201503001057193 ('Ebola ca suffit' FLWs)	Non-randomised, open-label, controlled trial (sub-study of the ring vaccination trial)	Participants who agreed to vaccination received one injection of vaccine (20 million PFU). Participants who refused vaccination were offered to participate as control group.	**2115** adults (FLWs)	Conakry (Guinea)	Over 70% of participants reported at least one AE at 3 days post vaccination. The most frequently reported symptoms were headache, fatigue, arthralgia, myalgia. Fever was reported by 15% of participants that completed fever diaries. A total of 8 SAEs were reported during follow-up, 2 of those related to pregnancy (1 miscarriage and 1 stillbirth).
Boum et al. 2020[48]	PACTR201503001057193 ('Ebola ca suffit' FLWs)	Same as Juan-Giner et al. 2019 (row above)	Same as Juan-Giner et al. 2019 (row above)	**1172** adults (FLWs)	Conakry (Guinea)	One dose of vaccine highly immunogenic at 28- and 180-days post vaccination. Vaccine antibody response in 86.4% at 28 days post vaccination. Among those, 90.7% still seropositive at 180 days. Significant correlation between binding and neutralising antibodies at 28 days post vaccination. Among samples analysed for cellular response, responses against the EBOV-GP detected in 10 (13.5%) at day 28 and 27 (48.2%) at day 180.
Legardy-Williams et al. 2020[49]	NCT02378753 PACTR201502001037220 (STRIVE)	Randomised, open-label, controlled trial with phased vaccine introduction	Randomised to immediate (within 7 days of enrolment) or deferred (18-24 weeks after enrolment) vaccination with one injection of vaccine (20 million PFU)	**84** adults (pregnant women)	Sierra Leone (7 sites)	Among immediate vaccinated women, 45% (14/31) reported pregnancy loss, compared with 33% (11/33) of unvaccinated women with contemporaneous pregnancies (relative risk 1.35, 95% CI 0.73-2.52; p=0.34). No congenital anomalies detected among 44 live-born infants examined.
PREVAC Study Team 2022[70]	NCT02876328 PACTR201712002760250 (PREVAC)	Randomised, double-blind, controlled trial	Randomised to placebo or rVSV-ZEBOV (94 million PFU, rVSV group) or rVSV-ZEBOV, with a rVSV-ZEBOV booster 56 days later (rVSV-booster group) or Ad26.ZEBOV (50 billion vp) followed by MVA-BN-Filo (100 million Inf.U.) after 56 days (Ad26-MVA group).	**1400** adults and **1401** children (aged 1-17 years)	Guinea, Liberia, Mali, Sierra Leone	Among both adults and children, incidence of injection-site reactions and symptoms (e.g., feverishness and headache) was higher in the week after receipt of an active vaccine than after placebo but not at later time points. AEs were mainly low-grade. At month 12, 41% of adults and 78% of children had a response in the Ad26-MVA group; 76% and 87% had a response in the rVSV group; 81% and 93% had a response in the rVSV-booster group; and 3% and 4% had a response in the placebo group (p<0.001 for all comparisons of vaccine with placebo). In both adults and children, antibody responses after active vaccine differed from those with placebo beginning on day 14.

rVSV-ZEBOV, single dose or 2-dose homologous regimen						
Phase 3 studies (continued)						
Study	**Registration**	**Design**	**Comparisons / vaccine dose**	**Participants**	**Sites**	**Key results**
Simon et al. 2022[50]	NCT02344407 (PREVAIL I) PACTR201503001057193 ('Ebola ca suffit' FLWs) NCT02378753 (STRIVE)	Post-hoc analysis of data from 3 trials	PREVAIL I: rVSV-ZEBOV and ChAd3-EBO-Z vs placebo (Kennedy et al. 2017) FLWs: all received rVSV-ZEBOV (Juan-Giner et al. 2019) STRIVE: phased rVSV-ZEBOV introduction (Samai et al. 2018)	**2199** adults	Guinea, Liberia and Sierra Leone	In the overall pooled population, in all subgroups receiving rVSV-ZEBOV, and in each trial independently, binding and neutralising antibody titres increased from baseline, generally peaking at day 28 and persisting through day 365. Immune responses were greater in women and participants with baseline GP-ELISA ≥200 Eu/mL, but did not differ across age groups.
Other studies						
Gsell et al. 2017[85]	NA	Compassionate use of ring vaccination	All rings immediately vaccinated with one dose of rVSV-ZEBOV (20 million PFU)	**1207** adults and **303** children (aged 6-17 years)	Guinea	No EVD cases among vaccinees. AEs post vaccination reported in 17% children (all mild) and 36% adults (98% mild). Fewer arthralgia events observed in children than in adults (<1% in children vs 7% in adults). No vaccine-related SAEs.
Carnino et al. 2021[86]	NA	Cohort of vaccinated FLWs	All received a single injection of rVSV-ZEBOV (dose >7.2E7 PFU/ml)	**124** adults (FLWs)	Geneva (Switzerland)	Most respondents (96.3%), had at least one early AE, such as injection site pain, fever, fatigue and myalgia. Delayed AEs were reported by 7.2% responders after a median of 11 days; half of them were joint-related AEs. Four SAEs reported: 2 high-grade fever, 1 rash and 1 arthritis. A case of recurrent transient dizziness and fatigue considered vaccine-related.
Hoff et al. 2022[87]	NA	Cohort of EVD exposed receiving vaccination	All received a single injection of rVSV-ZEBOV (20 million PFU)	**608** adolescents and adults (aged 12-82 years)	North Kivu (DRC)	87.2% of participants had an antibody response at 21 days after vaccination, and 95.6% demonstrated antibody persistence at 6 months.
Rupani et al. 2022[88]	NA	Retrospective cohort study	rVSV-ZEBOV vaccinated EVD patients compared to non-vaccinated EVD patients	**403** EVD patients (adults and children)	DRC	25% of patients vaccinated before symptom onset died compared with 63% of unvaccinated patients. Vaccination strongly associated with fewer deaths. Vaccinated had also fewer EVD-associated symptoms, reduced time to clearance of viral load, and reduced length of hospital stay.

Ad26.ZEBOV, MVA-BN-Filo 2-dose heterologous regimen						
Phase 1 studies						
Study	**Registration**	**Design**	**Comparisons / vaccine dose**	**Participants**	**Sites**	**Key results**
Milligan et al. 2016[59]	NCT02313077 (EBL1001)	Randomised, observer-blind, placebo-controlled trial	4 groups randomised to placebo or Ad26.ZEBOV (50 billion vp) or MVA-BN-Filo (100 million median tissue culture infective dose [TCID50]) (dose 1) and the alternative vaccine (dose 2), with 28 or 56-day intervals. One open-label group received Ad26.ZEBOV, MVA-BN-Filo with a 14-day interval.	**87** adults	Oxford (UK)	No vaccine-related SAEs. No febrile participants after MVA-BN-Filo vs 5% after Ad26.ZEBOV in the randomised groups and 27% in the open-label group. In the randomised groups, 97% of Ad26.ZEBOV recipients and 23% of MVA-BN-Filo recipients had detectable EBOV-GP-specific IgG antibodies 28 days after dose 1. All Ebola vaccine recipients had EBOV-GP-specific IgG antibodies 21 days post dose 2 and at 8-month follow-up. Within randomised groups, at least 86% of vaccine recipients showed Ebola-specific T-cell responses at 7 days post dose 2.
Shukarev et al. 2017[60]	NCT02313077 (EBL1001)	Same as Milligan et al. 2016 (above)	Same as Milligan et al. 2016 (above)	**87** adults	Oxford (UK)	At 8 months, 100% of individuals receiving the Ebola vaccine regimen, maintained Ebola-specific antibodies. The regimen was well-tolerated.
Winslow et al. 2017[61]	NCT02313077 (EBL1001)	Same as Milligan et al. 2016 (above)	Same as Milligan et al. 2016 (above)	**87** adults	Oxford (UK)	All of the active vaccine recipients had binding antibody responses at day 360. T-cell responses observed in at least 60% of vaccinees.
Anywaine et al. 2019[62]	NCT02376400 (EBL1004)	Randomised, observer-blind, placebo-controlled trial	Randomised to placebo or heterologous regimen with Ad26.ZEBOV (50 billion vp) or MVA-BN-Filo (100 million TCID50) as dose 1, followed by MVA-BN-Filo or Ad26.ZEBOV (dose 2), 28 or 56 days later.	**72** adults	Mwanza (Tanzania) Masaka (Uganda)	No vaccine-related SAEs. The most frequent solicited local and systemic AEs: injection site pain (70%, 66% and 42% per dose for MVA-BN-Filo, Ad26.ZEBOV and placebo) and headache (57%, 56%, and 46%). 21 days after dose 2, 100% of active vaccine recipients had EBOV-GP-specific binding antibody responses and 87%-100% had neutralizing antibody responses. Ad26.ZEBOV dose 1 induced higher initial binding antibody and cellular immune responses than MVA-BN-Filo dose 1.
Mutua et al. 2019[63]	NCT02376426 (EBL1003)	Randomised, observer-blind, placebo-controlled trial	Same as Anywaine et al. 2019 (row above)	**72** adults	Nairobi (Kenya)	No vaccine-related SAEs. The most frequent solicited systemic AE: headache (50%, 61%, and 42% per dose for MVA-BN-Filo, Ad26.ZEBOV and placebo). The most frequent solicited local AE: injection site pain (78%, 63%, and 33% per dose for MVA-BN-Filo, Ad26.ZEBOV, and placebo). Binding and neutralising anti-EBOV GP antibodies induced by all regimens and sustained to day 360 after dose 1.
Goldstein et al. 2022[64]	NCT02325050 (EBL1002)	Randomised, observer-blind, placebo-controlled trial	10 groups received placebo or standard (SD) or high dose (HD)* of Ad26.ZEBOV or MVA-BN-Filo in 2-dose regimens at 7-, 14-, 28-, or 56-day intervals; 8 groups received booster with Ad26.ZEBOV or MVA-BN-Filo on day 360.	**164** adults (aged 18-50 years)	Rockville Maryland (USA)	All regimens were well tolerated with no serious vaccine-related adverse events. Heterologous (Ad26.ZEBOV, MVA-BN-Filo or MVA-BN-Filo, Ad26.ZEBOV) and homologous (Ad26.ZEBOV, Ad26.ZEBOV) regimens induced humoral and cellular immune responses 21 days after dose 2; responses were higher after heterologous regimens. MVA-BN-Filo 2-dose homologous regimen was less immunogenic. Booster vaccination elicited anamnestic responses in all participants.
*Ad26.ZEBOV SD: 50 billion vp, HD: 100 billion vp; MVA-BN-Filo SD: 100 million TCID50, HD: 440 million TCID50.						

Ad26.ZEBOV, MVA-BN-Filo 2-dose heterologous regimen						
Phase 2 studies						
Study	**Registration**	**Design**	**Comparisons / vaccine dose**	**Participants**	**Sites**	**Key results**
Barry et al. 2021[65]	NCT02564523 (EBL2002)	Randomised, observer-blind, placebo-controlled trial	Randomised to placebo or Ad26.ZEBOV (50 billion vp) and MVA-BN-Filo (100 million Inf.U.) with 28, 56, or 84-day intervals.	**668** adults (aged 18-70 years) **142** HIV+ adults (aged 18-50 years)	Burkina Faso, Cote d'Ivoire, Kenya, Uganda	Ad26.ZEBOV, MVA-BN-Filo vaccination well tolerated and immunogenic in healthy and HIV-infected African adults. Increasing the interval between doses improved the magnitude of humoral immune responses. Antibody levels persisted to at least 1 year. Ad26.ZEBOV booster vaccination after 1 year was safe and induced an anamnestic response.
Bockstal et al. 2021[83]	NCT02543268 (Study 1, EBL3003); NCT02543567 (Study 2, EBL3002)	Randomised, double-blind, placebo-controlled, parallel-group, multicentre studies	Study 1: randomisation to placebo or different batches of Ad26.ZEBOV (50 billion vp) followed by MVA-BN-Filo (100 million Inf.U.) on day 57. Study 2: randomisation to placebo or various dosages of Ad26.ZEBOV (50, 20 or 8 billion vp) followed by MVA-BN-Filo (100 or 50 million Inf.U.) after 56 days.	**329** (Study 1) and **525** (Study 2) adults (aged 18-50 years)	USA	In Study 1, equivalence demonstrated for 2 of 3 batch comparisons post dose 1 and all 3 batches after dose 2. Study 2 demonstrated a dose-dependent response; however, non-inferiority of lower doses was not met against the full clinical dose. All regimens were well tolerated and immune responses were observed in all participants, regardless of manufacturing process or dose.
Pollard et al. 2021[67]	NCT02416453 (EBL2001)	Randomised, observer-blind, placebo-controlled trial	Participants enrolled to 4 cohorts. Cohorts I-III randomly assigned into 3 parallel groups, receiving Ad26.ZEBOV on day 1, followed by MVA-BN-Filo either 28, 56 or 84 days later. Within these 3 groups, participants in cohort II and III further randomised to either Ad26.ZEBOV or placebo on day 1, followed by either MVA-BN-Filo or placebo on days 28, 56, or 84. Cohort IV randomised to Ad26.ZEBOV or placebo on day 1 for vector shedding.	**423** adults (aged 18-65 years)	France (7 sites) UK (2 sites)	Vaccinations were generally well tolerated. Mild or moderate local AEs (mostly pain) were reported by 62% participants after Ad26.ZEBOV, 58% of participants after MVA-BN-Filo, and 15% after placebo. Systemic AEs (mostly mild or moderate fatigue, headache, or myalgia) were reported by 77% participants after Ad26.ZEBOV, 49% participants after MVA-BN-Filo, and 46% participants receiving placebo. Increasing the interval between vaccinations improved the magnitude of humoral immune responses. Antibody levels persisted to at least 1 year.
Afolabi et al. 2022[68]	NCT02509494 (EBOVAC-Salone)	Randomised, double-blind, controlled trial	Randomised to either Ad26.ZEBOV (50 billion vp) followed by MVA-BN-Filo (100 million Inf.U.) on day 57 (Ebola vaccine group), or a single dose of meningococcal quadrivalent conjugate vaccine (MenACWY) followed by placebo on day 57 (control group).	**576** children (aged 1-17 years)	Kambia district (Sierra Leone)	No vaccine-related SAE. At 7 days post dose 1 and 2, the most common solicited local AE was injection-site pain in all age groups. The most frequently observed solicited systemic AE was headache in the 12-17- and 4-11-years age cohorts and pyrexia in the 1-3 years age cohort. The most frequent unsolicited AE was malaria in all age cohorts. At 21 days post dose 2, EBOV GP-specific binding antibody responses observed in at least 98% of children in the Ebola vaccine group.

Ad26.ZEBOV, MVA-BN-Filo 2-dose heterologous regimen						
Phase 2 studies (continued)						
Study	**Registration**	**Design**	**Comparisons / vaccine dose**	**Participants**	**Sites**	**Key results**
Anywaine et al. 2022[66]	NCT02564523 (EBL2002)	Randomised, observer-blind, placebo-controlled trial	Randomised to placebo or Ad26.ZEBOV (50 billion vp) and MVA-BN-Filo (100 million Inf.U.) with 28, 56, or 84-day intervals.	**263** children (aged 4-17 years)	Burkina Faso, Cote d'Ivoire, Kenya, Uganda	Ebola vaccines were well tolerated with no vaccine-related SAEs. 21 days post-dose 2, binding antibody responses against EBOV GP observed in 100% of vaccinees. GMCs were higher after the 56-day interval than the 28-day interval. Antibody levels persisted to at least 1 year.
Ishola et al. 2022[30]	NCT02509494 (EBOVAC-Salone)	The trial had two stages: an open-label, non-randomised stage 1, and a randomised, double-blind, controlled stage 2.	Stage 1: participants allocated to Ad26.ZEBOV (50 billion vp) on day 1 (dose 1) followed by MVA-BN-Filo (100 million Inf.U.) on day 57 (dose 2), and offered an Ad26.ZEBOV booster 2 years after dose 1. Stage 2: randomisation to either the Ebola vaccine regimen (Ad26.ZEBOV, MVA-BN-Filo) or MenACWY (dose 1) followed by placebo on day 57 (dose 2; control group).	**443** adults (aged ≥18 years)	Kambia district (Sierra Leone)	No vaccine related SAE. Solicited local AEs (mostly mild or moderate injection-site pain) reported in up to 28% of participants after Ad26.ZEBOV, in up to 24% after MVA-BN-Filo, in 17% after MenACWY and in 9% after placebo. Solicited systemic AE (mostly mild or moderate headache, myalgia, fatigue, and arthralgia) reported in up to 54% after Ad26.ZEBOV, in up to 43% after MVA-BN-Filo, in 50% after MenACWY, and in 45% after placebo. The safety profile of the Ad26.ZEBOV booster and Ad26.ZEBOV dose 1 were similar. The Ebola vaccine regimen induced humoral immune responses in 98% of participants at 21 days post dose 2. Booster vaccination induced a strong anamnestic response within 7 days.
PREVAC Study Team 2022[70]	NCT02876328 PACTR201712002760250 (PREVAC)	Randomised, double-blind, controlled trial	Study already reported in the rVSV-ZEBOV section (page 222)	**1400** adults and **1401** children	Guinea, Liberia, Mali, Sierra Leone	Study already reported in the rVSV-ZEBOV section (page 222)
Manno et al. 2023[69]	NCT04711356 (EBOVAC-booster study in children)	Non-randomised, open-label trial	All participants had been previously vaccinated with the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen and received an Ad26.ZEBOV booster (50 billion vp)	**50** children (4-15 years)	Kambia Town (Sierra Leone)	Booster well tolerated. No SAE reported. Most common solicited local and systemic AEs at 7 days: injection site pain (36% of children) and headache (22% of children). Before the booster, 87% of participants still had a binding antibody response after 3.2 years from dose 1 of the Ad26.ZEBOV, MVA-BN-Filo regimen. The booster induced an increase in binding antibody GMC of 44 times at 7 days and of 101 times at 21 days after vaccination compared to pre-booster GMC.
Choi et al. 2023[93]	NCT03929757 (EBL2005) PACTR201905827924069	Randomised, double-blind, controlled trial	Infants randomised (1:1 in a sentinel cohort, 5:2 for the remaining infants) to receive Ad26.ZEBOV (50 billion vp) followed by MVA-BN-Filo (100 million Inf.U.) or two doses of meningococcal quadrivalent conjugate vaccine (control group) administered 56 days apart	**108** infants aged 4-11 months (75 in the Ebola vaccine group and 33 in the control group)	Kambia Town (Sierra Leone) Conakry (Guinea)	Most common solicited local AE: injection-site pain (20% in the Ebola vaccine regimen group and 12% in the control group). Solicited systemic AEs in the Ebola vaccine regimen group: irritability (35%), decreased appetite (24%), pyrexia (21%), and decreased activity (20%). In the control group: irritability (30%), decreased appetite (21%), pyrexia (9%), decreased activity (15%). No SAEs were considered vaccine-related. All the participants in the Ebola vaccine group had an immune response to the vaccine regimen.

Ad26.ZEBOV, MVA-BN-Filo 2-dose heterologous regimen						
Phase 2 studies (continued)						
Study	**Registration**	**Design**	**Comparisons / vaccine dose**	**Participants**	**Sites**	**Key results**
Larivière et al. 2023[94]	NCT04186000 (EBL2007)	Open-label, randomised trial	Participants allocated to Ad26.ZEBOV (50 billion vp) on day 1 (dose 1) followed by MVA-BN-Filo (100 million Inf.U.) on day 57 (dose 2), and randomised to an Ad26.ZEBOV booster at either 1 or 2 years after dose 1.	**699** health care providers and frontline workers	Boende (DRC)	This article presents the study results up to 6 months post dose 2. The vaccine regimen was well tolerated with no vaccine-related SAEs reported. Twenty-one days after dose 2, an EBOV GP-specific binding antibody response was observed in 95.2% of participants.
McLean et al. 2023[96]	NCT02564523 (EBL2002) NCT02509494 (EBOVAC-Salone) NCT02876328 (PREVAC)	See Barry et al. 2021 for EBL2002; Ishola et al. 2022 for EBOVAC-Salone; PREVAC Study Team 2022 for PREVAC	See Barry et al. 2021 for EBL2002; Ishola et al. 2022 for EBOVAC-Salone; PREVAC Study Team 2022 for PREVAC	**841** adults **71** HIV+ adults **1174** children	Burkina Faso, Cote d'Ivoire, Kenya, Uganda and Sierra Leone	Ad26.ZEBOV, MVA-BN-Filo induced a robust humoral immune response, with ≥95% of participants considered vaccine responders at 21 or 28 days post dose 2, irrespective of age and country. At month 12, the percent of responders decreased to 49%-88% among adults and 70%-100% among children.
Other studies						
Nyombayire et al. 2023[84]	NA	Vaccination campaign (UMURINZI)	All planned to receive Ad26.ZEBOV (50 billion vp) followed by MVA-BN-Filo (100 million Inf.U.) with 56 days interval between doses	**216,113** Adults and children (2-17 years of age)	Rwanda	Following dose 1, unsolicited AEs reported by 0.68% vaccinees and more common in younger children (aged 2-8 years, 1.2%) compared with older children (aged 9-17 years, 0.4%) and adults (aged ≥18 years, 0.7%). Fever and headache were the most reported symptoms. 17 SAEs considered related to the Ad26.ZEBOV vaccine occurred in children aged 2-8 years (10 postvaccination febrile convulsions ± gastroenteritis and 7 fever and/or gastroenteritis) within 24 hrs of vaccination. The incidence of febrile seizures was 0.031% prior to initiation of routine acetaminophen and 0.013% thereafter. No deaths considered related to vaccination. 94% of participants returned for their second dose.

b. Vaccines licensed only in the country of manufacture, not WHO-prequalified

rAd5.ZEBOV-GP, single dose regimen						
Phase 1-2 study						
Study	**Registration**	**Design**	**Comparisons / vaccine dose**	**Participants**	**Sites**	**Key results**
Zhu et al. 2015[72]	NCT02326194	Randomised, double-blind, placebo-controlled trial	Participants sequentially enrolled to receive low-dose vaccine (40 billion vp) or placebo (group 1) or high-dose vaccine (160 billion vp) or placebo (group 2)	**120** adults (aged 18-60 years)	China (1 site)	68% of participants reported a solicited adverse reaction (AR) at 7 days. Injection-site pain reported in 35% in the low-dose group and 73% in the high-dose group ($p<0.01$). No evidence of a difference in other ARs and laboratory tests across groups. No SAEs were reported. GP-specific binding antibody responses detected in 93% in low-dose group and 100% in high-dose group at day 14 and in 95% (low-dose) and 100% (high-dose) at day 28. T-cell responses peaked at day 14 and were higher in the high-dose group vs low-dose group.
Li et al. 2017[73]	NCT02326194 (single dose trial) NCT02533791 (booster trial)	Randomised, double-blind, placebo-controlled trial	Single dose trial: same as Zhu et al. 2015 (row above). Booster trial: participants re-recruited to receive a booster dose of the same vaccine in the same dose or placebo, at month 6.	**120** adults (aged 18-60 years)	China (1 site)	Solicited AR more frequent after active vaccine than placebo. Most common ARs: injection site pain and fever. EBOV GP binding antibodies peaked at day 28 after active vaccine, with 100% in the high-dose group and 93% in the low-dose group considered responders. High-dose group showed significantly higher and more sustained titres than the low-dose group during 6-month follow-up. At day 28 after booster, binding antibody GMT increase by 30 times in the low-dose group and 20 times in the high-dose group compared with pre-booster GMT.
Zhu et al. 2017[74]	PACTR201509001259869	Randomised, double-blind, placebo-controlled trial	Participants randomised to high-dose vaccine (160 billion vp), low-dose vaccine (80 billion vp), or placebo.	**500** adults (aged 18-50 years)	Sierra Leone (1 site)	Solicited injection-site AR more frequent in vaccine recipients (26% in high-dose group and 25% in low-dose group) than in placebo group (14%; $p=0.0169$). GP-specific antibody responses detected at day 14 in 96% and 97% of participants in the low-dose and high-dose group, respectively. GMT peaked at day 28, with 96% and 98% of participants considered responders in the low-dose and high-dose group, respectively. GMT declined at day 168.
Wu et al. 2017[75]	NCT02401373	Non-randomised, open-label, dose-escalation trial	Participants sequentially assigned to rAd5.ZEBOV-GP at 80 billion vp and 160 billion vp doses	**61** African adults	China (1 site)	87% participants reported ≥1 AR within 28 days of vaccination. Most common reactions: fever and mild pain at injection site (no difference between different doses). EBOV GP antibodies titres peaking at 28 days after vaccination. GMTs similar between the two dose groups. GP-specific T-cell responses peaked at 14 days post vaccination. Responses were more frequent in high-dose group vs low-dose group (60% vs 10%, $p=0.0014$). Pre-existing Ad5 neutralizing antibodies significantly reduced specific humoral immune response and cellular response.

rVSV-ZEBOV, rAd5.ZEBOV 2-dose heterologous regimens (GamEvac Combi)						
Phase 1-2 study						
Study	**Registration**	**Design**	**Comparisons / vaccine dose**	**Participants**	**Sites**	**Key results**
Dolzhikova et al. 2017[77]	grls. rosminzdrav.ru: No. 495 Zakupki.gov.ru: No. 0373100043215000055	Non-randomised, open-label, dose-escalation trial	Participants assigned to a full dose of rVSV-ZEBOV (25 million PFU) or rAd5.ZEBOV (250 billion) or sequential injections of rVSV-ZEBOV and rAd5.ZEBOV 21 days apart at half or full dose.	**84** adults (aged 18-55 years)	Russia	The most common AE was pain at the injection site. No clinically relevant lab abnormalities or SAEs were reported. EBOV GP-specific antibodies were detected at day 28 in 93% and 100% of volunteers immunised at half and full dose, respectively, and in 100% of volunteers at day 42, irrespective of dose. Antibody titres were higher after full dose than after half dose (p=0.0003) at day 28, but comparable at day 42 (p=0.26), indicating that the antibody titres increase more quickly at full dose. Neutralising antibodies were detected at day 28 in 93% of volunteers receiving the full dose. Antigen-specific response in PBMC was detected in 100% of participants. At day 28, 83% of volunteers receiving half dose and 83% of participants receiving full dose had an EBOV GP-specific CD4+T cell response, while 73% and 59%, respectively, had an EBOV GP-specific CD8+T cell response. At day 42, CD4+T cell response detected in 40% and 76%, while CD8+ T cell response detected in 43% and 62% of volunteers receiving half dose and full dose, respectively.

c. Vaccine candidates with safety and immunogenicity data from phase 1 and 2 studies, not licensed

ChAd3-EBO-Z, single dose regimen or 2-dose heterologous regimen with ChAd3-EBO-Z and MVA-BN Filo or MVA-EBO-Z						
Study	**Registration**	**Design**	**Comparisons / vaccine dose**	**Participants**	**Sites**	**Key results**
De Santis et al. 2016[52]	NCT02289027	Randomised, double-blind, placebo-controlled, dose-finding trial	Participants randomised to one injection of high-dose vaccine (50 billion vp), low-dose vaccine (25 billion vp), or placebo.	**120** adults (aged 18-65 years)	Lausanne (Switzerland)	No vaccine-related SAEs. Local and systemic AEs more frequent in active vaccine groups than in the placebo group, with no substantial differences between high and low doses. Binding antibody responses were detected in 96% of participants in the high-dose group, 96% in the low-dose group, and 5% in the placebo group. Binding antibody concentrations peaked at day 28 and decreased by day 180. 57% of participants given high-dose vaccine and 61% of participants given low-dose vaccine developed EBOV GP-specific CD4 cell responses, and 67% and 69%, respectively, developed CD8 cell responses.
Ewer et al. 2016[56]	NCT02240875	Non-randomised, open-label, dose-finding trial	Participants enrolled in 5 groups. Groups 1, 2 and 3, received one injection of ChAd3-EBO-Z at doses of 10, 25 or 50 billion vp, respectively. One injection of MVA-BN Filo (150 million or 300 million PFU) was given 3-10 weeks after dose 1 to half of the participants in these groups. Groups 4 and 5, received ChAd3-EBO-Z (25 billion vp) followed by MVA-BN Filo (150 million PFU) after 1 or 2 weeks.	**76** adults	Oxford (UK)	No safety issues were identified at any of the dose levels. Four weeks after vaccination with ChAd3-EBO-Z, EBOV-specific binding and neutralising antibody responses were similar to those induced by rVSV-ZEBOV in other studies. MVA-BN-Filo vaccination increased virus-specific antibodies by a factor of 12 and GP-specific CD8+T cells by a factor of 5. Neutralising antibodies were also significantly increased after MVA-BN-Filo vaccination in all 30 participants. Virus-specific antibody responses persisted for 6 months after ChAd3-EBO-Z vaccination and were significantly higher in those who had received also MVA-BN-Filo ($p<0.001$).
Tapia et al. 2016[57]	NCT02231866 (USA) NCT02267109 (Mali)	Randomised, single-blind, dose-escalation trial (USA) Combined open-label and double-blind, dose-escalation trial (Mali)	USA trial: participants randomised to ChAd3-EBO-Z at doses of 10 or 100 billion pu. Mali trial: initially designed to test two doses (25 and 50 billion pu). After a protocol amendment, participants randomised to 25 or 50 billion pu; open-label groups received 10 or 100 billion pu. In a nested study, participants vaccinated with ChAd3-EBO-Z were randomly allocated to dose 2 with MVA-BN-Filo or placebo.	**26** adults (USA) **91** adults (Mali)	Bethesda (USA) Bamako (Mali)	No safety concerns with either vaccine: 8% of participants in Mali (5% received 50 billion and 2% received 100 billion pu of ChAd3-EBO-Z) and 20% of participants in the USA (all received 100 billion pu) had fever lasting for less than 24h, and 56% of Malians receiving MVA-BN-Filo as dose 2 experienced injection-site pain or tenderness.

ChAd3-EBO-Z, single dose regimen or 2-dose heterologous regimen with ChAd3-EBO-Z and MVA-BN Filo or MVA-EBO-Z						
Study	**Registration**	**Design**	**Comparisons / vaccine dose**	**Participants**	**Sites**	**Key results**
Venkatraman et al. 2019[58]	NCT02451891 (phase 1a) NCT02485912 (phase 1b)	Non-randomised, open-label, dose-escalation trial (1a) Randomised, open-label trial (1b)	Phase 1a study: vaccination with single dose of MVA-EBO-Z at 100 or 150 million PFU or 2-dose regimen with ChAd3-EBO-Z (36 billion vp) and MVA-EBO-Z (100 million PFU) with either 7- or 28-days interval. Phase 1b study: randomisation to ChAd3-EBO-Z (36 billion vp) followed by MVA-EBO-Z (100 million PFU), 1 week later, either in the same or contralateral arm.	**80** adults	Oxford and London (UK), Dakar (Senegal)	The standard (28 days) and accelerated (1 week) ChAd3-EBO-Z, MVA-EBO-Z 2-dose heterologous regimens were well-tolerated and elicited robust humoral and cellular immune responses in both the UK and Senegal trials. EBOV-specific antibody titres at 1 week and 6 months after MVA vaccination were lower in Senegalese participants compared to UK participants ($p<0.01$ for both timepoints). Humoral antibody titres in the UK MVA-only group were significantly lower than those in groups that received the 2-dose regimen ($p=0.0048$). No significant differences in humoral immune responses between ipsilateral and contralateral arm groups, but cellular immune responses measured by flow cytometry were significantly greater in vaccinees receiving ChAd3 and MVA vaccines in the same rather than the contralateral arm.
Tapia et al. 2020 (A)[53]	NCT02485301	Randomised, observer-blind, placebo-controlled trial	Participants randomised to ChAd3-EBO-Z (100 billion pu) on day 0 vs placebo on day 0 and ChAd3-EBO-Z at month 6.	**3030** adults (≥18 years)	Cameroon, Mali, Nigeria and Senegal	Most common solicited local AE was pain, reported by 48% after ChAd3-EBO-Z and 8% after placebo; Most common solicited systemic AEs was headache (46% after ChAd3-EBO-Z vs 18% after placebo). Unsolicited AEs reported by 16% after ChAd3-EBO-Z and 16% after placebo. SAEs were reported in 1% of participants in both ChAd3-EBO-Z and placebo/ChAd3-EBO-Z groups; none considered vaccine-related. ChAd3-EBO-Z induced binding antibody response against EBOV GP and polyfunctional EBOV-GP specific CD4+ and CD8+ T-cell responses 30 days after vaccination, with antibody responses persisting up to 12 months.
Tapia et al. 2020 (B)[54]	NCT02548078	Randomised, observer-blind, controlled trial	ChAd3-EBO-Z (100 billion pu) on day 0 and a quadrivalent meningococcal tetanus toxoid conjugate vaccine (MenACWY-TT; month 6), or MenACWY-TT (day 0) and ChAd3-EBO-Z (month 6)	**600** children (aged 1-17 years)	Mali (1 site) Senegal (1 site)	Most common solicited local AEs was pain (42% after ChAd3-EBO-Z vs 20% after MenACWY-TT); Most common solicited systemic AE was fever (32% after ChAd3-EBO-Z group vs 9% after MenACWY-TT). Unsolicited AEs were reported by 14% after ChAd3-EBO-Z and 8% after MenACWY-TT. SAEs were reported in 1% of children in each comparison group; none considered vaccine-related. Binding antibody response against EBOV GP was observed after ChAd3-EBO-Z vaccination and persisted up to 12 months post-vaccination.

d. Vaccine candidates with safety and immunogenicity data from phase 1 studies, not licensed

Study	Registration	Design	Comparisons / vaccine dose	Participants	Sites	Key results
Ad26.Filo, MVA-BN-Filo 2-dose heterologous regimen						
Bockstal et al. 2022[71]	NCT02860650	Randomised, double-blind, placebo-controlled trial	Participants enrolled into 4 groups and randomised to an active regimen or placebo within each group. Active regimens: Group 1: Ad26.Filo (90 billion vp) followed by MVA-BN-Filo (500 million Inf.U.) 56 days later. Groups 2 and 3: MVA-BN-Filo followed by Ad26.Filo 56 days or 14 days later, respectively. In a Group 3 subset booster vaccination given on Day 92 either with Ad26.Filo or placebo. Group 4 (control group): Ad26.ZEBOV (50 billion vp) followed by MVA-BN-Filo (100 million Inf.U.) 56 days later.	**72** adults aged 18-50 years old	Rockville Maryland (USA)	All regimens were well tolerated with no deaths or vaccine-related SAEs. The most frequently reported solicited local AE was injection site pain/tenderness. Solicited systemic AEs most frequently reported: headache, fatigue, chills and myalgia; most solicited AEs were mild or moderate in severity. Solicited/unsolicited AE profiles were similar between regimens. 21 days post-dose 2, 100% of participants on active regimen responded to vaccination and exhibited binding antibodies against EBOV, SUDV, and MARV GPs; neutralising antibody responses were robust against EBOV (85.7-100%), but lower against SUDV (35.7-100%) and MARV (0-57.1%) GPs. An Ad26.Filo booster induced a rapid further increase in humoral responses.
ChAd3-EBO-Z + ChAd3-EBO-S, single dose containing both vaccines (1:1 ratio)						
Ledgerwood et al. 2017[55]	NCT02231866	Non-randomised, open-label, dose-escalation trial	Sequentially enrolled groups of 10 each, received one injection of vaccine at lower dose (20 billion pu) or higher dose (200 billion pu)	**20** adults	USA	Transient fever in 2 participants vaccinated with higher dose. GP-specific antibodies induced in all participants with significantly higher titres in the higher-dose group vs lower-dose group. GP-specific T-cell responses more frequent in the higher dose group compared to the lower dose group. Antibody titres remained high at week 48, with higher titres in the higher dose group.
cAd3-EBO S, single dose regimen						
Mwesigwa et al. 2023[95]	NCT04041570	Open-label, dose-escalation trial	Participants received one injection of vaccine at lower dose (10 billion pu) or higher dose (100 billion pu)	**40** adults	Uganda	No SAEs were reported. Symptoms after vaccination: injection site pain or tenderness, fatigue and headache. Binding antibody response against SUDV GP detected at 2 weeks in 78% of the participants. At 4 weeks, 85% of vaccinees were responders. At 48 weeks, 82% of participants were still responders. Binding antibody titres were higher in higher-dose group vs lower-dose group. GP-specific T-cell responses were detected at 4 weeks post vaccination.

EBOV GP nanoparticle vaccine, single and 2-dose homologous regimen						
Study	**Registration**	**Design**	**Comparisons / vaccine dose**	**Participants**	**Sites**	**Key results**
Fries et al. 2020[82]	NCT02370589	Randomised, observer-blind, placebo-controlled, dose-ranging trial	Participants randomised to 1 of 13 treatment groups of 1 of 4 escalating doses of EBOV GP nanoparticle vaccine (6.5, 13, 25, or 50 µg) given intramuscularly as a 1- or 2-dose regimen administered on days 0 and 21, formulated with or without Matrix-M adjuvant (50 µg).	**230** adults (aged 18-50 years)	Australia (3 sites)	All EBOV GP vaccine formulations were well tolerated. Two doses of EBOV GP with adjuvant induced a rapid increase in anti-EBOV GP IgG titres (peak titres observed on day 35); there was no evidence of an antigen dose response. Serum EBOV-neutralising and binding antibodies were 3- to 9-fold higher among recipients of 2-dose EBOV GP with adjuvant, compared with placebo on day 35 and persisted to 1 year.
Plasmid DNA vaccine, 3-dose homologous regimen						
Martin et al. 2006[79]	NCT00072605	Randomised, double-blind, placebo-controlled, dose escalation trial	Participants randomised to receive three injections of vaccine* at 2 mg, 4 mg, or 8 mg or placebo. *3-plasmid DNA vaccine expressing EBOV and SUDV GPs and EBOV NP	**27** adults (aged 18-44 years)	Bethesda (USA)	Vaccine well-tolerated. No significant AEs or coagulation abnormalities. Specific antibody responses to at least one of the three antigens encoded by the vaccine and CD4 T-cell GP-specific responses were detected in 20 of 20 vaccinees. CD8 T-cell GP-specific responses detected in 6 of 20 vaccinees.
Kibuuka et al. 2015[80]	NCT00997607	Randomised, double-blind, placebo-controlled trial	Participants randomised to active vaccine or placebo at weeks 0, 4, and 8, with vaccine allocations divided equally between 3 active vaccine groups: EBO vaccine only, MAR vaccine only, and both vaccines. EBO vaccine: plasmid expressing EBOV and SUDV GPs (VRC-EBODNA023-00-VP). MAR vaccine: plasmid expressing MARV GP (VRC-MARDNA025-00-VP).	**108** adults (aged 18-50 years)	Kampala (Uganda)	Vaccines were well tolerated. No significant differences in local or systemic reactions between groups. EBOV GP antibody response was detected in 57% (95% CI 37-75) after EBO vaccine and 47% (28-66) after both vaccines. SUDV GP antibody response was recorded in 50% (31-69) after EBO vaccine and 50% (31-69) after both vaccines. MARV GP antibody response was detected in 31% (15-51) after MAR vaccine and 23% (10-42) after both vaccines. T-cell response to the EBOV GP was detected in 63% (44-80) after EBO vaccine and 33% (17-53) after both vaccines. T-cell response to the SUDV GP was observed in 43% (25-63) after EBO vaccine group and 33% (17-53) after both vaccines. T-cell response to the MARV GP was detected in 52% (33-71) after MAR vaccine and 43% (25-63) after both vaccines.
Sarwar et al. 2015[81]	NCT00605514	non-randomised, open label trial	Group 1 enrolled first to receive MAR vaccine (VRC-MARDNA025-00-VP). Group 2 enrolled to receive the EBO vaccine (VRC-EBODNA023-00-VP). Each group received vaccine at weeks 0, 4, and 8, with an optional homologous booster offered at or after week 32.	**20** adults	Bethesda (USA)	No SAEs reported. At 4 weeks after the third dose, 80% in group 1 had MARV GP antibodies. In group 2, 89% had SUDV GP and 56% had EBOV GP antibodies. At 24 weeks after the third dose, 11% of subjects were positive for MARV GP and SUDV GP, and none were positive for EBOV GP. At 4 weeks post booster, 100% of subjects were positive for MARV GP, 75% for SUDV GP, and 63% for EBOV GP.

Study	Registration	Design	Comparisons / vaccine dose	Participants	Sites	Key results
rAd5.ZEBOV-GP + rAd5.SBOV-GP, single dose containing both vaccines (1:1 ratio)						
Ledgerwood et al. 2010[76]	NCT00374309	Randomised, double-blind, placebo-controlled, dose escalation trial	Participants randomised to active vaccine at lower dose (2 billion vp) or 20 billion vp (group 2, higher dose) or placebo.	**32** adults	Bethesda (USA)	The vaccine was well tolerated with only self-limited reactogenicity (injection site pain, malaise, myalgia, etc.). At 4 weeks post-vaccination, in the lower dose group, 58% of vaccinees were positive for SUDV GP binding antibodies and 50% for EBOV GP binding antibodies, while in the higher dose group, 100% of subjects had SUDV GP specific binding antibodies and 55% had antibodies to EBOV GP. Titres peaked at 4 weeks post-vaccination in both dose groups. At 48 weeks after vaccination, 42% were still positive for SUDV GP antibodies and 33% for EBOV GP antibodies in the lower dose group, while in the higher dose group, these percentages were 60% and 40%. When considering all vaccinees combined, Ad5 vector-seronegative subjects at baseline had a significantly higher response rate and a higher magnitude of response at 4 weeks post-vaccination compared to Ad5 vector-seropositive subjects at baseline. The vaccine induced EBOV and SUDV GP-specific T-cell responses.
rVSVN4CT1-EBOVGP1, 2-dose homologous regimens						
Clarke et al. 2020[78]	NCT02718469	Randomised, double-blind, placebo-controlled, dose-escalation trial	Participants enrolled in 3 cohorts and, within each cohort, randomised to vaccine or placebo in a homologous 2-dose regimen, with 4 weeks between doses. Vaccines given at low (25,000 PFU), intermediate (200,000 PFU), or high dose (1.8 million PFU).	**39** adults (18-60 years)	Melbourne Florida (USA)	The vaccine was well tolerated. No SAEs were observed. Solicited local AEs (mostly mild and moderate injection site tenderness and pain) occurred in 26% and 24% of participants after dose 1 and dose 2, respectively. Systemic AEs (mostly mild or moderate malaise or fatigue and headache) occurred in 33% and 21% of participants after dose 1 and dose 2, respectively. EBOV GP-specific IgG response was detected in 100% of vaccinees after two doses (100% after a single high dose), with increasing mean peak IgG titres with increasing doses at day 35. At day 182, mean responses were significantly higher after active vaccine than placebo, but not significantly different between different doses, with 100% of participants in the high-dose cohort still considered responders. Mean nAb titres were significantly higher after active vaccine than placebo at day 35, and decreased at day 182. GP-specific IFNγ ELISPOT responses peaked about 2 weeks after the second dose (day 42) in all cohorts. Mean responses decreased gradually by day 182.

APPENDIX 2 – Ebolavirus seroprevalence studies published from 01 January 2017 to 11 November 2023, ordered by country, study population and year of sample collection

Africa							
Democratic Republic of the Congo (DRC)							
Study population	**Sample collection period**	**Number of participants**	**Positive, n (%)**	**Study**	**Design**	**Test and cut-off used**	**Other relevant findings / notes**
General population (Healthy adults and children)	Aug-Sep 2007	3415	EBOV-NP IgG: 11% (number of positive not reported)	Mulangu et al. 2018.[132]	Cross-sectional study (CSS)	EBOV r-NP ELISA. Seropositivity cut-off: ≥5 SDs from the negative control mean (88 Congolese donors born and living in Kinshasa).	Odds of seropositivity higher for participants >15 years of age, males, resident in an area closer to an EVD outbreak site. Visits to the forest or hunting and exposure to rodents or duikers also associated with higher EBOV seropositivity.
General population (Healthy adults)	Sep 2015-Aug 2017	1366	EBOV-GP IgG: 113 (8.3%)	Bratcher et al. 2021.[122]	CSS	ELISA (Alpha Diagnostic Int.) with seropositivity cut-off: 4.7 units/mL.	Associations of EBOV seropositivity with contact with bats and consumption of NHP meat.
Contacts of EVD cases	2014	182 (48 households)	EBOV-GP IgG: 2 (1.1%)	Mbala et al. 2017.[133]	CSS	ELISA (Public Health Agency of Canada). The study used a Bayesian mixture model to identify positive samples.	No difference in seroprevalence between EVD affected and unaffected households during the 2014 EVD outbreak in Equateur province. Age significantly associated with high antibody titres.
HCWs	Sep-Nov 2015	582	EBOV-GP IgG: 131 (22.5%)	Doshi et al. 2020.[123]	CSS	ELISA (Alpha Diagnostic Int.) with seropositivity cut-off: 2.5 units/mL.	Using any form of personal protective equipment when caring for a confirmed, probable, or suspect EVD case negatively associated with seroreactivity.
HCWs	Sep-Nov 2015	565	Reactive to at least 1 EBOV protein: 234 (41.4%) EBOV-GP IgG: 159 (28.1%) EBOV-NP IgG: 89 (15.8%) VP40 reactive: 54 (9.5%) Neutralising antibodies: 16 (2.8%)	Hoff et al. 2019.[124]	CSS	ELISA (Alpha Diagnostic Int.) for EBOV-GP and NP IgG (cut-off: 2.5 units/mL). Luciferase Immunoprecipitation System (LIPS) for VP40 reactivity (cut-off: 3 SDs above background signal). psVNA for neutralising antibodies. (Positivity = neutralisation of 50% virus at 1:50 dilution).	A significant proportion of HCWs of Boende Health Zone in DRC have EBOV binding and neutralising antibodies, despite never having developed EVD symptoms.

Africa							
DRC (continued)							
Study population	**Sample collection period**	**Sample size**	**Positive, n (%)**	**Study**	**Design**	**Test and cut-off used**	**Relevant findings / notes**
HCWs	June-July 2018	539	EBOV complete GP ectodomain IgG: 25 (4.6%) EBOV mucin-like domain-deleted GP (GPΔMuc): 15 (2.8%) BDBV GPΔMuc: 13 (2.4%) SUDV GPΔMuc: 12 (2.2%)	Shaffer et al. 2022.[129]	CSS	Multi-antigen ELISAs (in-house). Sample titre/positive control EC_{50}: 1<2 for Weak Reactivity, 2≤10 for Moderate Reactivity, and 10+ for Strong Reactivity.	Community health volunteers in Mbandaka Health Region more likely to be seroreactive against each antigen than nurses. HCWs with indirect patient contact had higher anti-EBOV GP IgG levels than those with direct contact. Findings attributed to lack of PPE in HCWs with indirect patient contact.
HCWs and FLWs	Dec 2019-Oct 2022	698	Luminex-based assay: GP-EBOV + VP40-EBOV (Mayinga): 10 (1.4%, 95% CI: 0.7-2.6) VP40-EBOV + NP-EBOV (Mayinga): 2 (0.3%, 95% CI: 0.0-1.0) FANG ELISA: GP-EBOV-Kikwit: 59/693 (8.5%, 95%CI: 6.5-10.9) Both Luminex and FANG ELISA positive: 6/693 (0.8%, 95%CI: 0.1-1.5)	Zola Matuvanga et al. 2023.[144]	CSS nested in a vaccine RCT	Luminex-based assay with 4 commercially available antigens: GP-EBOV-Kissidougou/Makona 2014 strain; GP-EBOV, NP-EBOV and VP40-EBOV of the Mayinga 1976 strain. Positivity = reactivity to ≥2 EBOV antigens. FANG ELISA (Q2 Solutions Vaccine Testing Laboratory). Seropositivity cut-off: >607 ELISA units (EU)/mL.	Low seroprevalence was found in HCWs and FLWs participating in an Ebola vaccine trial in Boende, DRC. Participants with previous contact with an Ebola case were less likely to be EBOV-seropositive than those who never became into contact. Weak correlation between the FANG ELISA and Luminex-based assay results.
Bushmeat Vendors	Nov 2018	19	1 (5.3%)	Lucas et al. 2020.[130]	CSS	ELISA (in-house). Positivity cut-off: 3 times background absorption (no antigen) or negative wells (whichever higher).	Antibodies against EBOV found in one participant with no reported history of EVD.
Febrile patients negative for filoviruses PCR	May 2017-Apr 2018	272	EBOV-GP IgG: 29 (10.7%) BOMV-GP IgG: 1 (0.4%) No positive samples for SUDV, BDBV, RESTV, TAFV and MARV	Goldstein et al. 2020.[131]	CSS	ELISA (in-house). Positivity cut-off: 3 times background absorption (no antigen) or negative wells (whichever higher).	Women were significantly more likely to be positive than man and the majority of positives were in February 2018.
Suspected EVD patients negative for EBOV PCR	2018-2020	488	Reactivity to at least 2 EBOV antigens: 11 (2.3%). EBOV-NP IgG: 7 (1.4%) EBOV-GP IgG: 54 (11.1%) EBOV-VP40 IgG: 39 (8%)	Nkuba-Ndaye et al. 2022.[136]	Retrospective CSS	Luminex-based assay (Luminex Corp, Austin, TX, USA) using dried blood spots (DBS). Positivity=simultaneous reactivity to ≥2 EBOV antigens.	Simultaneous reactivity to at least 2 EBOV antigens was detected in 11 of 488 (2.3%; 95% CI: 1.1-4.0) suspected EVD patients who were discharged as negative after 2 consecutive negative PCR tests during the 2018-20 EVD outbreak in DRC.

Africa							
Guinea							
Study population	**Sample collection period**	**Sample size**	**Positive, n (%)**	**Study**	**Design**	**Test and cut-off used**	**Relevant findings / notes**
General population (DHS survey)	Jun-Oct 2012	1483	At least one EBOV antigen: 154 (10.4%) EBOV-GP IgG (Makona): 49 (3.3%) EBOV-GP IgG (Mayinga): 81 (5.5%) EBOV-NP IgG: 9 (0.6%) VP40 reactive: 62 (4.2%) NP+GP: 1 (0.07%) GP+VP40: 7 (0.47%) NP+GP+VP40: 0 (0%)	Keita et al. 2018.[137]	Retrospective serosurvey	Luminex analysis of DBS. Positivity = simultaneous and repeated reactivity to EBOV-NP and GP.	The study found differences in seropositivity by geographical site (i.e. 4.1% in Conakry vs 19.4% in Guinée Forestière for at least one EBOV antigen).
General population (adults from all the households at the index site of the 2014-16 EVD Ebola epidemic)	Jun-Jul 2017	237 (27 households)	8/224 (3.6%) seropositive survivors not previously identified. 2/224 (0.9%) mild or asymptomatic infections.	Timothy et al. 2019.[18]	CSS	Oral fluid anti-glycoprotein IgG capture assay (Kalon Biological, Guildford, UK). Samples with normalised optical density (NOD) values >1.1 considered seropositive.	Study used interviews and oral fluid test to determine the occurrence of previously undocumented EVD infections. Study identified higher number of deaths than initially reported (13 vs 11) and 8 seropositive survivors. Mild or asymptomatic forms of EBOV infection occurred in 2 (11.1%) of 18 total adult infections.
Contacts of EVD cases (adults and children)	May 2016-Sept 2017	1721	18 (8.3%) of 216 paucisymptomatic contacts 39 (3.3%) of 1174 asymptomatic contacts	Diallo et al. 2019.[138]	Retrospective CSS	Luminex-based assay (Luminex Corp, Austin, TX, USA) using DBS. Seropositivity cut-offs: 501 median fluorescence intensity (MFI) for GP, 950 MFI for NP, and 580 MFI for VP40.	Seropositivity increased with participation in burial rituals (adjusted OR 2.30, 95% CI 1.21-4.17; p=0.0079) and exposure to blood or vomit (adjusted OR 2.15, 1.23-3.91; p=0.0090).
Mali							
General population (Healthy volunteers)	2015	600	EBOV-GP IgG: 22 (3.7%) EBOV-NP IgG: 24 (4.0%)	Bane et al. 2021.[125]	CSS	ELISA (Alpha Diagnostic International). Positive result = positive reaction at >1:400 serum dilution.	Low seroprevalence in the general population, indicating local exposure to EBOV or closely related ebolaviruses. Age of participants not reported.

Africa							
Sierra Leone							
Study population	**Sample collection period**	**Sample size**	**Positive, n (%)**	**Study**	**Design**	**Test and cut-off used**	**Relevant findings / notes**
Suspected Lassa fever patients and contacts	2007-2014	675	EBOV-GP or VP40 IgG: 35/672 (5.2%) MARV-VP40 IgG: 71/663 (10.7%)	O'Hearn et al. 2016.[139]	Serosurvey	Luminex-based assay (Luminex Corp, Austin, TX, USA). Results with a z-score of ≥3 standard errors above zero considered positive. Samples testing positive for ≥1 virus targets were considered positive for the virus.	Significant presence of filoviruses actively circulating in the Sierra Leone region over 7-year period.
Healthy adults and children (not previous EVD diagnosis or Ebola vaccination)	Mar 2016-Jun 2018	1282	EBOV GP IgG: 107 (8.4%, 95%CI: 7.0%-10.0%)	Manno et al. 2022.[134]	CSS nested in a vaccine RCT	FANG ELISA (Q2 Solutions Vaccine Testing Laboratory). Seropositivity cut-off: >607 ELISA units (EU)/mL.	Antibody concentration increased with age. Both EBOV antibody seropositivity and concentration independently associated with residence in a household compound with one or more pigs during the 2014-16 EVD epidemic.
Healthy contacts of EVD cases and HCWs not caring for EVD patients	Feb-Mar 2015	105 contacts 79 HCW	12/105 (11.4%) in community contacts 3/79 (3.8%) in HCW	Mafopa et a. 2017.[126]	CSS	ELISA (Alpha Diagnostic Int.). Seropositivity cut-off not specified.	EBOV responsible of the 2014-16 epidemic in West Africa may have caused mild or asymptomatic infection in a proportion of the population.
Household contacts of EVD survivors (adults and children)	Nov 2014-Mar 2015	481	EBOV-GP IgG: 11 (12.0%) of 92 contacts with symptoms. 10 (2.6%) of 388 asymptomatic contacts	Glynn et al. 2017.[142]	CSS	Oral fluid anti-glycoprotein IgG capture assay. Samples with NOD values >1.1 considered positive.	Seropositivity detected among asymptomatic and paucisymptomatic contacts of EVD cases. Among asymptomatic contacts, seropositivity was weakly correlated with exposure level to an EVD case.
Close contacts of EVD survivors (adult relatives and HCWs)	Mar 2017	267	IgG against: ≥1 antigen 107 (40.1%) 1 antigen: 69 (25.8%) 2 antigens: 20 (7.5%) 3 antigens: 18 (6.7%) EBOV-GP: 34 (12.7%) of those, 31 (91.2%) had also neutralising antibodies against EBOV.	Halfmann et al. 2019.[127]	CSS nested in a cohort study	ELISA (Alpha Diagnostic Int.) for EBOV GP, NP and VP40. Seropositivity cut-off not reported. Replication-defective EBOVΔVP30 system (in-house) for neutralising antibodies. Titres defined as the highest plasma dilution with a 50% reduction in relative light units compared to a plasma control.	Up to 12.7% of close contacts of EVD survivors may have experienced a subclinical virus infection.

Africa							
Uganda							
Study population	**Sample collection period**	**Sample size**	**Positive, n (%)**	**Study**	**Design**	**Test and cut-off used**	**Relevant findings / notes**
Miners and their household members. Non-miners living close to a mine. Community members living far from mines (control group).	Not specified	724	IgG anti-bodies against filoviruses: 19 (2.6%). SUDV IgG: 17 (2.3%) MARV virus IgG: 1 (0.1%) SUDV IgG and BDBV virus IgG: 1 (0.1%) EBOV IgG: 0 (0%)	Nyakarahuka et al. 2020.[135]	CSS	ELISA validated by US CDC. A sample was considered positive when the adjusted optical density (OD) value of either the 1:400, 1:1600 or 1:6400 dilution was greater than 0.2 and the sum OD value was greater than 0.95.	Miners in western Uganda were 5.4 times more likely to be filovirus seropositive to SUDV, BDBV and MARV, compared to control group in central Uganda (Risk ratio [RR]=5.4; 95% CI 1.5-19.7) whereas people living in high-risk areas close to the mine were 3.6 more likely to be seropositive compared to the control group (RR=3.6; 95% CI 1.1-12.2). None of the samples was positive for EBOV IgG.
Febrile patients negative for filoviruses PCR	Mar-Jun 2013	331	SUDV GP IgG: 14/300 (4.7%) EBOV-GP IgG: 16/301 (5.3%) BDBV-partial GP IgG: 27/303 (8.9%)	Smiley Evans et al. 2018.[141]	CSS	Western blot. Samples were considered positive after visual comparison to positive and negative control samples.	Touching duikers was a risk factor associated with EBOV seropositivity, while hunting primates and touching and/or eating cane rats were risk factors for SUDV seropositivity.
Multiple country in Equatorial Africa: Uganda, Cameroon, Ghana, Republic of the Congo (ROC) and DRC							
Research study participants (Deidentified serum samples from previous studies)	1997-2012	2430	EBOV neutralising antibodies: 33 (1.4%) EBOV-VP40 antibodies: 108 (4.4%) EBOV-confirmed (reactivity in >2 different assay): 53 (2.2%)	Steffen et al. 2019.[128]	Retrospective serosurvey	psVNA for neutralising antibodies. Positivity = reduction of infection with EBOV pseudoviruses by >50% compared with negative control. LIPS for VP40 Reactivity. Cut-off based on mean plus 3 SDs of 10 presumed negative samples from Kinshasa. ELISA (Alpha Diagnostic Int.) for EBOV-NP IgG. Cut-off not specified.	Specimens sero-reactive for EBOV were confirmed with ELISA. Difference in seroprevalence by country. Prevalence of 2%-3.5% in the Republic of the Congo and the DRC. Seroprevalence of 1.3% in southern Cameroon, which indicated a low risk for exposure in this region.

Other continents							
Asia (India)							
Study population	**Sample collection period**	**Sample size**	**Positive, n (%)**	**Study**	**Design**	**Test and cut-off used**	**Relevant findings / notes**
Bat harvesters (adults)	2017	85	Filovirus-reactive sera: 5 (5.9%)	Dovih et al. 2019.[140]	CSS	Bio-Plex (Bio-Rad, Hercules, CA, USA) bead-based multiplex assay. Positive samples defined as exceeding two thresholds: 95th percentile of the log-normal distribution of background subtracted MFI and 3-fold change above the arithmetic mean of the background-adjusted scaled MFI.	Samples were tested for antibodies against the GPs of EBOV, BDBV, TAFV, SUDV, RESTV, MARV and other viruses. Four individual sera were reactive to EBOV-, BDBV-, and SUDV-GPs and one serum reactive to MARV-GP.
Europe (UK and Ireland)							
Returned responders to the 2014-16 EVD epidemic in West Africa	December 2015 to June 2016	268	2 (0.7%)	Houlihan et al. 2017.[143]	CSS	Oral fluid anti-glycoprotein IgG capture assay. NOD >1 was considered reactive.	Two seropositive participants (0.7%) who never tested positive for EBOV by PCR and were not vaccinated against EBOV.

APPENDIX 3 – EBOV GP-specific binding antibody response in different clinical trials of the Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen, with a 56-day interval between doses, listed by geographical location and age of participants

Location	Study	Age group	Ebola virus glycoprotein IgG antibodies in GMC in Eu/ml (95% CI)		
			D57	D78 (D21 post dose 2)	D360
Africa					
Uganda, Tanzania	Anywaine 2019,[62] EBL1004	Adults (≥18 years)	N=15 323 (170, 616)	N=15 10,613 (6092, 18,492)	N=15 550 (296, 1022)
Kenya	Mutua 2019,[63] EBL1003	Adults (≥18 years)	N=15 413 (225, 757)	N=15 16,341 (10,812, 24,697)	N=15 403 (214, 756)
Burkina Faso, Côte d'Ivoire, Kenya, Uganda	Barry 2021,[65] EBL2002*	Adults (18-50 years)	N=114 365 (308, 433)	N=115 8113 (6875, 9573)	N=112 349 (291, 417)
		Adults (>50 years)	N=22 337 (211, 538)	N=21 4956 (3564, 6891)	N=21 307 (211, 446)
	Anywaine 2022,[66] EBL2002	Children 12-17 years	N=53 562 (460, 686)	N=53 13,532 (10,732, 17,061)	N=52 541 (433, 678)
		Children 4-11 years	N=54 658 (556, 780)	N=53 17,388 (12,973, 23,306)	N=54 637 (529, 767)
Sierra Leone	Ishola 2022,[30] EBL3001 (stage 1)	Adults (≥18 years)	N=43 269 (208, 347)	N=42 4784 (3736, 6125)	N=31 325 (238, 445)
	Ishola 2022,[30] EBL3001 (stage 2)	Adults (≥18 years)	N=190 236 (206, 270)	N=182 3810 (3312, 4383)	N=168 259 (223, 301)
	Afolabi 2022,[68] EBL3001 (stage 2)	Children 12-17 years	N=142 314 (269, 366)	N=134 9929 (8172, 12,064)	N=132 386 (326, 457)
		Children 4-11 years	N=133 390 (334, 456)	N=124 10,212 (8419, 12,388)	N=123 436 (375, 506)
		Children 1-3 years	N=125 693 (591, 812)	N=125 22,568 (18,426, 27,642)	N=125 750 (629, 894)
Guinea, Liberia, Mali, Sierra Leone	PREVAC Study Team 2022[70]	Adults (≥18 years)	N=369 387 (NA)	NA	N=374 401 (NA)
		Children 1-17 years	N=389 679 (NA)	NA	N=381 828 (NA)
Guinea, Sierra Leone	Choi 2023[93] EBL2005	Infants 4-11 months	N=74 <LLOQ (<LLOQ, <LLOQ)	N=74 24,309 (19,695, 30,005)	N=74 1466 (1090, 1971)
America					
USA	Bockstal 2021[83] EBL3002	Adults (18-50 years)	N=140 793 (698, 902)	N=135 11,054 (9673, 12,633)	NA
	Bockstal 2021[83] EBL3003	Adults (18-50 years)	N=85 (Group 1) 813 (632, 1046)	N=81 (Group 1) 11,089 (9323, 13,189)	NA
			N=88 (Group 2) 745 (603, 921)	N=87 (Group 2) 10,337 (8660, 12,339)	NA
			N=88 (Group 3) 851 (720, 1006)	N=86 (Group 3) 11,790 (9701, 14,328)	NA
Europe					
UK	Milligan 2016,[59] EBL1001	Adults (18-50 years)	N=14 854 (556, 1312)	N=14 7553 (5114, 11,156)	NA
France, UK	Pollard 2021,[67] EBL2001	Adults (≥18 years)	N=69 880 (709, 1093)	N=69 10,131 (8554, 11,999)	N=50 1205 (971, 1497)

*The study recruited also HIV+ participants but these are not included in this table.
NA=not available, LLOQ=36.11 Eu/ml.

References

1. Centers for Disease Control and Prevention (CDC). 2014-2016 Ebola Outbreak in West Africa. https://www.cdc.gov/vhf/ebola/history/2014-2016-outbreak/index.html (Accessed on 11 November 2023).

2. Centers for Disease Control and Prevention (CDC). What is Ebola Virus Disease? https://www.cdc.gov/vhf/ebola/about.html (Accessed on 11 November 2023).

3. Feldmann H, Sprecher A, Geisbert TW. Ebola. The New England journal of medicine 2020;382(19):1832-1842. DOI: 10.1056/NEJMra1901594.

4. Centers for Disease Control and Prevention (CDC). Ebola virus disease (EVD) Signs and Symptoms. https://www.cdc.gov/vhf/ebola/symptoms/index.html (Accessed on 11 November 2023).

5. World Health Organization (WHO). Ebola virus disease. https://www.who.int/news-room/fact-sheets/detail/ebola-virus-disease (Accessed on 11 November 2023).

6. Science. This bat species may be the source of the Ebola epidemic that killed more than 11,000 people in West Africa. https://www.science.org/content/article/bat-species-may-be-source-ebola-epidemic-killed-more-11000-people-west-africa (Accessed on 11 November 2023).

7. Keita AK, Koundouno FR, Faye M, et al. Resurgence of Ebola virus in 2021 in Guinea suggests a new paradigm for outbreaks. Nature 2021;597(7877):539-543. DOI: 10.1038/s41586-021-03901-9.

8. Thorson A, Formenty P, Lofthouse C, Broutet N. Systematic review of the literature on viral persistence and sexual transmission from recovered Ebola survivors: evidence and recommendations. BMJ Open. 2016;6(1):e008859. DOI: 10.1136/bmjopen-2015-008859.

9. Pratt C. Oct 2021 EVD case in DRC linked to 2018-2020 Nord Kivu EVD outbreak. https://virological.org/t/oct-2021-evd-case-in-drc-linked-to-2018-2020-nord-kivu-evd-outbreak/762 (Accessed on 11 November 2023).

10. Arias A, Watson SJ, Asogun D, et al. Rapid outbreak sequencing of Ebola virus in Sierra Leone identifies transmission chains linked to sporadic cases. Virus Evol 2016;2(1):vew016. DOI: 10.1093/ve/vew016.

11. Sissoko D, Keita M, Diallo B, et al. Ebola Virus Persistence in Breast Milk After No Reported Illness: A Likely Source of Virus Transmission From Mother to

Child. Clinical infectious diseases 2017;64(4):513-516. DOI: 10.1093/cid/ciw793.

12. Bausch DG. The need for a new strategy for Ebola vaccination. Nat Med 2021;27(4):580-581. DOI: 10.1038/s41591-021-01313-w.

13. Olivero J, Fa JE, Real R, et al. Recent loss of closed forests is associated with Ebola virus disease outbreaks. Sci Rep 2017;7(1):14291. DOI: 10.1038/s41598-017-14727-9.

14. Redding DW, Atkinson PM, Cunningham AA, et al. Impacts of environmental and socio-economic factors on emergence and epidemic potential of Ebola in Africa. Nat Commun 2019;10(1):4531. DOI: 10.1038/s41467-019-12499-6.

15. Bower H. (2022). Ebola virus transmission and disease severity in Sierra Leone 2013-2016. PhD (research paper style) thesis, London School of Hygiene & Tropical Medicine. DOI: https://doi.org/10.17037/PUBS.04668986 (Accessed on 11 November 2023).

16. Centers for Disease Control and Prevention (CDC). February 2021 Democratic Republic of the Congo, North Kivu Province 2021. https://www.cdc.gov/vhf/ebola/outbreaks/drc/2021-february.html (Accessed 11 November 2023).

17. Mari Saez A, Weiss S, Nowak K, et al. Investigating the zoonotic origin of the West African Ebola epidemic. EMBO Mol Med 2015;7(1):17-23. DOI: 10.15252/emmm.201404792.

18. Timothy JWS, Hall Y, Akoi-Bore J, et al. Early transmission and case fatality of Ebola virus at the index site of the 2013-16 west African Ebola outbreak: a cross-sectional seroprevalence survey. Lancet Infect Dis 2019;19(4):429-438. DOI: 10.1016/S1473-3099(18)30791-6.

19. World Health Organization (WHO). Ground zero in Guinea: the Ebola outbreak smoulders – undetected – for more than 3 months. https://www.who.int/news/item/04-09-2015-ground-zero-in-guinea-the-ebola-outbreak-smoulders-undetected-for-more-than-3-months (Accessed on 11 November 2023).

20. Baize S, Pannetier D, Oestereich L, et al. Emergence of Zaire Ebola virus disease in Guinea. The New England journal of medicine 2014;371(15):1418-25. DOI: 10.1056/NEJMoa1404505.

21. World Health Organization (WHO). Factors that contributed to undetected spread of the Ebola virus and impeded rapid containment. https://www.who.int/news-room/spotlight/one-year-into-the-ebola-epidemic/factors-that-contributed-to-undetected-spread-of-the-ebola-virus-and-impeded-rapid-containment (Accessed on 11 November 2023).

22. Kamradt-Scott A, Harman S, Wenham C, Smith F, 3rd. Civil-military cooperation in Ebola and beyond. Lancet 2016;387(10014):104-5. DOI: 10.1016/S0140-6736(15)01128-9.

23. Dalziel BD, Lau MSY, Tiffany A, et al. Unreported cases in the 2014-2016 Ebola epidemic: Spatiotemporal variation, and implications for estimating transmission. PLoS Negl Trop Dis 2018;12(1):e0006161. DOI: 10.1371/journal.pntd.0006161.

24. Scarpino SV, Iamarino A, Wells C, et al. Epidemiological and viral genomic sequence analysis of the 2014 ebola outbreak reveals clustered transmission. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2015;60(7):1079-82. DOI: 10.1093/cid/ciu1131.

25. Gignoux E, Idowu R, Bawo L, et al. Use of Capture-Recapture to Estimate Underreporting of Ebola Virus Disease, Montserrado County, Liberia. Emerg Infect Dis 2015;21(12):2265-7. DOI: 10.3201/eid2112.150756.

26. Huber C, Finelli L, Stevens W. The Economic and Social Burden of the 2014 Ebola Outbreak in West Africa. The Journal of infectious diseases 2018;218(suppl_5):S698-S704. DOI: 10.1093/infdis/jiy213.

27. Centers for Disease Control and Prevention (CDC). Ebola (Ebola Virus Disease) Treatment. https://www.cdc.gov/vhf/ebola/treatment/index.html (Accessed 11 November 2023).

28. Mulangu S, Dodd LE, Davey RT, Jr., et al. A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics. The New England journal of medicine 2019;381(24):2293-2303. DOI: 10.1056/NEJMoa1910993.

29. Gross L, Lhomme E, Pasin C, Richert L, Thiebaut R. Ebola vaccine development: Systematic review of pre-clinical and clinical studies, and meta-analysis of determinants of antibody response variability after vaccination. Int J Infect Dis 2018;74:83-96. DOI: 10.1016/j.ijid.2018.06.022.

30. Ishola D, Manno D, Afolabi MO, et al. Safety and long-term immunogenicity of the two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in adults in Sierra Leone: a combined open-label, non-randomised stage 1, and a randomised, double-blind, controlled stage 2 trial. Lancet Infect Dis 2022;22(1):97-109. DOI: 10.1016/S1473-3099(21)00125-0.

31. Bache BE, Grobusch MP, Agnandji ST. Safety, immunogenicity and risk-benefit analysis of rVSV-DeltaG-ZEBOV-GP (V920) Ebola vaccine in Phase I-III clinical trials across regions. Future Microbiol 2020;15:85-106. DOI: 10.2217/fmb-2019-0237.

32. Diallo A, Carlos-Bolumbu M, Cervantes-Gonzalez M, et al. Immunogenicity and safety of Ebola virus vaccines in healthy adults: a systematic review and network meta-analysis. Hum Vaccin Immunother 2021;17(10):3771-3783. DOI: 10.1080/21645515.2021.1932214.

33. Huttner A, Dayer JA, Yerly S, 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(10):1156-1166. DOI: 10.1016/S1473-3099(15)00154-1.

34. Agnandji ST, Huttner A, Zinser ME, et al. Phase 1 Trials of rVSV Ebola Vaccine in Africa and Europe. The New England journal of medicine 2016;374(17):1647-60. DOI: 10.1056/NEJMoa1502924.

35. Agnandji ST, Fernandes JF, Bache EB, et al. Safety and immunogenicity of rVSVDeltaG-ZEBOV-GP Ebola vaccine in adults and children in Lambarene, Gabon: A phase I randomised trial. PLoS Med 2017;14(10):e1002402. DOI: 10.1371/journal.pmed.1002402.

36. ElSherif MS, Brown C, MacKinnon-Cameron D, et al. Assessing the safety and immunogenicity of recombinant vesicular stomatitis virus Ebola vaccine in healthy adults: a randomized clinical trial. CMAJ 2017;189(24):E819-E827. DOI: 10.1503/cmaj.170074.

37. Heppner DG, Jr., Kemp TL, Martin BK, 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(8):854-866. DOI: 10.1016/S1473-3099(17)30313-4.

38. Regules JA, Beigel JH, Paolino KM, et al. A Recombinant Vesicular Stomatitis Virus Ebola Vaccine. The New England journal of medicine 2017;376(4):330-341. DOI: 10.1056/NEJMoa1414216.

39. Huttner A, Agnandji ST, Combescure C, et al. Determinants of antibody persistence across doses and continents after single-dose rVSV-ZEBOV vaccination for Ebola virus disease: an observational cohort study. Lancet Infect Dis 2018;18(7):738-748. DOI: 10.1016/S1473-3099(18)30165-8.

40. Ehrhardt SA, Zehner M, Krahling V, et al. Polyclonal and convergent antibody response to Ebola virus vaccine rVSV-ZEBOV. Nat Med 2019;25(10):1589-1600. DOI: 10.1038/s41591-019-0602-4.

41. Henao-Restrepo AM, Longini IM, Egger M, et al. Efficacy and effectiveness of an rVSV-vectored vaccine expressing Ebola surface glycoprotein: interim results from the Guinea ring vaccination cluster-randomised trial. Lancet 2015;386(9996):857-66. DOI: 10.1016/S0140-6736(15)61117-5.

42. Henao-Restrepo AM, Camacho A, Longini IM, 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 Ca Suffit!). Lancet 2017;389(10068):505-518. DOI: 10.1016/S0140-6736(16)32621-6.

43. Halperin SA, Arribas JR, Rupp R, et al. Six-Month Safety Data of Recombinant Vesicular Stomatitis Virus-Zaire Ebola Virus Envelope Glycoprotein Vaccine in a Phase 3 Double-Blind, Placebo-Controlled Randomized Study in Healthy Adults. The Journal of infectious diseases 2017;215(12):1789-1798. DOI: 10.1093/infdis/jix189.

44. Samai M, Seward JF, Goldstein ST, et al. The Sierra Leone Trial to Introduce a Vaccine Against Ebola: An Evaluation of rVSVΔG-ZEBOV-GP Vaccine Tolerability and Safety During the West Africa Ebola Outbreak. The Journal of infectious diseases 2018;217(suppl_1):S6-S15. DOI: 10.1093/infdis/jiy020.

45. Bolay FK, Grandits G, Lane HC, et al. PREVAIL I Cluster Vaccination Study With rVSVDeltaG-ZEBOV-GP as Part of a Public Health Response in Liberia. The Journal of infectious diseases 2019;219(10):1634-1641. DOI: 10.1093/infdis/jiy698.

46. Halperin SA, Das R, Onorato MT, et al. Immunogenicity, Lot Consistency, and Extended Safety of rVSVDeltaG-ZEBOV-GP Vaccine: A Phase 3 Randomized, Double-Blind, Placebo-Controlled Study in Healthy Adults. The Journal of infectious diseases 2019;220(7):1127-1135. DOI: 10.1093/infdis/jiz241.

47. Juan-Giner A, Tchaton M, Jemmy JP, et al. Safety of the rVSV ZEBOV vaccine against Ebola Zaire among frontline workers in Guinea. Vaccine 2019;37(48):7171-7177. DOI: 10.1016/j.vaccine.2018.09.009.

48. Boum Y, Juan-Giner A, Hitchings M, et al. Humoral and cellular immune response induced by rVSVDeltaG-ZEBOV-GP vaccine among frontline workers during the 2013-2016 West Africa Ebola outbreak in Guinea. Vaccine 2020;38(31):4877-4884. DOI: 10.1016/j.vaccine.2020.04.066.

49. Legardy-Williams JK, Carter RJ, Goldstein ST, et al. Pregnancy Outcomes among Women Receiving rVSVDelta-ZEBOV-GP Ebola Vaccine during the Sierra Leone Trial to Introduce a Vaccine against Ebola. Emerg Infect Dis 2020;26(3):541-548. DOI: 10.3201/eid2603.191018.

50. Simon JK, Kennedy SB, Mahon BE, et al. Immunogenicity of rVSVDeltaG-ZEBOV-GP Ebola vaccine (ERVEBO®) in African clinical trial participants by age, sex, and baseline GP-ELISA titer: A post hoc analysis of three Phase 2/3 trials. Vaccine 2022;40(46):6599-6606. DOI: 10.1016/j.vaccine.2022.09.037.

51. Kennedy S, Bolay F, Kieh M, et al. Phase 2 placebo-controlled trial of two vaccines to prevent ebola in Liberia. New England Journal of Medicine 2017;377(15):1438-1447. DOI: 10.1056/NEJMoa1614067.

52. De Santis O, Audran R, Pothin E, et al. Safety and immunogenicity of a chimpanzee adenovirus-vectored Ebola vaccine in healthy adults: a randomised, double-blind, placebo-controlled, dose-finding, phase 1/2a study. Lancet Infect Dis 2016;16(3):311-20. DOI: 10.1016/S1473-3099(15)00486-7.

53. Tapia MD, Sow SO, Ndiaye BP, et al. Safety, reactogenicity, and immunogenicity of a chimpanzee adenovirus vectored Ebola vaccine in adults in Africa: a randomised, observer-blind, placebo-controlled, phase 2 trial. Lancet Infect Dis 2020;20(6):707-718. DOI: 10.1016/S1473-3099(20)30016-5.

54. Tapia MD, Sow SO, Mbaye KD, 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(6):719-730. DOI: 10.1016/S1473-3099(20)30019-0.

55. Ledgerwood JE, DeZure AD, Stanley DA, et al. Chimpanzee Adenovirus Vector Ebola Vaccine. The New England journal of medicine 2017;376(10):928-938. DOI: 10.1056/NEJMoa1410863.

56. Ewer K, Rampling T, Venkatraman N, et al. A Monovalent Chimpanzee Adenovirus Ebola Vaccine Boosted with MVA. The New England journal of medicine 2016;374(17):1635-46. DOI: 10.1056/NEJMoa1411627.

57. Tapia MD, Sow SO, Lyke KE, et al. Use of ChAd3-EBO-Z Ebola virus vaccine in Malian and US adults, and boosting of Malian adults with MVA-BN-Filo: a phase 1, single-blind, randomised trial, a phase 1b, open-label and double-blind, dose-escalation trial, and a nested, randomised, double-blind, placebo-controlled trial. Lancet Infect Dis 2016;16(1):31-42. DOI: 10.1016/S1473-3099(15)00362-X.

58. Venkatraman N, Ndiaye BP, Bowyer G, et al. Safety and Immunogenicity of a Heterologous Prime-Boost Ebola Virus Vaccine Regimen in Healthy Adults in the United Kingdom and Senegal. The Journal of infectious diseases 2019;219(8):1187-1197. DOI: 10.1093/infdis/jiy639.

59. Milligan ID, Gibani MM, Sewell R, et al. Safety and Immunogenicity of Novel Adenovirus Type 26- and Modified Vaccinia Ankara-Vectored Ebola Vaccines: A Randomized Clinical Trial. JAMA 2016;315(15):1610-23. DOI: 10.1001/jama.2016.4218.

60. Shukarev G, Callendret B, Luhn K, Douoguih M, consortium E. A two-dose heterologous prime-boost vaccine regimen eliciting sustained immune responses to Ebola Zaire could support a preventive strategy for future outbreaks. Hum Vaccin Immunother 2017;13(2):266-270. DOI: 10.1080/21645515.2017.1264755.

61. Winslow RL, Milligan ID, Voysey M, et al. Immune Responses to Novel Adenovirus Type 26 and Modified Vaccinia Virus Ankara-Vectored Ebola Vaccines at 1 Year. JAMA 2017;317(10):1075-1077. DOI: 10.1001/jama.2016.20644.

62. Anywaine Z, Whitworth H, Kaleebu P, et al. Safety and Immunogenicity of a 2-Dose Heterologous Vaccination Regimen With Ad26.ZEBOV and MVA-BN-Filo Ebola Vaccines: 12-Month Data From a Phase 1 Randomized Clinical Trial in Uganda and Tanzania. The Journal of infectious diseases 2019;220(1):46-56. DOI: 10.1093/infdis/jiz070.

63. Mutua G, Anzala O, Luhn K, et al. Safety and Immunogenicity of a 2-Dose Heterologous Vaccine Regimen With Ad26.ZEBOV and MVA-BN-Filo Ebola Vaccines: 12-Month Data From a Phase 1 Randomized Clinical Trial in Nairobi, Kenya. The Journal of infectious diseases 2019;220(1):57-67. DOI: 10.1093/infdis/jiz071.

64. Goldstein N, Bockstal V, Bart S, et al. Safety and Immunogenicity of Heterologous and Homologous 2-Dose Regimens of Adenovirus Serotype 26- and Modified Vaccinia Ankara-Vectored Ebola Vaccines: A Randomized, Controlled Phase 1 Study. The Journal of infectious diseases 2022;226(4):595-607. DOI: 10.1093/infdis/jiaa586.

65. Barry H, Mutua G, Kibuuka H, et al. Safety and immunogenicity of 2-dose heterologous Ad26.ZEBOV, MVA-BN-Filo Ebola vaccination in healthy and HIV-infected adults: A randomised, placebo-controlled Phase II clinical trial in Africa. PLoS Med 2021;18(10):e1003813. DOI: 10.1371/journal.pmed.1003813.

66. Anywaine Z, Barry H, Anzala O, 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 Medicine 2022;19(1):e1003865. DOI: 10.1371/journal.pmed.1003865.

67. Pollard AJ, Launay O, Lelievre JD, et al. Safety and immunogenicity of a two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in adults in Europe (EBOVAC2): a randomised, observer-blind, participant-blind, placebo-controlled, phase 2 trial. Lancet Infect Dis 2021;21(4):493-506. DOI: 10.1016/S1473-3099(20)30476-X.

68. Afolabi MO, Ishola D, Manno D, 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(1):110-122. DOI: 10.1016/S1473-3099(21)00128-6.

69. Manno D, Bangura A, Baiden F, 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 2023;23(3):352-360. DOI: 10.1016/S1473-3099(22)00594-1.

70. PREVAC Study Team, Kieh M, Richert L et al. Randomized Trial of Vaccines for Zaire Ebola Virus Disease. The New England journal of medicine 2022;387(26):2411-2424. DOI: 10.1056/NEJMoa2200072.

71. Bockstal V, Shukarev G, McLean C, et al. First-in-human study to evaluate safety, tolerability, and immunogenicity of heterologous regimens using the multivalent filovirus vaccines Ad26.Filo and MVA-BN-Filo administered in different sequences and schedules: A randomized, controlled study. PLoS ONE 2022;17(10):e0274906. DOI: 10.1371/journal.pone.0274906.

72. Zhu FC, Hou LH, Li JX, et al. Safety and immunogenicity of a novel recombinant adenovirus type-5 vector-based Ebola vaccine in healthy adults in China: preliminary report of a randomised, double-blind, placebo-controlled, phase 1 trial. Lancet 2015;385(9984):2272-9. DOI: 10.1016/S0140-6736(15)60553-0.

73. Li JX, Hou LH, Meng FY, et al. Immunity duration of a recombinant adenovirus type-5 vector-based Ebola vaccine and a homologous prime-boost immunisation in healthy adults in China: final report of a randomised, double-blind, placebo-controlled, phase 1 trial. Lancet Glob Health 2017;5(3):e324-e334. DOI: 10.1016/S2214-109X(16)30367-9.

74. Zhu FC, Wurie AH, Hou LH, et al. Safety and immunogenicity of a recombinant adenovirus type-5 vector-based Ebola vaccine in healthy adults in Sierra Leone: a single-centre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet 2017;389(10069):621-628. DOI: 10.1016/S0140-6736(16)32617-4.

75. Wu L, Zhang Z, Gao H, et al. Open-label phase I clinical trial of Ad5-EBOV in Africans in China. Hum Vaccin Immunother 2017;13(9):2078-2085. DOI: 10.1080/21645515.2017.1342021.

76. Ledgerwood JE, Costner P, Desai N, et al. A replication defective recombinant Ad5 vaccine expressing Ebola virus GP is safe and immunogenic in healthy adults. Vaccine 2010;29(2):304-13. DOI: 10.1016/j.vaccine.2010.10.037.

77. Dolzhikova IV, Zubkova OV, Tukhvatulin AI, et al. Safety and immunogenicity of GamEvac-Combi, a heterologous VSV- and Ad5-vectored Ebola vaccine: An open phase I/II trial in healthy adults in Russia. Hum Vaccin Immunother 2017;13(3):613-620. DOI: 10.1080/21645515.2016.1238535.

78. Clarke DK, Xu R, Matassov D, et al. Safety and immunogenicity of a highly attenuated rVSVN4CT1-EBOVGP1 Ebola virus vaccine: a randomised, double-blind, placebo-controlled, phase 1 clinical trial. Lancet Infect Dis 2020;20(4):455-466. DOI: 10.1016/S1473-3099(19)30614-0.

79. Martin JE, Sullivan NJ, Enama ME, et al. A DNA vaccine for Ebola virus is safe and immunogenic in a phase I clinical trial. Clin Vaccine Immunol 2006;13(11):1267-77. DOI: 10.1128/CVI.00162-06.

80. Kibuuka H, Berkowitz NM, Millard M, et al. Safety and immunogenicity of Ebola virus and Marburg virus glycoprotein DNA vaccines assessed separately and concomitantly in healthy Ugandan adults: a phase 1b, randomised, double-blind, placebo-controlled clinical trial. Lancet 2015;385(9977):1545-54. DOI: 10.1016/S0140-6736(14)62385-0.

81. Sarwar UN, Costner P, Enama ME, et al. Safety and immunogenicity of DNA vaccines encoding Ebolavirus and Marburgvirus wild-type glycoproteins in a phase I clinical trial. The Journal of infectious diseases 2015;211(4):549-57. DOI: 10.1093/infdis/jiu511.

82. Fries L, Cho I, Krahling V, et al. Randomized, Blinded, Dose-Ranging Trial of an Ebola Virus Glycoprotein Nanoparticle Vaccine With Matrix-M Adjuvant in Healthy Adults. The Journal of infectious diseases 2020;222(4):572-582. DOI: 10.1093/infdis/jiz518.

83. Bockstal V, Gaddah A, Goldstein N, et al. Assessments of different batches and dose levels of a two-dose Ad26.ZEBOV and MVA-BN-Filo vaccine regimen. NPJ Vaccines 2021;6(1):157. DOI: 10.1038/s41541-021-00402-8.

84. Nyombayire J, Ingabire R, Magod B, 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. The Journal of infectious diseases 2023;227(2):268-277. DOI: 10.1093/infdis/jiac283.

85. Gsell PS, Camacho A, Kucharski AJ, et al. Ring vaccination with rVSV-ZEBOV under expanded access in response to an outbreak of Ebola virus disease in Guinea, 2016: an operational and vaccine safety report. Lancet Infect Dis 2017;17(12):1276-1284. DOI: 10.1016/S1473-3099(17)30541-8.

86. Carnino L, Vetter P, Peyraud N, et al. Feasibility and safety of rVSV-ZEBOV vaccination of humanitarian health workers against Ebola virus disease: an observational study. J Travel Med 2021;28(8). DOI: 10.1093/jtm/taab086.

87. Hoff NA, Bratcher A, Kelly JD, et al. Immunogenicity of rVSVDeltaG-ZEBOV-GP Ebola vaccination in exposed and potentially exposed persons in the Democratic Republic of the Congo. Proc Natl Acad Sci U S A 2022;119(6). DOI: 10.1073/pnas.2118895119.

88. Rupani N, Ngole ME, Lee JA, 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(6):1180-1188. DOI: 10.3201/eid2806.212223.

89. World Health Organization (WHO). Ebola virus disease: Vaccines. https://www.who.int/news-room/questions-and-answers/item/ebola-vaccines (Accessed on 11 November 2023).

90. Woolsey C, Geisbert TW. Current state of Ebola virus vaccines: A snapshot. PLoS Pathog 2021;17(12):e1010078. DOI: 10.1371/journal.ppat.1010078.

91. Finch CL, Martinez C, Leffel E, et al. Vaccine Licensure in the Absence of Human Efficacy Data. Vaccines (Basel) 2022;10(3). DOI: 10.3390/vaccines10030368.

92. World Health Organization (WHO). Overview of candidate Ebola vaccines as of August 19, 2019. https://www.nesi.be/wp-content/uploads/2021/03/WHO.-Ebola-Candidate-Vaccines_August-2019.pdf (Accessed on 11 November 2023).

93. Choi EM, Lacarra B, Afolabi MO, et al. Safety and immunogenicity of the two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in infants: a phase 2, randomised, double-blind, active-controlled trial in Guinea and Sierra Leone. Lancet Glob Health 2023;11(11):e1743-e1752. DOI: 10.1016/S2214-109X(23)00410-2.

94. Lariviere Y, Garcia-Fogeda I, Zola Matuvanga T, et al. Safety and Immunogenicity of the Heterologous 2-Dose Ad26.ZEBOV, MVA-BN-Filo Vaccine Regimen in Health Care Providers and Frontliners of the Democratic Republic of the Congo. The Journal of infectious diseases 2023. DOI: 10.1093/infdis/jiad350.

95. Mwesigwa B, Houser KV, Hofstetter AR, et al. Safety, tolerability, and immunogenicity of the Ebola Sudan chimpanzee adenovirus vector vaccine (cAd3-EBO S) in healthy Ugandan adults: a phase 1, open-label, dose-escalation clinical trial. Lancet Infect Dis 2023. DOI: 10.1016/S1473-3099(23)00344-4.

96. McLean C, Barry H, Kieh M, et al. Immune response of a two-dose heterologous Ebola vaccine regimen: summary of three African clinical trials using a single validated Filovirus Animal Nonclinical Group enzyme-linked

immunosorbent assay in a single accredited laboratory. EBioMedicine 2023;91:104562. DOI: 10.1016/j.ebiom.2023.104562.

97. Huttner A, Agnandji ST, Engler O, 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. DOI: 10.1016/j.cmi.2023.08.026.

98. Marzi A, Feldmann H, Geisbert TW, Falzarano D. Vesicular Stomatitis Virus-Based Vaccines for Prophylaxis and Treatment of Filovirus Infections. J Bioterror Biodef 2011;S1(4). DOI: 10.4172/2157-2526.S1-004.

99. European Commission. Vaccine against Ebola: Commission grants first-ever market authorisation. https://ec.europa.eu/commission/presscorner/detail/en/ip_19_6246 (Accessed on 11 November 2023).

100. Merck. ERVEBO® (Ebola Zaire Vaccine, Live) now registered in four African countries, within 90 days of reference country approval and WHO prequalification. https://www.merck.com/news/ervebo-ebola-zaire-vaccine-live-now-registered-in-four-african-countries-within-90-days-of-reference-country-approval-and-who-prequalification/ (Accessed on 11 November 2023).

101. Burki T. Ebola virus vaccine receives prequalification. Lancet 2019;394(10212):1893. DOI: 10.1016/S0140-6736(19)32905-8.

102. US Food and Drug Administration (FDA). First FDA-approved vaccine for the prevention of Ebola virus disease, marking a critical milestone in public health preparedness and response. https://www.fda.gov/news-events/press-announcements/first-fda-approved-vaccine-prevention-ebola-virus-disease-marking-critical-milestone-public-health (Accessed on 11 November 2023).

103. US Food and Drug Administration (FDA). Ervebo. https://www.fda.gov/vaccines-blood-biologics/ervebo (Accessed on 11 November 2023).

104. Callendret B, Vellinga J, Wunderlich K, et al. A prophylactic multivalent vaccine against different filovirus species is immunogenic and provides protection from lethal infections with Ebolavirus and Marburgvirus species in non-human primates. PLoS ONE 2018;13(2). DOI: 10.1371/journal.pone.0192312.

105. Johnson & Johnson. Johnson & Johnson joins World Health Organization in efforts to prevent spread of Ebola in West Africa. 13 May 2021. https://www.jnj.com/johnson-johnson-joins-world-health-organization-in-

efforts-to-prevent-spread-of-ebola-in-west-africa (Accessed on 11 November 2023).

106. European Commission. Vaccine against Ebola: Commission grants new market authorisations. https://ec.europa.eu/commission/presscorner/detail/%20en/ip_20_1248 (Accessed on 11 November 2023).

107. Johnson & Johnson. Ebola. https://healthforhumanityreport.jnj.com/2022/global-health-equity/pandemics-epidemics/ebola.html. (Accessed on 11 November 2023).

108. World Health Organization (WHO). Strategic Advisory Group of Experts (SAGE) on immunization interim recommendations on vaccination against Ebola virus disease (EVD). https://cdn.who.int/media/docs/default-source/immunization/ebola/interim-ebola-recommendations-may-2019.pdf?sfvrsn=c54ce264_9 (Accessed on 11 November 2023).

109. World Health Organization (WHO). Meeting of the strategic advisory group of experts on immunization, 22–24 March 2021: conclusions and recommendations. Weekly Epidemiological Record 2021, 22, 197-216.

110. Innovative Medicine Initiative (IMI). EBOVAC1. https://www.imi.europa.eu/projects-results/project-factsheets/ebovac1 (Accessed on 11 November 2023).

111. EBOVAC Projects. The Ebola Vaccine Projects, https://www.ebovac.org (Accessed on 11 November 2023).

112. Cutts FT, Hanson M. Seroepidemiology: an underused tool for designing and monitoring vaccination programmes in low- and middle-income countries. Trop Med Int Health 2016;21(9):1086-98. DOI: 10.1111/tmi.12737.

113. World Health Organization (WHO). Ebola situation report 30 March 2016. https://apps.who.int/iris/handle/10665/204714 (Accessed on 11 November 2023).

114. Heffernan RT, Pambo B, Hatchett RJ, Leman PA, Swanepoel R, Ryder RW. Low seroprevalence of IgG antibodies to Ebola virus in an epidemic zone: Ogooue-Ivindo region, Northeastern Gabon, 1997. The Journal of infectious diseases 2005;191(6):964-8. DOI: 10.1086/427994.

115. Leroy EM, Baize S, Volchkov VE, et al. Human asymptomatic Ebola infection and strong inflammatory response. Lancet 2000;355(9222):2210-5. DOI: 10.1016/s0140-6736(00)02405-3.

116. Sonnenberg P, Field N. Sexual and mother-to-child transmission of Ebola virus in the postconvalescent period. Clinical infectious diseases : an official

publication of the Infectious Diseases Society of America 2015;60(6):974-5. DOI: 10.1093/cid/ciu981.

117. Christie A, Davies-Wayne GJ, Cordier-Lasalle T, et al. Possible sexual transmission of Ebola virus - Liberia, 2015. MMWR Morbidity and mortality weekly report 2015;64(17):479-81.

118. Bellan SE, Pulliam JR, Dushoff J, Meyers LA. Ebola control: effect of asymptomatic infection and acquired immunity. Lancet 2014;384(9953):1499-500. DOI: 10.1016/S0140-6736(14)61839-0.

119. Bower H, Glynn J. A systematic review and meta-analysis of seroprevalence surveys of ebolavirus infection. Sci Data 2017;4:160133. DOI: 10.1038/sdata.2016.133.

120. Dean NE, Halloran ME, Yang Y, Longini IM. Transmissibility and Pathogenicity of Ebola Virus: A Systematic Review and Meta-analysis of Household Secondary Attack Rate and Asymptomatic Infection. Clinical Infectious Diseases 2016;62(10):1277-1286.

121. Nyakarahuka L, Kankya C, Krontveit R, et al. How severe and prevalent are Ebola and Marburg viruses? A systematic review and meta-analysis of the case fatality rates and seroprevalence. BMC Infect Dis 2016;16(1):708. DOI: 10.1186/s12879-016-2045-6.

122. Bratcher A, Hoff NA, Doshi RH, et al. Zoonotic risk factors associated with seroprevalence of Ebola virus GP antibodies in the absence of diagnosed Ebola virus disease in the Democratic Republic of Congo. PLoS Negl Trop Dis 2021;15(8):e0009566. DOI: 10.1371/journal.pntd.0009566.

123. Doshi RH, Hoff NA, Bratcher A, et al. Risk Factors for Ebola Exposure in Health Care Workers in Boende, Tshuapa Province, Democratic Republic of the Congo. The Journal of infectious diseases 2022;226(4):608-615. DOI: 10.1093/infdis/jiaa747.

124. Hoff NA, Mukadi P, Doshi RH, et al. Serologic Markers for Ebolavirus Among Healthcare Workers in the Democratic Republic of the Congo. The Journal of infectious diseases 2019;219(4):517-525. DOI: 10.1093/infdis/jiy499.

125. Bane S, Rosenke K, Maiga O, et al. Ebola Virus IgG Seroprevalence in Southern Mali. Emerg Infect Dis 2021;27(6):1681-1684. DOI: 10.3201/eid2706.203510.

126. Mafopa NG, Russo G, Wadoum REG, et al. Seroprevalence of Ebola virus infection in Bombali District, Sierra Leone. J Public Health Afr 2017;8(2):732. DOI: 10.4081/jphia.2017.732.

127. Halfmann PJ, Eisfeld AJ, Watanabe T, et al. Serological analysis of Ebola virus survivors and close contacts in Sierra Leone: A cross-sectional study. PLoS Negl Trop Dis 2019;13(8):e0007654. DOI: 10.1371/journal.pntd.0007654.

128. Steffen I, Lu K, Yamamoto LK, et al. Serologic Prevalence of Ebola Virus in Equatorial Africa. Emerg Infect Dis 2019;25(5):911-918. DOI: 10.3201/eid2505.180115.

129. Shaffer KCL, Hui S, Bratcher A, et al. Pan-ebolavirus serology study of healthcare workers in the Mbandaka Health Region, Democratic Republic of the Congo. PLoS Negl Trop Dis 2022;16(3):e0010167. DOI: 10.1371/journal.pntd.0010167.

130. Lucas A, Kumakamba C, Lange CE, et al. Serology and Behavioral Perspectives on Ebola Virus Disease Among Bushmeat Vendors in Equateur, Democratic Republic of the Congo, After the 2018 Outbreak. Open Forum Infect Dis 2020;7(8):ofaa295. DOI: 10.1093/ofid/ofaa295.

131. Goldstein T, Belaganahalli MN, Syaluha EK, et al. Spillover of ebolaviruses into people in eastern Democratic Republic of Congo prior to the 2018 Ebola virus disease outbreak. One Health Outlook 2020;2(1):21. DOI: 10.1186/s42522-020-00028-1.

132. Mulangu S, Alfonso VH, Hoff NA, et al. Serologic Evidence of Ebolavirus Infection in a Population With No History of Outbreaks in the Democratic Republic of the Congo. The Journal of infectious diseases 2018;217(4):529-537. DOI: 10.1093/infdis/jix619.

133. Mbala P, Baguelin M, Ngay I, et al. Evaluating the frequency of asymptomatic Ebola virus infection. Philos Trans R Soc Lond B Biol Sci 2017;372(1721). DOI: 10.1098/rstb.2016.0303.

134. Manno D, Ayieko P, Ishola D, et al. Ebola Virus Glycoprotein IgG Seroprevalence in Community Previously Affected by Ebola, Sierra Leone. Emerg Infect Dis 2022;28(3):734-738. DOI: 10.3201/eid2803.211496.

135. Nyakarahuka L, Schafer IJ, Balinandi S, et al. A retrospective cohort investigation of seroprevalence of Marburg virus and ebolaviruses in two different ecological zones in Uganda. BMC Infect Dis 2020;20(1):461. DOI: 10.1186/s12879-020-05187-0.

136. Nkuba-Ndaye A, Mukadi-Bamuleka D, Bulabula-Penge J, et al. Added Value of an Anti-Ebola Serology for the Management of Clinically Suspected Ebola Virus Disease Patients Discharged as Negative in an Epidemic Context. The Journal of infectious diseases 2022;226(2):352-356. DOI: 10.1093/infdis/jiac057.

137. Keita AK, Butel C, Thaurignac G, et al. Serological Evidence of Ebola Virus Infection in Rural Guinea before the 2014 West African Epidemic Outbreak. Am J Trop Med Hyg 2018;99(2):425-427. DOI: 10.4269/ajtmh.18-0105.

138. Diallo MSK, Rabilloud M, Ayouba A, et al. Prevalence of infection among asymptomatic and paucisymptomatic contact persons exposed to Ebola virus in Guinea: a retrospective, cross-sectional observational study. Lancet Infect Dis 2019;19(3):308-316. DOI: 10.1016/S1473-3099(18)30649-2.

139. O'Hearn AE, Voorhees MA, Fetterer DP, et al. Serosurveillance of viral pathogens circulating in West Africa. Virol J 2016;13(1):163. DOI: 10.1186/s12985-016-0621-4.

140. Dovih P, Laing ED, Chen Y, et al. Filovirus-reactive antibodies in humans and bats in Northeast India imply zoonotic spillover. PLoS Negl Trop Dis 2019;13(10):e0007733. DOI: 10.1371/journal.pntd.0007733.

141. Smiley Evans T, Tutaryebwa L, Gilardi KV, et al. Suspected Exposure to Filoviruses Among People Contacting Wildlife in Southwestern Uganda. The Journal of infectious diseases 2018;218(suppl_5):S277-S286. DOI: 10.1093/infdis/jiy251.

142. Glynn JR, Bower H, Johnson S, et al. Asymptomatic infection and unrecognised Ebola virus disease in Ebola-affected households in Sierra Leone: a cross-sectional study using a new non-invasive assay for antibodies to Ebola virus. Lancet Infect Dis 2017;17(6):645-653. DOI: 10.1016/S1473-3099(17)30111-1.

143. Houlihan CF, McGowan CR, Dicks S, et al. Ebola exposure, illness experience, and Ebola antibody prevalence in international responders to the West African Ebola epidemic 2014-2016: A cross-sectional study. PLoS Med 2017;14(5):e1002300. DOI: 10.1371/journal.pmed.1002300.

144. Zola Matuvanga T, Marien J, Lariviere Y, et al. Low seroprevalence of Ebola virus in health care providers in an endemic region (Tshuapa province) of the Democratic Republic of the Congo. PLoS ONE 2023;18(9):e0286479. DOI: 10.1371/journal.pone.0286479.

145. Kelly JD, Barrie MB, Mesman AW, et al. Anatomy of a Hotspot: Chain and Seroepidemiology of Ebola Virus Transmission, Sukudu, Sierra Leone, 2015-16. The Journal of infectious diseases 2018;217(8):1214-1221. DOI: 10.1093/infdis/jiy004.

146. Mulangu S, Borchert M, Paweska J, et al. High prevalence of IgG antibodies to Ebola virus in the Efe pygmy population in the Watsa region, Democratic Republic of the Congo. BMC Infectious Diseases 2016;16(1). DOI: 10.1186/s12879-016-1607-y.

147. Gonzalez J, Nakoune E, Slenczka W, Vidal P, Morvan J. Ebola and Marburg virus antibody prevalence in selected populations of the Central African Republic. Microbes Infect 2000;2(1):39-44. DOI: 10.1016/S1286-4579%2800%2900287-2.

148. Busico K, Marshall K, Ksiazek TG, et al. Prevalence of IgG antibodies to Ebola virus in individuals during an Ebola outbreak, Democratic Republic of the Congo, 1995. Journal of Infectious Diseases 1999;179(SUPPL. 1):S102-S107.

149. Bouree P, Bergmann JF. Ebola virus infection in man: a serological and epidemiological survey in the Cameroons. Am J Trop Med Hyg 1983;32(6):1465-6.

150. Allela L, Boury O, Pouillot R, et al. Ebola virus antibody prevalence in dogs and human risk. Emerg Infect Dis 2005;11(3):385-90.

151. Haun BK, Kamara V, Dweh AS, et al. Serological evidence of Ebola virus exposure in dogs from affected communities in Liberia: A preliminary report. PLoS Negl Trop Dis 2019;13(7):e0007614.

152. Gumusova S, Sunbul M, Leblebicioglu H. Ebola virus disease and the veterinary perspective. Annals of Clinical Microbiology and Antimicrobials 2015;14(1). DOI: 10.1186/s12941-015-0089-x.

153. Fischer K, Camara A, Troupin C, et al. Serological evidence of exposure to ebolaviruses in domestic pigs from Guinea. Transbound Emerg Dis 2020;67(2):724-732.

154. Fischer K, Jabaty J, Suluku R, et al. Serological Evidence for the Circulation of Ebolaviruses in Pigs From Sierra Leone. The Journal of infectious diseases 2018;218(suppl_5):S305-S311.

155. Weingartl HM, Embury-Hyatt C, Nfon C, Leung A, Smith G, Kobinger G. Transmission of Ebola virus from pigs to non-human primates. Sci rep 2012;2:811.

156. Rudge TL, Jr., Sankovich KA, Niemuth NA, 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(4):e0215457. DOI: 10.1371/journal.pone.0215457.

157. Logue J, Tuznik K, Follmann D, et al. Use of the Filovirus Animal Non-Clinical Group (FANG) Ebola virus immuno-assay requires fewer study participants to power a study than the Alpha Diagnostic International assay. J Virol Methods 2018;255:84-90. DOI: 10.1016/j.jviromet.2018.02.018.

158. Brainard J, Hooper L, Pond K, Edmunds K, Hunter P, Email Hunter P. Risk factors for transmission of Ebola or Marburg virus disease: A systematic review and meta-analysis. International Journal of Epidemiology 2016;45(1):102-116. DOI: 10.1093/ije/dyv307.

159. Luiselli L, Hema, E., Segniagbeto, G. et al. Bushmeat consumption in large urban centres in West Africa. Oryx 2018; 1-4. doi:10.1017/S0030605318000893.

160. Milner-Gulland EJ, Bennett EL, Abernethy K, et al. Wild meat: the bigger picture. Trends in Ecology and Evolution 2003;18 (7): 351-357.

161. Barrette RW, Metwally SA, Rowland JM, et al. Discovery of swine as a host for the Reston ebolavirus. Science 2009;325(5937):204-6.

162. Ogawa H, Miyamoto H, Nakayama E, et al. Seroepidemiological Prevalence of Multiple Species of Filoviruses in Fruit Bats (Eidolon helvum) Migrating in Africa. The Journal of infectious diseases 2015;212 Suppl 2:S101-8. DOI: 10.1093/infdis/jiv063.

163. Macneil A, Reed Z, Rollin PE. Serologic cross-reactivity of human IgM and IgG antibodies to five species of Ebola virus. PLoS Negl Trop Dis 2011;5(6):e1175. DOI: 10.1371/journal.pntd.0001175.

164. O'Hara GA, Duncan CJ, Ewer KJ, et al. Clinical assessment of a recombinant simian adenovirus ChAd63: a potent new vaccine vector. The Journal of infectious diseases 2012;205(5):772-81. DOI: 10.1093/infdis/jir850.

165. Reyes-Sandoval A, Berthoud T, Alder N, et al. Prime-boost immunization with adenoviral and modified vaccinia virus Ankara vectors enhances the durability and polyfunctionality of protective malaria CD8+ T-cell responses. Infect Immun 2010;78(1):145-53. DOI: 10.1128/IAI.00740-09.

166. Sheehy SH, Duncan CJ, Elias SC, et al. Phase Ia clinical evaluation of the safety and immunogenicity of the Plasmodium falciparum blood-stage antigen AMA1 in ChAd63 and MVA vaccine vectors. PLoS ONE 2012;7(2):e31208. DOI: 10.1371/journal.pone.0031208.

167. Barouch DH, Tomaka FL, Wegmann F, et al. Evaluation of a mosaic HIV-1 vaccine in a multicentre, randomised, double-blind, placebo-controlled, phase 1/2a clinical trial (APPROACH) and in rhesus monkeys (NHP 13-19). Lancet 2018;392(10143):232-243. DOI: 10.1016/S0140-6736(18)31364-3.

168. Swadling L, Capone S, Antrobus RD, et al. A human vaccine strategy based on chimpanzee adenoviral and MVA vectors that primes, boosts, and sustains functional HCV-specific T cell memory. Sci Transl Med 2014;6(261):261ra153. DOI: 10.1126/scitranslmed.3009185.

169. Camacho A, Kucharski A, Aki-Sawyerr Y, et al. Temporal Changes in Ebola Transmission in Sierra Leone and Implications for Control Requirements: a Real-time Modelling Study. PLoS Curr 2015;7. DOI: 10.1371/currents.outbreaks.406ae55e83ec0b5193e30856b9235ed2.

170. Gomes MF, de la Fuente-Nunez V, Saxena A, Kuesel AC. Protected to death: systematic exclusion of pregnant women from Ebola virus disease trials. Reprod Health 2017;14(Suppl 3):172. DOI: 10.1186/s12978-017-0430-2.

171. Roozendaal R, Hendriks J, van Effelterre T, et al. Nonhuman primate to human immunobridging to infer the protective effect of an Ebola virus vaccine candidate. npj Vaccines 2020;5(1):112. DOI: 10.1038/s41541-020-00261-9.

172. Bockstal. V. EBOVAC1 Work Package 3: Immune Monitoring. EBOVAC1 annual meeting 29-30 January 2019. Nairobi, Kenya.

173. World Health Organization (WHO). World malaria report 2022. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2022 (Accessed on 11 November 2023).

174. Portugal S, Tipton CM, Sohn H, et al. Malaria-associated atypical memory B cells exhibit markedly reduced B cell receptor signaling and effector function. Elife 2015;4. DOI: 10.7554/eLife.07218.

175. Usen S, Milligan P, Ethevenaux C, Greenwood B, Mulholland K. Effect of fever on the serum antibody response of Gambian children to Haemophilus influenzae type b conjugate vaccine. Pediatr Infect Dis J 2000;19(5):444-9. DOI: 10.1097/00006454-200005000-00010.

176. Greenwood BM, Bradley AK, Blakebrough IS, Whittle HC, Marshall TF, Gilles HM. The immune response to a meningococcal polysaccharide vaccine in an African village. Trans R Soc Trop Med Hyg 1980;74(3):340-6. DOI: 10.1016/0035-9203(80)90095-4.

177. Bradley-Moore AM, Greenwood BM, Bradley AK, et al. Malaria chemoprophylaxis with chloroquine in young Nigerian children. II. Effect on the immune response to vaccination. Ann Trop Med Parasitol 1985;79(6):563-73. DOI: 10.1080/00034983.1985.11811963.

178. Brown J, Baisley K, Kavishe B, et al. Impact of malaria and helminth infections on immunogenicity of the human papillomavirus-16/18 AS04-adjuvanted vaccine in Tanzania. Vaccine 2014;32(5):611-7. DOI: 10.1016/j.vaccine.2013.11.061.

179. Nakalembe M, Banura C, Namujju PB, Mirembe FM. Immunogenicity to the bivalent HPV-16/18 vaccine among adolescent African students exposed to

helminths and malaria. J Infect Dev Ctries 2015;9(2):197-205. DOI: 10.3855/jidc.5719.

180. National Malaria Control Programme (NMCP) [Sierra Leone], Statistics Sierra Leone, University of Sierra Leone,Catholic Relief Services, and ICF International. 2013. Sierra Leone Malaria Indicator Survey. Freetown, Sierra Leone: NMCP, SSL, CRS, and ICF International. https://dhsprogram.com/pubs/pdf/MIS15/MIS15.pdf (Accessed on 11 November 2023).

181. National Malaria Control Programme (NMCP) [Sierra Leone], Statistics Sierra Leone, University of Sierra Leone, Catholic Relief Services, and ICF. 2016. Sierra Leone Malaria Indicator Survey 2016. Freetown, Sierra Leone: NMCP, SSL, CRS, and ICF. https://dhsprogram.com/pubs/pdf/MIS25/MIS25.pdf (Accessed on 11 November 2023).

182. Manno D., Patterson C, Drammeh A, et al. The Effect of Previous Exposure to Malaria Infection and Clinical Malaria Episodes on the Immune Response to the Two-Dose Ad26.ZEBOV, MVA-BN-Filo Ebola Vaccine Regimen. Vaccines (Basel) 2023;11(8) (https://doi.org/10.3390/vaccines11081317).

183. Achan J, Reuling IJ, Yap XZ, et al. Serologic Markers of Previous Malaria Exposure and Functional Antibodies Inhibiting Parasite Growth Are Associated With Parasite Kinetics Following a Plasmodium falciparum Controlled Human Infection. Clinical infectious diseases 2020;70(12):2544-2552. DOI: 10.1093/cid/ciz740.

184. Wu L, Hall T, Ssewanyana I, et al. Optimisation and standardisation of a multiplex immunoassay of diverse Plasmodium falciparum antigens to assess changes in malaria transmission using sero-epidemiology. Wellcome Open Res 2019;4:26. DOI: 10.12688/wellcomeopenres.14950.2.

185. Triglia T, Healer J, Caruana SR, et al. Apical membrane antigen 1 plays a central role in erythrocyte invasion by Plasmodium species. Mol Microbiol 2000;38(4):706-18. DOI: 10.1046/j.1365-2958.2000.02175.x.

186. Moss DK, Remarque EJ, Faber BW, et al. Plasmodium falciparum 19-kilodalton merozoite surface protein 1 (MSP1)-specific antibodies that interfere with parasite growth in vitro can inhibit MSP1 processing, merozoite invasion, and intracellular parasite development. Infect Immun 2012;80(3):1280-7. DOI: 10.1128/IAI.05887-11.

187. Obiero JM, Shekalaghe S, Hermsen CC, et al. Impact of malaria preexposure on antiparasite cellular and humoral immune responses after controlled human malaria infection. Infect Immun 2015;83(5):2185-96. DOI: 10.1128/IAI.03069-14.

188. van den Hoogen LL, Stresman G, Presume J, et al. Selection of Antibody Responses Associated With Plasmodium falciparum Infections in the Context of Malaria Elimination. Front Immunol 2020;11:928. DOI: 10.3389/fimmu.2020.00928.

189. Theisen M, Soe S, Oeuvray C, et al. The glutamate-rich protein (GLURP) of Plasmodium falciparum is a target for antibody-dependent monocyte-mediated inhibition of parasite growth in vitro. Infect Immun 1998;66(1):11-7. DOI: 10.1128/IAI.66.1.11-17.1998.

190. Reiling L, Richards JS, Fowkes FJ, et al. Evidence that the erythrocyte invasion ligand PfRh2 is a target of protective immunity against Plasmodium falciparum malaria. J Immunol 2010;185(10):6157-67. DOI: 10.4049/jimmunol.1001555.

191. Helb DA, Tetteh KK, Felgner PL, et al. Novel serologic biomarkers provide accurate estimates of recent Plasmodium falciparum exposure for individuals and communities. Proc Natl Acad Sci USA 2015;112(32):E4438-47. DOI: 10.1073/pnas.1501705112.

192. Ishola D. and the EBOVAC-Salone Malaria Infection Sub-Study Team. Asymptomatic Malaria Infection and the Immune Response to the 2-Dose Ad26.ZEBOV, MVA-BN-Filo Ebola Vaccine Regimen in Adults and Children. Clinical infectious diseases 2022;75(9):1585-1593. DOI: 10.1093/cid/ciac209.

193. Government of Sierra Leone, Ministry of Health and Sanitation, National Malaria Control Programme. Guidelines for Case Management of Malaria. Fourth Edition, 2015. Available online: https://www.afro.who.int/publications/guidelines-case-management-malaria-2015 (Accessed on 11 November 2023).

194. Hennekens C, Buring J, Mayrent SL. Epidemiology in Medicine: Lippincott Williams & Wilkins, 1987.

195. Mahon BE, Simon J, Widdowson MA, et al. Baseline Asymptomatic Malaria Infection and Immunogenicity of Recombinant Vesicular Stomatitis Virus-Zaire Ebola Virus Envelope Glycoprotein. The Journal of infectious diseases 2021;224(11):1907-1915. DOI: 10.1093/infdis/jiab243.

196. World Health Organization (WHO). Ebola virus disease - Democratic Republic of the Congo - 2019-DON149. https://www.who.int/emergencies/disease-outbreak-news/item/2019-DON149. (Accessed on 11 November 2023).

197. Pigott DM, Golding N, Mylne A, et al. Mapping the zoonotic niche of Ebola virus disease in Africa. eLife Journal 2014;3:e04395. DOI: 10.7554/eLife.04395.

198. Funk S, Piot P. Mapping Ebola in wild animals for better disease control. eLife Journal 2014;3:e04565. DOI: 10.7554/eLife.04565.

199. World Health Organization (WHO). Factors that contributed to undetected spread of the Ebola virus and impeded rapid containment. https://www.who.int/news-room/spotlight/one-year-into-the-ebola-epidemic/factors-that-contributed-to-undetected-spread-of-the-ebola-virus-and-impeded-rapid-containment (Accessed on 11 November 2023).

200. European Medicines Agency - European Union. Zabdeno. https://www.ema.europa.eu/en/medicines/human/EPAR/zabdeno (Accessed on 11 November 2023).

201. World Health Organization (WHO). Ebola vaccination starts in Sierra Leone to prevent new outbreak - 11 May 2021. https://www.afro.who.int/news/ebola-vaccination-starts-sierra-leone-prevent-new-outbreak. (Accessed on 11 November 2023).

202. McLean C, Dijkman K, Gaddah A, et al. Persistence of immunological memory as a potential correlate of long-term, vaccine-induced protection against Ebola virus disease in humans. Front Immunol 2023;14:1215302. DOI: 10.3389/fimmu.2023.1215302.

203. Watson-Jones D, Kavunga-Membo H, Grais RF, et al. Protocol for a phase 3 trial to evaluate the effectiveness and safety of a heterologous, two-dose vaccine for Ebola virus disease in the Democratic Republic of the Congo. BMJ Open 2022;12(3):e055596. DOI: 10.1136/bmjopen-2021-055596.

204. Karita E, Nyombayire J, Ingabire R, et al. Safety, reactogenicity, and immunogenicity of a 2-dose Ebola vaccine regimen of Ad26.ZEBOV followed by MVA-BN-Filo in healthy adult pregnant women: study protocol for a phase 3 open-label randomized controlled trial. Trials 2022;23(1):513. DOI: 10.1186/s13063-022-06360-3.

205. World Health Organization (WHO). Ebola Virus Disease Consolidated Preparedness Checklist, Revision 1 - 15 January 2015. https://iris.who.int/bitstream/handle/10665/137096/WHO_EVD_Preparedness_14_eng.pdf?sequence=1 (Accessed on 11 November 2023).