Evaluating an Ebola Vaccine Regimen and Booster Dose among Healthcare Personnel of the Democratic Republic of the Congo: a Step Towards Outbreak Prevention

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Thesis submitted for the degree of doctor of Medical Sciences at the University of Antwerp to be defended by

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“Choose a job you love, and you will never have to work a day in your life.”
— Confucius

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Summary

In **Part I, Chapter 1**, a short background on Ebola virus disease (EVD), its epidemiology, clinical signs and symptoms, the available preventive measures, and the rationale for the thesis are discussed. EVD is a severe and often deadly disease, caused by orthoebolaviruses. Overall, six orthoebolaviruses have been identified within the *Filoviridae* family, four of which have caused disease in humans. The species *Orthoebolavirus zairense*, otherwise known as Ebola virus (EBOV), has led to the most outbreaks in humans with the highest case fatality rates (CFRs); ranging between 40-90% depending on the outbreak. However, the observed CFR depends on the outbreak and is influenced by factors such as the healthcare infrastructure, public health response (national and international), and access to medical care.

Healthcare providers (HCPs) and frontliners are at high risk of contracting infectious diseases as first contacts of infected patients, and can afterwards contribute to spreading them within the community. Especially for a disease like EVD, that is very deadly but generally presents itself with flu-like symptoms at early onset of the disease, HCP and frontliners are most at risk. Therefore, finding methods to protect this vulnerable population is crucial.

Though first discovered in 1976, EBOV was not considered a real global health threat until 2014 during the West Africa epidemic. In this epidemic, more than 28,600 people became infected and more than 11,300 died. While Guinea, Liberia, and Sierra Leone were most affected during this epidemic, several import cases were also reported in other African countries, European countries, and the United States of America (USA). As an international response to this epidemic, treatment and vaccine development against EBOV has been fast-tracked in the past decade.

One of the vaccines that was fast-tracked in response to the West Africa epidemic was the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen. Over the years, this regimen has been assessed in Phase 1, 2 and 3 trials, conducted in healthy adults, adolescents (12-17 years old), children (1-11 years old), infants younger than one year old, HIV-infected adults with well-controlled infection and on highly active antiretroviral therapy, and children and adults with asymptomatic malaria, or symptomatic malaria before or shortly after vaccination. Within these trials, the vaccination interval, safety, tolerability, and humoral and cellular immunogenicity in response to vaccination were assessed. However, information on the persistence of the binding antibody response after vaccination with the regimen and on the possibility to induce a humoral immune memory response with a booster dose was limited. To assess these aspects, we conducted a large Ebola vaccine trial in Boende, an Ebola endemic region of the Democratic Republic of the Congo (DRC). To prepare this area against a potential future outbreak, we vaccinated HCP and frontliners with the two-dose heterologous Ad26.ZEBOV, MVA-BN-Filo vaccine regimen (administered at a 56-day interval) followed by an Ad26.ZEBOV booster dose one or two years after the first dose (depending on the randomisation arm). The protocol of this trial is published and addresses its methods in **Part I, Chapter 2,** of this doctoral thesis.

Results of the trial are addressed in **Part II,** Chapters 3 and 4. **Chapter 3** includes the publication on the safety and immunogenicity of the heterologous Ad26.ZEBOV, MVA-BN-Filo vaccine regimen and assesses whether certain factors (e.g., age, sex, profession) influence the immune response. The safety was assessed from enrolment until six months after the second dose through the collection of serious adverse events (SAEs). The humoral immune response was assessed until 21 days after the second dose through the measurement of EBOV glycoprotein (GP)-specific immunoglobulin G (IgG). The latter was measured using the Filovirus Animal Nonclinical Group human anti-EBOV GP IgG enzyme-linked immunosorbent assay (FANG ELISA). **Chapter 4** comprises the publication that describes the long-term persistence (i.e., up to two years after vaccination) of EBOV GP-specific IgG binding antibodies after vaccination with the heterologous vaccine regimen, and the safety and immune memory response capabilities of an Ad26.ZEBOV booster dose

administered either one or two years after the first dose. Additionally, the long-term binding antibody persistence after booster vaccination was assessed for the vaccination arm that was boosted one year after the first dose. To assess the safety of these booster doses, SAEs were collected until six months after booster vaccination and (un)solicited adverse events (AEs) were collected for seven days after booster vaccination using a participant journal. The immunogenicity before and after booster vaccination (i.e., at seven days post-booster for both study arms and at one year post-booster for those boosted one year after the first dose only) was measured using FANG ELISA.

While setting-up and conducting the Ebola vaccine trial in Boende, a remote, resource-constrained area in the DRC, we encountered several challenges (e.g., logistical, organisational, financial, healthcare, etc.). These challenges, how we mitigated them, and the lessons that were learned throughout the vaccine trial could be meaningful to other researchers planning to conduct trials in similar settings. Therefore, **Chapters 5 and 6** contain the published articles on the experienced challenges, mitigations and lessons learned from setting up and conducting the Ebola vaccine trial.

As one of the major challenges of the trial, we were confronted with the limited quality healthcare services available to our participants. While a study pharmacy was implemented from the start of the trial to help provide more healthcare options, many medical events of participants were addressed ad hoc. Therefore, a trial-specific ancillary care[1] (AC) algorithm and policy became paramount to ensure and provide equal and systematic care to all trial participants for any medical event. A trial-specific AC algorithm and policy were thus developed, approved by the national ethics committee (EC) in the DRC, published, and implemented by the research team at the start of the third year of the trial. In **Chapter 7**, we present the evaluation of this algorithm and policy, as well as recommendations to take into account when implementing a similar approach in a resource-limited setting. This is

[1] Ancillary care is defined as healthcare provided to research participants that goes beyond the scope and aims of the research being conducted.

the only article in the thesis that has not yet been published. However, it was submitted to the journal *BMJ Global Health* early February 2024 and is currently under review. Chapters 5 to 7 were grouped under **Part III** – Research challenges in low- and middle-income countries (LMICs) – of this doctoral thesis.

Finally, in **Part IV, Chapter 8**, all chapters were combined into a general discussion and conclusion. Overall, the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen, administered at a 56-day interval, was generally well tolerated, led to a persistent immune response, and a similar and robust anamnestic response was elicited with an Ad26.ZEBOV booster vaccination in adults one and two years after the first dose. These findings support the use of the regimen for prophylactic vaccination in at risk populations such as HCP and frontliners living and working in Ebola endemic areas, and show flexibility in booster dose administration timing up to two years after the regimen. Unfortunately, current recommendations only foresee reactive (ring or population based) vaccination strategies during outbreaks. To conclude this chapter, we propose next steps for this vaccine regimen as well as for Ebola vaccination in general, and address some crucial key messages on setting-up and conducting a vaccine trial in a remote area of a LMIC.

Dutch Summary

In **Deel I, Hoofdstuk 1**, wordt kort ingegaan op de achtergrond, epidemiologie, klinische verschijnselen en symptomen, beschikbare preventieve maatregelen van Ebola, en de aanleiding voor dit doctoraatsproefschrift. De ebolavirusziekte is een ernstige en vaak dodelijke ziekte, veroorzaakt door orthoëbolavirussen. In totaal zijn er zes orthoëbolavirussen geïdentificeerd binnen de *Filoviridae* familie, waarvan er vier ziekte veroorzaken bij mensen. Het genus *Orthoebolavirus zairense*, ook bekend als het Ebola virus (EBOV), heeft tot nu toe geleid tot de meeste uitbraken bij mensen met de hoogste sterftecijfers; variërend tussen 40 en 90%, afhankelijk van de uitbraak. De waargenomen sterftecijfers hangen echter af van de uitbraak en worden beïnvloed door factoren zoals de gezondheidszorginfrastructuur, de reactie op een uitbraak (nationaal en internationaal) en de toegang tot medische zorg.

Zorgverleners en eerstelijnswerkers lopen vaak hoog risico om infectieziekten op te lopen als eerste contact van geïnfecteerde patiënten en kunnen daarna bijdragen aan de verspreiding ervan binnen de gemeenschap. Vooral voor een ziekte als Ebola, die zeer dodelijk is maar zich meestal presenteert met griepachtige symptomen in het begin van de ziekte, lopen zorgverleners en eerstelijnswerkers het meeste risico. Daarom is het van cruciaal belang om methoden te vinden om deze kwetsbare groep te beschermen.

Hoewel EBOV voor het eerst werd ontdekt in 1976, werd het pas in 2014, tijdens de epidemie in West-Afrika, beschouwd als een echte bedreiging voor de wereldgezondheid. Tijdens deze epidemie raakten meer dan 28.600 mensen geïnfecteerd en stierven er meer dan 11.300 van hen. Guinee, Liberia en Sierra Leone werden het zwaarst getroffen tijdens deze epidemie, maar er werden ook verschillende importgevallen gemeld in andere Afrikaanse landen, Europese landen en de Verenigde Staten van Amerika. Als internationale reactie op deze epidemie is de ontwikkeling van behandelingen en vaccins tegen EBOV het afgelopen decennium in een stroomversnelling geraakt.

Eén van de vaccins die sneller werden doorgevoerd als reactie op de West-Afrikaanse epidemie was het Ad26.ZEBOV, MVA-BN-Filo vaccinregime. Sinds de epidemie in West-Afrika is dit vaccin geëvalueerd in Fase 1, 2 en 3 studies, uitgevoerd bij kinderen jonger dan één jaar, kinderen (1-11 jaar), adolescenten (12-17 jaar), gezonde volwassenen, Hiv-geïnfecteerde volwassenen met een goed gecontroleerde infectie en onder actieve antiretrovirale therapie, en kinderen en volwassenen met asymptomatische malaria of symptomatische malaria voor of kort na vaccinatie. Binnen deze studies werden het vaccinatie-interval, de veiligheid, verdraagbaarheid, en humorale en cellulaire immunogeniciteit als reactie op vaccinatie beoordeeld. Bestaande informatie over (1) de duurzaamheid van de bindende antilichaamrespons na vaccinatie met het regime en (2) de mogelijkheid om een humorale immuungeheugenrespons op te wekken met een boosterdosis, was echter beperkt. Om deze aspecten te beoordelen, hebben we een grootschalige vaccinatiestudie uitgevoerd in Boende, een ebola-endemische regio in de Democratische Republiek Congo (DRC). Om dit gebied voor te bereiden op een mogelijke toekomstige uitbraak, vaccineerden we zorgverleners en eerstelijnswerkers met het heterologe Ad26.ZEBOV, MVA-BN-Filo vaccinregime (toegediend met een interval van 56 dagen), gevolgd door een Ad26.ZEBOV boosterdosis één of twee jaar na de eerste dosis (afhankelijk van de randomisatiearm). Het protocol van deze studie is gepubliceerd en de methoden worden behandeld in D**eel I, Hoofdstuk 2**, van dit doctoraatsproefschrift.

De resultaten van het onderzoek worden behandeld in D**eel II, H**oofdstukken 3 en 4. **Hoofdstuk 3** bevat de publicatie over de veiligheid en immunogeniciteit van het heterologe Ad26.ZEBOV, MVA-BN-Filo vaccinregime en beoordeelt of bepaalde factoren (bijv. leeftijd, geslacht, beroep) de immuunrespons beïnvloeden. De veiligheid werd beoordeeld vanaf het ondertekenen van het formulier voor geïnformeerde toestemming tot zes maanden na de tweede dosis door het verzamelen van ernstig ongewenste voorvallen. De humorale immuunrespons werd beoordeeld tot 21 dagen na de tweede dosis door het meten van EBOV-glycoproteïne (GP)-specifiek immunoglobuline G (IgG). Dit laatste werd gemeten met behulp van de Filovirus Animal Nonclinical Group human anti-EBOV GP IgG enzyme-linked immunosorbent assay (FANG ELISA). **Hoofdstuk 4** bevat de publicatie die de duurzaamheid

(d.w.z. tot twee jaar na vaccinatie) beschrijft van EBOV GP-specifieke IgG-bindende antilichamen na vaccinatie met het heterologe vaccinregime, en de veiligheid en immuungeheugenrespons van een Ad26.ZEBOV boosterdosis die één of twee jaar na de eerste dosis werd toegediend. Daarnaast werd de duurzaamheid van antilichamen (d.w.z. tot één jaar na boostervaccinatie) beoordeeld voor de vaccinatiearm die één jaar na de eerste dosis een booster kreeg. Om de veiligheid van de boosterdosis te beoordelen, werden ernstig ongewenste voorvallen verzameld tot zes maanden na de boostervaccinatie en werden (on)bevraagde ongewenste voorvallen verzameld tot zeven dagen na de boostervaccinatie met behulp van een participantendagboek. De immunogeniciteit voor en na de boostervaccinatie (d.w.z. zeven dagen na de boostervaccinatie voor beide studiearmen en één jaar na de boostervaccinatie voor degenen die één jaar na de eerste dosis werden gevaccineerd) werd gemeten met behulp van FANG ELISA.

Tijdens het opzetten en uitvoeren van de klinische studie met het ebolavaccin in Boende, een afgelegen gebied in de DRC met beperkte middelen, werden we geconfronteerd met verschillende uitdagingen (bijv. logistiek, organisatorisch, financieel, gezondheidszorg, enz.). Deze uitdagingen, de manier waarop we ze hebben aangepakt en de lessen die we hebben geleerd tijdens de vaccinatiestudie kunnen waardevol zijn voor andere onderzoekers die klinische studies willen uitvoeren in soortgelijke omgevingen. Daarom bevatten de **Hoofdstukken 5 en 6** de gepubliceerde artikelen over de ervaren uitdagingen, mitigaties en geleerde lessen van het opzetten en uitvoeren van de Ebola vaccinatiestudie.

Een van de grootste uitdagingen van de studie was de beperkte kwaliteit van de gezondheidszorg die beschikbaar was voor onze deelnemers. Hoewel er vanaf het begin van het onderzoek een studieapotheek werd geïmplementeerd om meer gezondheids-zorgmogelijkheden te bieden, werden veel medische voorvallen van deelnemers ad hoc

behandeld. Een studie-specifiek algoritme voor aanvullende zorg (AZ)[2] en bijbehorend beleid werd van het grootste belang waren om te zorgen voor een gelijke en systematische zorg voor alle deelnemers van de studie voor om het even welk medisch voorval. Een studie-specifiek AZ-algoritme en -beleid werden daarom ontwikkeld, goedgekeurd door de nationale ethische commissie in de DRC, gepubliceerd en geïmplementeerd door het onderzoeksteam aan het begin van het derde jaar van de vaccinatiestudie. In **Hoofdstuk 7** presenteren we de evaluatie van dit algoritme en beleid, evenals aanbevelingen om rekening mee te houden bij het implementeren van een vergelijkbare aanpak in een omgeving met beperkte middelen. Dit is het enige artikel in het proefschrift dat nog niet is gepubliceerd. Het is echter wel ingediend bij het tijdschrift *BMJ Global Health* begin februari 2024 en wordt momenteel beoordeeld door de reviewers. De hoofdstukken 5 tot en met 7 werden gegroepeerd onder **Deel III** – Onderzoeks-uitdagingen in lage- en middeninkomenslanden – van dit proefschrift.

Tot slot werden in **Deel IV**, **Hoofdstuk 8**, alle hoofdstukken samengevoegd tot een algemene discussie en conclusie. Over het algemeen werd het Ad26.ZEBOV, MVA-BN-Filo vaccinregime, toegediend met een interval van 56 dagen, goed verdragen, leidde het tot een persistente immuunrespons en werd een vergelijkbare en robuuste humorale immuungeheugenrespons opgewekt met een Ad26.ZEBOV boostervaccinatie bij volwassenen één en twee jaar na de eerste dosis. Deze bevindingen ondersteunen het gebruik van het schema voor profylactische vaccinatie bij risicopopulaties zoals zorgverleners en eerstelijnswerkers die wonen en werken in gebieden waar Ebola endemisch is, en tonen flexibiliteit in de timing van de toediening van de boosterdosis tot twee jaar na vaccinatie met het vaccinregime. Helaas omvatten de huidige vaccinatierichtlijnen tegen Ebola enkel reactieve (ring- of populatiegebaseerde) vaccinatiestrategieën tijdens een uitbraak. Ter afsluiting van dit hoofdstuk doen we enkele

[2] Aanvullende zorg wordt gedefinieerd als zorg die wordt verleend aan deelnemers aan klinisch onderzoek dat verder gaat dan de reikwijdte en doelen van het onderzoek dat wordt uitgevoerd.

voorstellen voor volgende stappen voor het vaccinregime en voor ebolavaccinatie in het algemeen, en behandelen we enkele cruciale kernboodschappen over het opzetten en uitvoeren van een vaccinatiestudie in afgelegen gebieden van lage- en middeninkomenslanden.

List of abbreviations

Abbreviations	Full description
AC	Ancillary care
ACE Research	Africa Contract Research Organization
Ad26	Adenovirus type 26
Ad26.ZEBOV	Monovalent, recombinant, replication-incompetent, adenovirus type 26-vector based vaccine, encoding the Ebola virus Glycoprotein of the Mayinga variant
Ad5	Adenovirus type 5
Ad5-EBOV	Recombinant, replication-incompetent, adenovirus type 5-vector vaccine encoding the GP antigens from the Zaire strain of Ebola virus and from the Gulu strain of Sudan virus
AE	Adverse event
AIDS	Acquired immunodeficiency syndrome
ASTMH	American Society of Tropical Medicine and Hygiene
AZ	Aanvullende zorg
BDBV	Bundibugyo virus
BMJ	British medical journal
BOMV	Bombali virus
CEPI	Coalition for Epidemic Preparedness Innovations
CFR	Case fatality rate
ChAd	Chimpanzee adenovirus
ChAd3-EBO-Z	Replication-incompetent, chimpanzee adenovirus 3 vector vaccine expressing Zaire Ebola virus glycoprotein
CI	Confidence interval
COMAHS	College of Medicine and Allied Health Sciences
COVID-19	Coronavirus disease 2019
CRF	Case report form
CRO	Clinical Research Organization
CTA	Clinical Trial Applications
CV	Curriculum vitae
DALY	Disability-Adjusted Life Year
Dfnet Research	Healthcare technology company that provides eClinical solutions including electronic data capture, eSource, and data management services
DNA	Deoxyribonucleic acid
DPM	Direction de la Pharmacie et de Medicament
DRC	Democratic Republic of the Congo

Abbreviations	Full description
DSI	Data Science Institute
EBL2007	Healthcare provider Ebola vaccine study, conducted in the DRC
EBL3001	EBOVAC-Salone Ebola vaccine study, conducted in Sierra Leone
EBOV	Ebola virus
EBOVAC1	Development of a prophylactic Ebola vaccine using an heterologous prime-boost regimen: phase I
EBOVAC2	Development of a prophylactic Ebola vaccine using an heterologous prime-boost regimen: phase II
EBOVAC3	Development of a prophylactic Ebola vaccine using an heterologous prime-boost regimen: phase III
EC	Ethics committee
EFPIA	European Federation of Pharmaceutical Industries and Associations
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EPI	Expanded Programme on Immunization
Ervebo	Marketing name for rVSV△G-ZEBOV-GP
EU/mL	ELISA unit per millilitre
EU-IMI	European Union - Innovative Medicines Initiative
EVD	Ebola virus disease
FANG ELISA	Filovirus Animal Non-clinical Group enzyme-linked immunosorbent assay
FAS	Full analysis set
FDA	United States Food and Drug Administration
GAMLSS	Generalized additive model for location, scale and shape
GCLP	Good clinical and laboratory practices
GCP	Good clinical practice
GIBS	Group Inter Bailleurs Santé
GMC	Geometric mean concentration
GMR	Geometric mean ratio
GP	Glycoprotein
GRH	General reference hospital
HCP	Healthcare provider
HIC	High income country
HIV	Human immunodeficiency virus
ICF	Informed consent form
ICMJE	International committee of medical journal editors
ICP	Infection prevention and control
IgG	Immunoglobulin G
IMI	Innovative Medicines Initiative
IMI-EU	Innovative Medicines Initiative - European Union

Abbreviations	Full description
IMVAMUNE	Third-generation smallpox vaccine also known as MVA-BN, JYNNEOS, and IMVANEX
IMVANEX	Third-generation smallpox vaccine also known as MVA-BN, JYNNEOS, and IMVAMUNE
INSERM	Institut National de la Santé et de la Recherche Médicale
IP	Investigational product
IQR	Interquartile range
JYNNEOS	Third-generation smallpox vaccine also known as MVA-BN, IMVANEX, and IMVAMUNE
LLOQ	Lower limit of quantification
LMIC	Low- or middle-income country
LMICs	Low- and middle-income countries
LSHTM	London School of Hygiene and Tropical Medicine
LUMINEX	A bead-based multiplexed immunoassay system in a microplate format
MedDRA	Medical Dictionary for Regulatory Activities
mRNA	Messenger ribonucleic acid
MVA	Modified Vaccinia Ankara
Mvabea	Marketing name for MVA-BN-Filo
MVA-BN	Third-generation smallpox vaccine also known as JYNNEOS, IMVANEX, and IMVAMUNE
MVA-BN-Filo	Non-replicating, multivalent, modified vaccinia Ankara vaccine, encoding the Ebola virus Mayinga glycoprotein, the Tai Forest ebolavirus nucleoprotein, the Sudan ebolavirus Gulu glycoprotein, and the Marburg virus Musoke glycoprotein
NA	Not applicable
NGO	Non-governmental organizations
NHP	Non-human primate
PCR	Polymerase chain reaction
PI	Principal investigator
PPS	Per protocol set
PPS1	Per protocol set of all participants that received the Ad26.ZEBOV, MVA-BN-Filo regimen
PPS2	Per protocol set of all participants that received the Ad26.ZEBOV booster dose
PREVAC	Partnership for Research on Ebola Vaccinations
PRNT	Plaque Reduction Neutralization Test
Q1	Quartile 1
Q3	Quartile 3
R&D	Research and development

Abbreviations	Full description
RESTV	Reston virus
RNA	Ribonucleic acid
rVSV	recombinant Vesicular Stomatitis Virus
rVSV△G-ZEBOV-GP	Single-dose, live, attenuated recombinant Vesicular Stomatitis Virus (Indiana strain) vaccine encoding the Ebola virus Kikwit 1995 strain surface glycoprotein
SAE	Serious adverse event
SAGE	Strategic Advisory Group of Experts on immunization
SARS	Severe acute respiratory syndrome
SOC	System organ class
SOP	Standard operating procedure
SUDV	Sudan virus
TAFV	Taï Forest virus
TOU	Test of understanding
UAntwerp	University of Antwerp
UK	United Kingdom
ULOQ	Upper limit of quantification
UMURINZI	Unified Rwandan initiative for national Ebola virus immunization
UNICEF	United Nations Children's Fund
UNIKIN	University of Kinshasa
US	United States
USA	United States of America
USD	United States dollar
VAC52150	Reference to the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen
VAXINFECTIO	Vaccine & Infectious Disease Institute
VLP	Virus like particle
VNA	Virus Neutralization Assay
VSAT	Very-small-aperture terminal
WHO	World Health Organization
Zabdeno	Marketing name for Ad26.ZEBOV
β-HCG	β-human chorionic gonadotropin

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Chapter 1 General introduction

Ebola virus disease

1.1 Background and epidemiology

Ebola disease is a severe viral illness caused by viruses of the *Orthoebolavirus*[3] genus, *Filoviridae* family [1]. Since their discovery in 1976, these viruses have caused 38 outbreaks in humans, mainly across Central and Western Africa (Figure 1) [2]. So far, six orthoebolaviruses have been identified: Ebola virus (EBOV)[4], Sudan virus (SUDV), Bundibugyo virus (BDBV), Taï Forest virus (TAFV), Reston virus (RESTV), and Bombali virus (BOMV). Among these, EBOV is the most common, followed by SUDV and BDBV, and most lethal, with a case fatality rate ranging between 40% and 90% in humans, depending on the outbreak [2, 3]. To date, TAFV has caused only one nonlethal human infection, infection with RESTV in humans has led to the development of antibodies but not disease, and no cases of infection or disease in humans have been reported for BOMV [4-6]. The orthoebolavirus causing Ebola disease determines the disease terminology. Therefore, Ebola disease caused by EBOV is known as Ebola virus disease (EVD), by BDBV as Bundibugyo virus disease, and by SUDV as Sudan virus disease [7]. There is evidence suggesting that there may be some cross-reactivity and cross-protection among different species within the genus *Orthoebolavirus* [8].

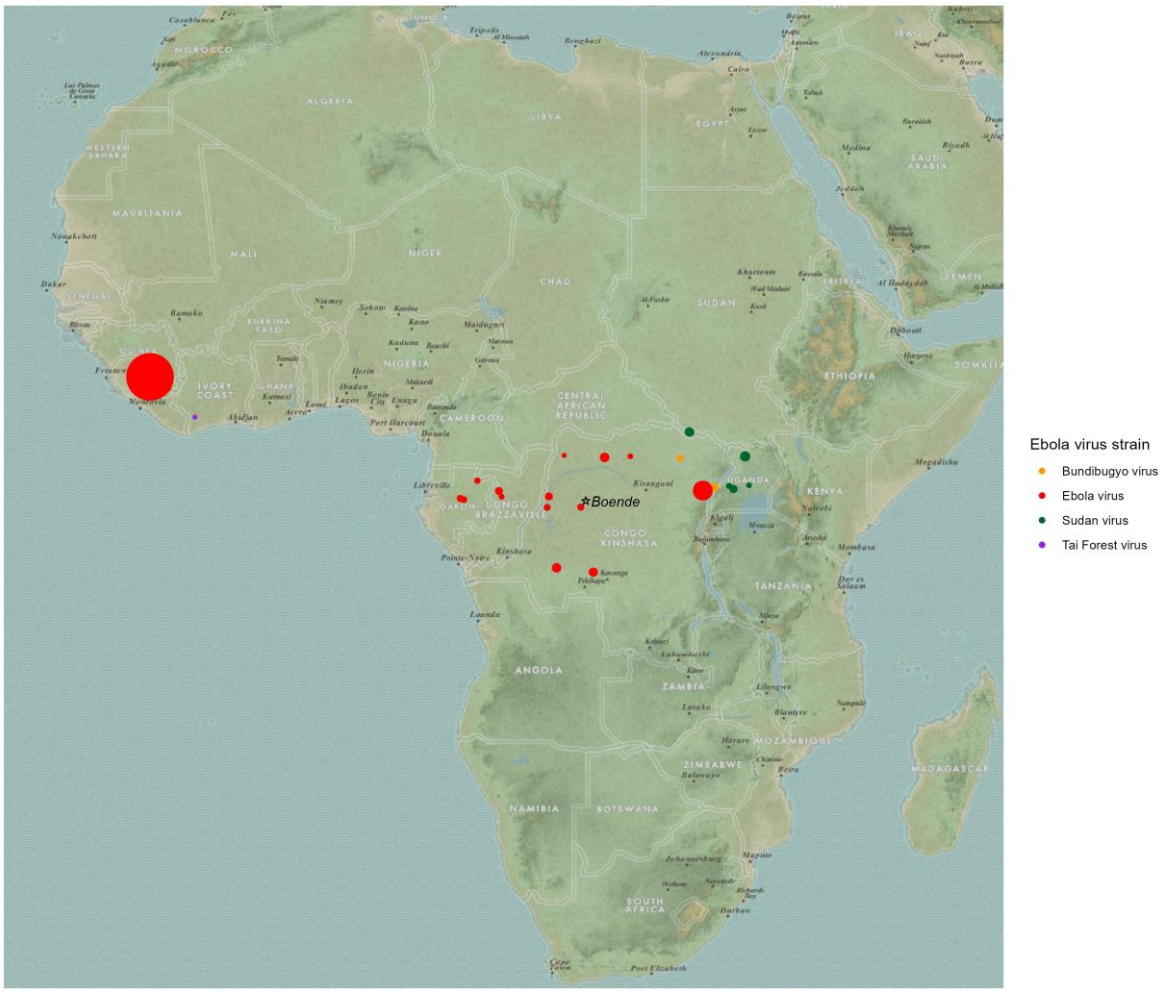
In outbreak epidemiology, an outbreak of a disease is defined as any increase in disease occurrence as per the norm in a certain location, population or time frame [9]. Therefore, when a disease is uncommon, like Ebola disease, an outbreak can be declared when only

[3] Genus taxonomy was changed from *Ebolavirus* to *Orthoebolavirus* in April 2023 [1].
[4] Formerly know as Zaire ebolavirus.

one case has been identified. Between 1976, when Ebola disease was first recognised, and 2013, outbreaks mainly occurred sporadically in Central and East Africa (i.e., Democratic Republic of the Congo (DRC), Uganda, South-Sudan, Republic of the Congo, and Gabon) [2]. However, the unexpected appearance of the virus in West Africa in December 2013, led to the largest (i.e., >28,600 cases and >11,300 deaths, CFR 40%) and longest-lasting (from 2013 until 2016) EVD outbreak to date [2]. Until then, 22 outbreaks had occurred with in sum 2,378 cases and 1,601 deaths (overall CFR of 67%) [2], making the West Africa Ebola epidemic unprecedented in size and location. As a consequence of this epidemic, Ebola viruses went from tropical pathogens with a negligible global health threat, to the focus of global health research as a pathogen of international concern [10]. As EBOV is most commonly the cause of an Ebola outbreak in humans (Figure 1), and leads to the highest morbidity and mortality compared to other orthoebolaviruses, most research towards prevention and treatment has been directed towards EBOV.

Most EVD outbreaks can be traced back to a zoonotic origin, initiated by the spillover of EBOV from an animal host or reservoir to a human [11]. While human index cases of EVD outbreaks have been associated with spillover events from for example chimpanzees, gorillas, and duikers, they are considered improbable EBOV reservoirs due to high mortality rates and fast disease progression associated with EBOV infection in these animals [12, 13]. They are more likely bridge hosts in transmission to humans and play an intermediate role between the EBOV reservoir and human infection. Until today, despite extensive research efforts, the animal reservoir of EBOV remains unknown. However, certain bat species are strongly suspected [14]. Once among humans, an outbreak is sustained by direct human-to-human contact or through contact with infected tissues, bodily fluids, or fomites [15]. Provision of care to infected individuals and taking part in traditional burial practices have been associated with an increased risk of infection [16, 17].

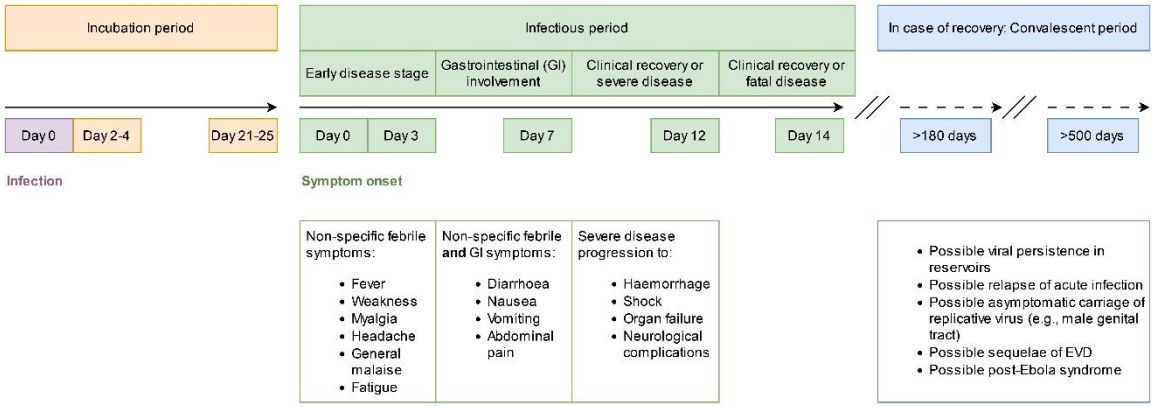
Figure 1. Ebola virus outbreaks in Africa from 1976-2023 by strain and size.



The size of the dots is determined by the number of cases in the outbreak. The colour is determined by the Ebola strain. The dots are centralized at the outbreak starting point. The spread of the outbreak, if applicable, is not accounted for. The trial site location is indicated with a star and labelled (i.e., Boende). This figure was created in R v4.3.1 using the package "OpenStreetMap" v0.3.4, with map type "apple-iphoto". Information on outbreaks and number of cases was obtained from [2].

1.2 Clinical signs and symptoms

Figure 2. Ebola virus disease infection and disease progression.



The mean incubation period, defined as the time between infection and the onset of symptoms, following direct contact with an infected individual or animal, has ranged from 5-13 days, depending on the outbreak [18, 19]. The incubation period is generally shorter when infection occurred percutaneously (e.g., as a consequence of a needle-stick accident) [18]. However, according to a review and meta-analysis of seven EVD outbreaks, not all EBOV infections are symptomatic and approximately 27% (95% CI, 15%–40%) are asymptomatic [20]. Fortunately for outbreak management, there is no evidence that people experiencing asymptomatic EBOV infections can transmit the virus to others. In general, and based on previous outbreaks, a minimum incubation period of 2-4 days and a maximum incubation period of 21 days is assumed [18, 21, 22]. Before declaring the end of an EVD outbreak, the World Health Organization waits twice this maximum incubation period (i.e., 42 days) after the detection of the last confirmed or probable EVD case [23]. This seems prudent as one study calculated the risk of developing EVD 21 days after infection to be approximately 4%, which decreases to 1% when a 25-day incubation period has passed [24].

Once EBOV-infected individuals become symptomatic, a wide range of clinical presentation of EVD (mild to severe) has been reported. It is during this symptomatic stage that individuals also become infectious to others. Individuals remain contagious as long as they

remain viraemic or the virus persists in bodily fluids (e.g. semen, breast milk, tears, urine, etc.) [25]. At onset of symptoms, patients typically present themselves with non-specific flu-like symptoms such as fever, weakness, headache, and fatigue [26]. As these symptoms are also seen in more prevalent infectious diseases in Sub-Saharan Africa (e.g., malaria), patients are often misdiagnosed in these early stages, allowing continued transmission of the virus in the community. After a few days, these non-specific flu-like symptoms are usually followed by gastrointestinal symptoms such as anorexia, nausea, vomiting, and diarrhoea [26]. Within the first week some patients also develop a non-specific maculopapular rash or hiccups [27, 28]. While some EVD patients recover after gastro-intestinal symptoms, for others it leads to considerable fluid loss, hypovolemia, shock, organ failure, and potentially death. Individuals who die of EVD remain infectious [29], making safe burials highly important.

Unfortunately, many EVD survivors often have substantial and long-term medical sequelae. One study, looking into the long-term effects of EVD in survivors from Guinea found that approximately 50% of EVD survivors experienced sequelae up to two years after discharge [30]. While this decreased over time, 25% of survivors still reported sequelae after four years. Investigated sequelae were neurological (i.e., headache, dizziness, and behavioural, neuro-sensitive, or neuromotor disorders), abdominal (i.e., pelvic pain, gastritis), ocular (i.e., vision problems, ocular pain, conjunctivitis, glaucoma, iridocyclitis, cataract), musculoskeletal (i.e., neck, back, or joint pain, and myalgia), and general (i.e., anorexia, fatigue, fever) [30]. Alarmingly, next to long-term sequelae, it has been shown that viable EBOV can persist in certain immunologically protected sites of the human body (e.g., male gonads and chambers of the eyes) [31, 32]. For example, because of viral persistence in semen of a male survivor, a new EVD cluster as a consequence of sexual transmission was reported more than 500 days after disease onset in the survivor [33]. In addition to the resurgence of EVD via sexual transmission, there have been documented cases of survivors experiencing a relapse of acute EVD infection, leading to a new transmission chain for up to six months post-recovery [34]. For these reasons, continued surveillance with fast response teams are necessary in areas where EVD outbreaks previously occurred and

preventive vaccination of at risk individuals, such as the partner of male survivors, may be indicated.

To ensure a fast and rapid response to a suspected EVD case, a suspected case definition of EVD is required. However, suspected case definitions can rarely be both specific and sensitive [35]. When a suspected case definition has a low sensitivity, true EBOV-positive cases may be missed. These cases will then be re-introduced into the community and allow EBOV to spread. However, when a suspected case definition has a low specificity, EBOV-negative cases may be considered positive. During a confirmed outbreak, these cases will be admitted to an Ebola treatment unit, exposing them to EBOV-positive cases. Depending on the EBOV incidence in a community and the progress of the outbreak, the case definition may thus need to be updated. Unfortunately, depending on the outbreak, clinical signs and symptoms have varied across EVD outbreaks, making a universal case definition more difficult [36]. For example, while the World Health Organization (WHO) does provide a case definition for suspected and probable EVD cases, they indicate that "during an outbreak, case definitions are likely to be adapted to new clinical presentation(s) or different modes of transmission related to the local event" [37].

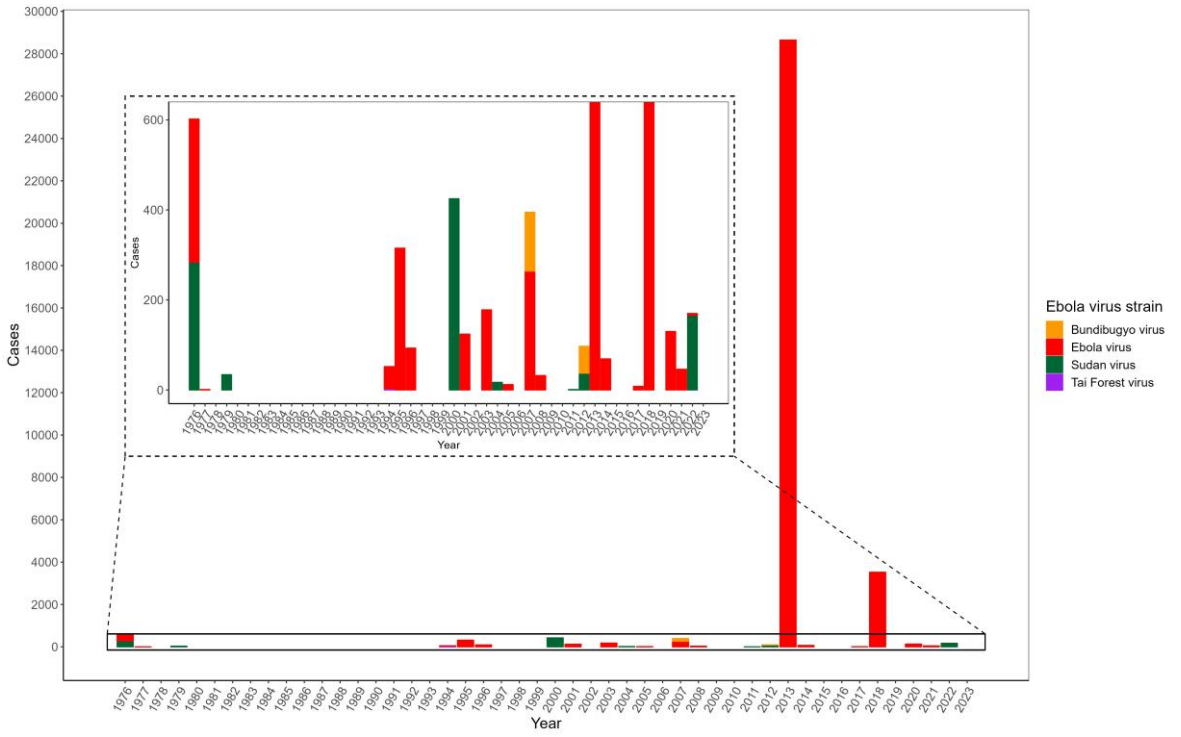
1.3 Preventive strategies

EVD control remains a challenge in Sub-Saharan Africa [38]. Since the first recognized EVD outbreak in 1976, no substantial changes in case fatality rates have been observed [38]. However, since its discovery, EVD outbreaks have become more frequent (Figure 3). This is most likely due to increased deforestation events in West and Central Africa [39], where EBOV is endemic, and an overlap of the human and animal ecosystems as a consequence. ***Preventing deforestation***, could therefore help prevent future EVD outbreaks.

Next to an increased frequency, recent outbreaks have a greater potential global health risk and are more difficult to maintain, due to evolving national and international travel and transport possibilities of humans and goods (e.g., bush meet) [40, 41]. As an example, the arrival of EVD in densely populated capital cities during the West Africa Ebola epidemic

(e.g., Conakry, Guinea; Freetown, Sierra Leone; Monrovia, Liberia) allowed the disease to spread at an unprecedented speed and made outbreak control more difficult [42]. During the West Africa Ebola epidemic, the EVD CFR of patients treated in Europe or the United States was considerably lower than the CFR in West African countries (19% versus 40%, respectively) [43]. Healthcare accessibility, healthcare personnel trained in infection prevention and control (ICP), and the availability of adequate medical supplies have shown to be crucial determinants of EVD survival [44]. ***Strengthening local health systems, surveillance, and diagnostic capabilities*** of Ebola endemic areas in West and Central Africa can therefore considerably decrease the risk of the next Ebola outbreak becoming epidemic or even pandemic [44].

Figure 3. Overview of outbreak year, strain and number of cases.



The colour of the bar is determined by the Ebola strain. This figure was created in R v4.3.1. Information on the outbreak year, strain, and number of cases was obtained from [2]. The largest EVD outbreak with the most cases, occurred in West Africa between 2013 and 2016. The second largest EVD outbreak to date, occurred in the DRC between 2018 and 2020.

Community engagement and education on effective prevention of Ebola can help raise awareness about the virus, its transmission and prevention [45]. For community engagement and education to be successful, community-based interventions need to be adapted to specific regions and contexts, and trusted community figures and local health agents need to be involved [45, 46]. Strategies that have been implemented in Ebola affected communities include (but are not limited to) educating on the symptoms of EVD, the importance of early detection and medical care, safe burials, community-based surveillance, survivor reintegration programs, and community care centres [45]. Finally, community engagement in EVD research (e.g., in study design, the informed consent process, and study implementation) is crucial, to address myths and misconceptions about investigational products developed to treat (e.g., antibody therapy) or prevent disease (e.g., vaccination) [7, 47].

While strengthening local healthcare systems is crucial and should remain a priority in countries where EBOV is endemic, the non-specific febrile symptoms at early onset of EVD leave HCP and frontliners disproportionately at risk to acquire the disease [48, 49]. Therefore, protecting them against this deadly virus is crucial. Modelling studies have shown that ***prophylactic vaccination of HCP and frontliners*** would be the most effective way to reduce EVD outbreaks and its related morbidity and mortality [50-52], even at a 30% vaccine coverage and 50-60% vaccine efficacy. However, key factors influencing the use of existing vaccines against EBOV principally differ depending on geographic location, and uncertainties persist regarding their durability of protection, particularly in light of the re-emerging outbreaks – either through relapse or sexual transmission – in Central and West Africa, occurring several months or even years after the recovery of an infected individual.

1.4 Ebola vaccination

Since the West Africa epidemic, vaccine development against EBOV was fast-tracked with several candidate vaccines going into accelerated clinical development stages [53]. More recently (2018-2020) the second largest EVD outbreak (3,470 cases and 2,287 deaths [2]) in the DRC further highlighted the global health threat that EVD continues to pose and the need for effective vaccines. As the viral surface GP is the only exposed protein on the surface of a mature EBOV particle, vaccine development (and the development of monoclonal antibodies) has focused on the viral GP as a crucial target [54]. Additionally, due to the high conservation of the GP nucleotide sequence among strains within a species, older EBOV strains are more inclined to offer cross-protection within that species. Consequently, these older strains are frequently employed in the formulation of developed EBOV vaccines [25]. While original vaccines against Ebola were DNA vaccines, this vaccine type often generated a limited immune response, leading to the need for several boosters at high doses to achieve a strong response and ensure longevity [25]. At present, the majority of the developed EBOV vaccines are based on the use of recombinant viral vectors (e.g. recombinant Vesicular Stomatitis Virus (rVSV), adenovirus type 5 (Ad5)) expressing the Ebola virus surface GP [25]. As an alternative to Ad5-vector based vaccines, that hold a higher risk of pre-existing immunity against the Ad5-vector among humans and consequently lead to a lower vaccine efficacy, other (rarer) circulating adenovirus serotypes in humans have been used in vaccine development against EBOV (e.g., adenovirus type 26 (Ad26); Ad26.ZEBOV) [55, 56]. As another alternative, chimpanzee adenovirus (ChAd) serotypes have also been used (e.g., ChAd3-EBO-Z) [57, 58]. Next to recombinant viral vectors, non-replicating vaccinia virus vectors (e.g. Modified Vaccinia Ankara (MVA) vectors) have shown promising results, especially as a booster dose after Ad26.ZEBOV or ChAd3-EBO-Z [25]. Some virus like particle (VLP)-vaccines against Ebola are also under development and under investigation in phase 1 clinical trials [25]. Vaccines utilizing VLP-technology are created using bioinspired nanostructures [59]. These structures incorporate repetitive and densely packed antigens derived from various

virulent agents, contributing to the induction of a robust immune response. Finally, while messenger ribonucleic acid (mRNA) vaccines have proven very effective during the COVID-19 pandemic [60], further research is needed on whether this technology can also be applied to obtain a safe and effective Ebola vaccine [61]. The development of an mRNA Ebola vaccine is currently still in pre-clinical stages but there is some evidence in guinea pigs indicating that mRNA vaccination against Ebola may be possible [62].

While several candidate vaccines have reached human trials, only two have received broad regulatory approval and have been implemented to help fight recent EVD outbreaks in Africa. The first, rVSV△G-ZEBOV-GP (Ervebo®; developed by Merck) is a single-dose, live, attenuated rVSV (Indiana strain) vaccine, where the VSV envelope GP was replaced by the EBOV Kikwit 1995 strain surface GP [63]. This vaccine has proven to be 97.5-100% effective during reactive ring vaccination (i.e., vaccination of contacts and contacts of contacts during an EVD outbreak) in Guinea and the DRC [64, 65] and was first licensed for use in adults older than 18 years of age by the European Medicines Agency (EMA) and pre-qualified by the WHO in November 2019 [63, 66]. It was later also approved by the FDA in December 2019 and by the regulatory authorities of several African countries [67, 68]. However, some safety concerns have been reported as related to this vaccine (e.g., anaphylaxis, arthralgia, arthritis) [69]. The second, consists of a two-dose heterologous vaccine regimen, with Ad26.ZEBOV (Zabdeno®; developed by Janssen Vaccines & Prevention B.V) as first dose, and MVA-BN-Filo (Mvabea®; developed by Bavarian Nordic and licensed to Janssen) as second dose. The Ad26.ZEBOV vaccine is a monovalent, recombinant, replication-incompetent, Ad26-vector based vaccine, encoding the EBOV GP of the Mayinga variant [70]. The MVA-BN-Filo vaccine is a multivalent, recombinant, replication-incompetent, MVA-vector based vaccine, encoding GPs from the EBOV Mayinga variant, SUDV Gulu variant, and Marburg Musoke variant and the nucleoprotein from the TAFV [71]. Though determining clinical vaccine efficacy or effectiveness has not been possible through vaccine trials in humans for the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen, this heterologous vaccine regimen has shown to be protective in challenged non-human primates [72]. Additionally, through immunobridging analysis, researchers

discovered that the vaccine regimen is likely to confer protection against EVD in humans [73, 74]. This analysis involves inferring the vaccine's potential protective effect in humans from its observed efficacy in animals during challenge models. Based on this immunobridging analysis, this regimen was approved by EMA for use in epidemic emergencies against EBOV in July 2020 for adults and children older than one year old [75, 76]. The vaccine regimen was later also approved for use under exceptional circumstances by several African regulatory authorities (i.e., Ghana, Côte d'Ivoire, Rwanda [77], Uganda, Sierra Leone, Nigeria, and Gabon) in 2022 or 2023.

Other vaccines approved against EBOV have been approved "for emergency use" in China (Ad5-EBOV) and Russia (GamEvac Combi; rVSV/Ad5) [78, 79]. These vaccines were licensed based on animal model studies and data from phase 1 and 2 clinical trials on human immunogenicity, which included studies in African populations [80]. The Ad5-EBOV vaccine is a recombinant, replication-incompetent Ad5-vector vaccine that contains two recombinant Ad5 vectors expressing GP antigens in a 1:1 ratio of viral particles from the Zaire strain of EBOV and from the Gulu strain of SUDV [81]. The GamEvac Combi vaccine is a heterologous live-attenuated rVSV- and Ad5-vaccine encoding the Ebola virus GP (Makona strain) [82].

1.5 Rationale of the thesis

This doctoral thesis is focussed on the results of a phase 2, open-label, monocentric, randomised Ebola vaccine trial conducted in Boende, a remote and resource-poor setting in the DRC. Within the trial, approximately 700 HCP and frontliners were recruited to receive the two-dose heterologous Ad26.ZEBOV, MVA-BN-Filo vaccine regimen followed by an Ad26.ZEBOV booster dose either one or two years after the first dose, depending on the randomisation arm (ratio 1:1) [83].

Below, the "Trial in context" section, first outlines how this trial fits into the already existing literature and knowledge on the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen by describing its research and development (R&D) and manufacturing path. Subsequently, it provides a

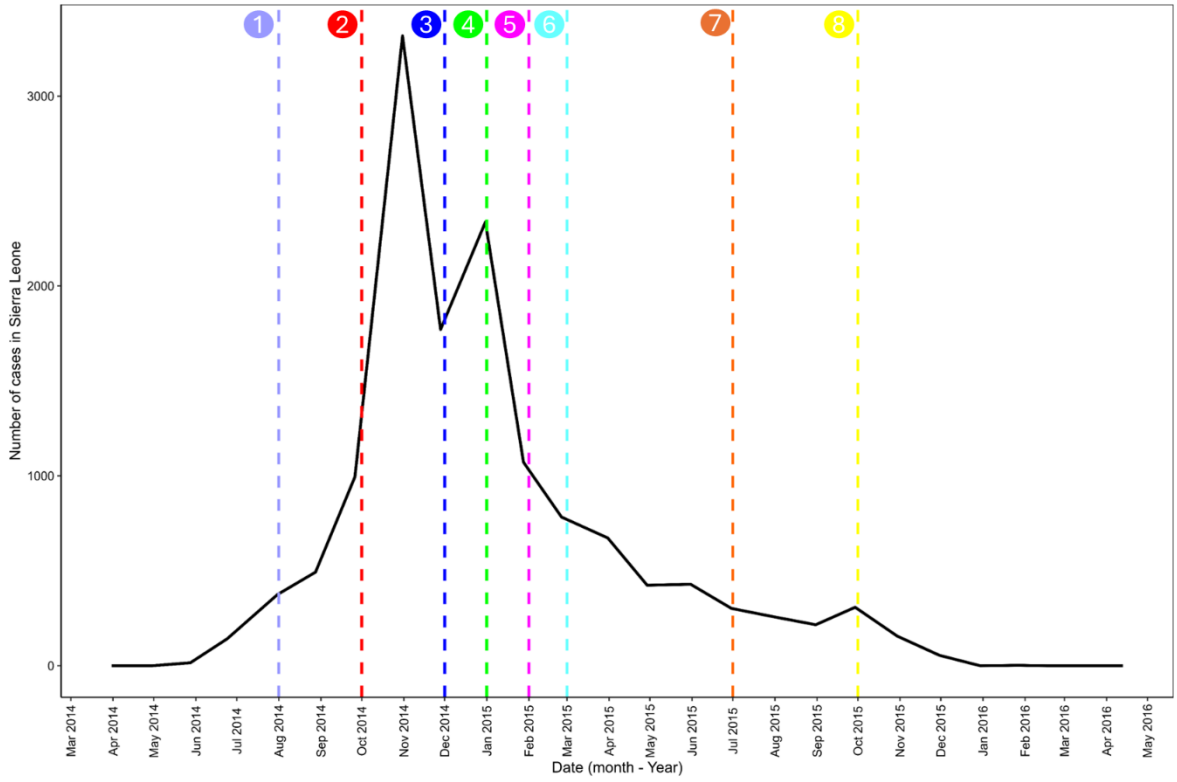
comprehensive overview of all published trial results for the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen (excluding our own publications as this is taken up in the general discussion). The section concludes by highlighting how the Ebola vaccine trial discussed in this doctoral thesis addresses identified knowledge gaps. Next, the section titled "Vaccine research challenges in LMICs" delves into the importance of transparency regarding the logistical, financial, and healthcare obstacles (among others) encountered when setting-up and conducting the vaccine trial in Boende, a remote and resource-poor location in the DRC.

1.5.1 Trial in context

1.5.1.1 *R&D and manufacturing timeline*

The severity of the 2013-2016 West Africa Ebola epidemic underscored the pressing need for safeguards against EBOV, and prompted the accelerated R&D and manufacturing of a heterologous two-dose vaccine regimen, incorporating Ad-vector and MVA-vector-based vaccines against this virus, driven by the successful proof of concept demonstrated in two non-human primates (NHPs) [84]. Additionally, to achieve an accelerated development track, the different phases of the clinical development were conducted in parallel [84]. To visualise these different phases, Figure 4 portrays the monthly number of cases reported during the West Africa Ebola epidemic in Sierra Leone and the different fast-tracked R&D and manufacturing steps.

Figure 4. Number of cases per month in Sierra Leone as reported by the WHO during the West Africa Ebola Epidemic.



Number of monthly cases obtained from [85]. 1 *WHO declares the West Africa Ebola Epidemic a Public Health Emergency of International Concern;* 2 *Janssen Pharmaceuticals and Bavarian Nordic sign a commitment to invest in Ebola vaccine development and to manufacture two million vaccine doses by the end of 2015;* 3 *First in human study with the Ad26.ZEBOV, MVA-BN-Filo vaccines starts in the United Kingdom;* 4 *A second phase 1 trial starts in the United States;* 5 *A phase 1 study starts in Kenya;* 6 *A phase 1 study starts in Uganda and Tanzania;* 7 *A phase 2 study starts in Europe;* 8 *A phase 3 trial was intended to start in Sierra Leone. The figure was created in R v4.3.1.*

On August 8th, 2014, the WHO declared the West Africa Ebola Epidemic a Public Health Emergency of International Concern [86]. Emphasizing the urgency of the situation within the Ebola Response Roadmap, the WHO urged the accelerated development of Ebola vaccines, underscoring the critical importance of this effort for public health [87]. In the hope to help fight the West Africa epidemic with an effective vaccine regimen, the first in human study with the Ad26.ZEBOV, MVA-BN-Filo vaccines started December 2014 in the United Kingdom (UK) [88], just two months after Janssen Pharmaceuticals and Bavarian Nordic signed a commitment to invest in Ebola vaccine development and to manufacture two million vaccine doses by the end of 2015 [84]. Shortly after, in January 2015, a second

phase 1 trial commenced in the United States (US) [77]. While these two studies were set-up to establish preliminary safety and immunogenicity results, identify the optimal regimen schedule, and assess the durability of the immune response [89], two additional phase 1 trials were started in February and March of 2015; one in Kenya (low malaria endemicity), and one Uganda and Tanzania (high malaria endemicity) [90, 91]. These trials were intended to replicate data of the first in human trials in the UK and US in participants from African countries unaffected by the West Africa epidemic and confirm initial safety and immunogenicity results [89]. In July 2015, a phase 2 trial was established in Europe and in October 2015 a phase 3 trial was intended to start in Sierra Leone to assess the efficacy of the vaccine regimen [92, 93]. Unfortunately, the latter trial was implemented around the tail end of the West Africa epidemic (Figure 4) and proving efficacy was no longer possible in the context of a classical clinical trial design [93]. The protocol was amended and the trial design and outcomes were changed from a cluster-randomized trial intending to assess vaccine effectiveness, to a two-stage, randomised trial focussing on safety and immunogenicity of the two-dose regimen in an area previously affected by an Ebola outbreak [94]. The first stage of the trial would assess the safety and immunogenicity of the vaccine regimen in a small cohort of adult participants, and was expanded into the second stage of the trial where a larger adult cohort and subsequently also adolescents and children above the age of one were vaccinated [94]. Additionally, in stage 1 participants, a booster dose was administered for the first time at two years after the first dose to assess the regimen's capability to induce an anamnestic response[5] [94].

Following this limitation of not being able to assess the regimen's effectiveness, the question remained as to how the Ebola vaccine regimen (that had proven to be safe and immunogenic in phase 1 and phase 2 trials) could be licensed without demonstrated clinical efficacy. However, for diseases where establishing clinical efficacy is unethical and field

[5] The term *anamnestic response* is used interchangeably with *(humoral) immune memory response* throughout the doctoral thesis.

trials are unfeasible, the US Food and Drug Administration (FDA) implemented the 'animal rule' in 2002 [95]. This allows for the approval of drugs or vaccines when efficacy can be established based on well-characterized animal models of the human disease, while clinical trials indicate the safety and tolerability of the drugs in humans. Therefore, based on immunogenicity data available from five Ad26.ZEBOV, MVA-BN-Filo vaccine trials (N=764), Bockstal et al. determined the likely protective effect of human antibody concentrations using immunobridging of EBOV GP binding antibody responses between non-human primates and humans [73]. In agreement with the FDA and EMA, for the protective effect to be demonstrated, the lower limit of the 95% CI of the mean survival probability had to be above the pre-specified success criterion of 20%. Overall, the study calculated a mean predicted survival probability of 53.4% (95%CI: 36.7-67.4), fulfilling the prespecified success criterion [73]. However, due to the strictness of the parameters under which the model was built, this is expected to be an underestimation of the actual vaccine efficacy in humans. The actual quantification of the protective effect in humans will still need to be determined using a field study. Based on this 'animal rule'-method, the EMA was the first to approve the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen for marketing authorisation under exceptional circumstances in July 2020 [75, 96].

1.5.1.2 *Overall Ad26.ZEBOV, MVA-BN-Filo vaccine trial results*

Phase 1 and 2 trials in healthy adults initially evaluated in which order the two components of the vaccine regimen could best be administered (Ad26.ZEBOV or MVA-BN-Filo first), and at which interval they should be administered; 28-, 56-, or 84-day interval [77, 88, 90-92, 97, 98]. While safety profiles remained consistent across various schedules, there was a notable increase in EBOV-specific binding antibody geometric mean concentrations (GMCs) when MVA-BN-Filo was administered after Ad26.ZEBOV at a 56-day interval compared to a 28-day interval [88, 98]. No significant differences were observed between the 56- and 84-day intervals [98]. Additionally, evidence from Ebola challenged NHP showed a 75% protection generated by Ad26.ZEBOV as a single dose [56], which increased to 100% in challenged animals that received a MVA-BN-Filo vaccine as a second dose [72]. Furthermore, NHP boosted with MVA-BN-Filo showed to be protected for longer [72, 99].

The licenced version of the regimen therefore recommends a 56-day interval between with Ad26.ZEBOV as first dose, followed by MVA-BN-Filo as second dose to confer a more rapid and long-lasting immunity [71].

Vaccine trials assessing the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen have been conducted in healthy adults (18 or older), adolescents (12-17 years old), children (1-11 years old), infants less than one year old, HIV-infected adults, and children and adults with asymptomatic malaria, or with malaria exposure before or shortly after vaccination leading to symptomatic infection [77, 88, 90-92, 94, 97, 98, 100-104]. In all populations the two-dose heterologous vaccine regimen was generally well-tolerated with mostly mild to moderate transient adverse events. In all subgroups (except infants <1 year old and children of 1-3 years old), injection-site pain was the most frequently reported local solicited adverse event and in most, headache was the most common systemic solicited AE, whereas in some, this was fatigue or myalgia. In children (1-3 years old), a decreased appetite, decreased activity, and fever have been described [100]. In infants (<1 year old), a decreased appetite, decreased activity, and irritability were most common [101]. One study reported two serious adverse events (one participant was diagnosed with Fisher Miller syndrome; and one with intermittent episodes of paraesthesia of the palms and soles) both possibly related to vaccination [92]. However, no specific safety concern was raised by an external expert panel of neurologists.

One vaccination campaign, vaccinated 216,113 nonpregnant persons, ≥2 years old, in Rwandan residents living on the border with the DRC, with the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen (56-day interval) [105]. Overall, 0.68% unsolicited AEs were reported (fever and headache most commonly reported) and 17 SAEs were considered related to the investigational product [105]. All 17 related SAE occurred on the eve of vaccination or the day after in children 2-8 years old. In ten cases, a child experienced febrile convulsions with or without fever and/or diarrhoea. In the seven other cases, the child experienced fever and/or diarrhoea/vomiting. All children responded to appropriate therapy with hospital discharge within 2-4 days. In response to the febrile convulsion cases in young

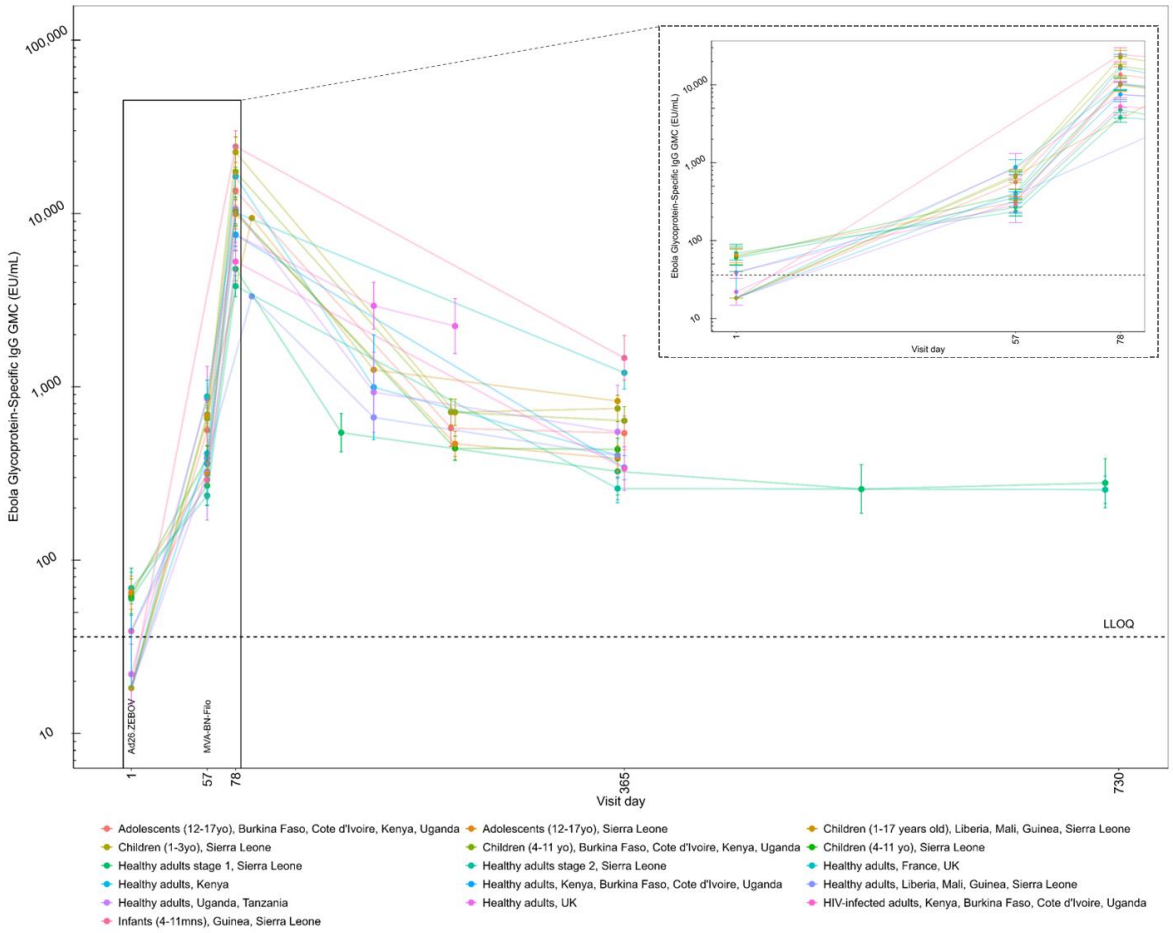
vaccinees (2-8 years old), a routine acetaminophen administration was implemented via a 250mg suppository at the time of vaccination and again six hours later at home. Once this mitigation was implemented, febrile convulsions seemed to decrease with only two cases occurring after implementation of this strategy. Noteworthy, for these cases the second dose of acetaminophen, six hours after vaccination, had not been administered. The safety of the vaccines in pregnant or lactating women is currently lacking. However, several studies are currently assessing the maternal/foetal safety profile after vaccinating pregnant women with the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen [106, 107].

To ensure binding antibody responses were comparable across different trials and populations, the same validated assay (i.e., Filovirus Animal Nonclinical Group (FANG) enzyme-linked immunosorbent assay (ELISA)) was conducted at the same laboratory (Q^2 Solutions, United States) [108, 109]. Figure 5 depicts the EBOV GP-specific binding antibody GMCs of all published phase 1 and 2 results whereby the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen was administered at a 56-day interval [88, 90-92, 94, 97, 98, 100, 101, 103].

The humoral immune responder rate (defined as having a ≥2·5-fold increase in EBOV GP-specific binding antibody concentration over baseline, i.e., before administration of the first dose) 21 days after vaccination with the full regimen, administered at a 56-day interval, was ≥95% for all assessed study populations. In healthy adults, this corresponded with a pooled EBOV GP-specific IgG GMC of 6,758 EU/mL (95% CI 5,547-8,349) [88, 90-92, 94, 98], in adolescents (12-17 years old) with a pooled GMC of 10,950 EU/mL (95% CI 8,898-13,480) [97, 100], in children (1-11 years old) with a pooled GMC of 16,566 EU/mL (95% 13,343-20,594) [97, 100], and in infants (<1 years old) with a GMC of 24,309 Elisa Units (EU)/mL (95% CI 19,695-30,005) [101]. In general, the younger the vaccinated individual, the higher the observed magnitude of the humoral response. However, differences in immune response within age groups seem to be present across studies and geographical locations [109]. Reasons for this observation could be numerous (e.g., socio-economic status, concomitant disease burdens, poor nutritional status, pathogen exposure, etc.) [110]. Nevertheless, these possible explanations have not been formally studied, and additional

research is needed to assess whether any of these aspects contributed to the observed differences in the humoral immune response across studies and countries.

Figure 5. Ebola-specific geometric mean concentrations (GMCs) of all Ad26.ZEBOV, MVA-BN-Filo clinical trials that have been conducted and published (excluding our trial results).



Colours represent the different studies that have been conducted in different populations. Some studies were conducted in multiple populations (e.g., adults and children); results of these are presented separately. Only results of the Ad26.ZEBOV, MVA-BN-Filo regimen administered at a 56-day interval have been included. This figure was created in R v4.3.1. Data was obtained from several phase 1 and 2 studies reporting on the immunogenicity of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen administered at a 56-day [88, 90-92, 94, 97, 98, 100, 101, 103].

While the Ad26.ZEBOV, MVA-BN-Filo regimen has been administered in different countries (e.g., UK, France, US, Sierra Leone, Liberia, Guinea, Kenya, Uganda, etc.), individuals of different races, and different ages, the vaccines' safety and immunogenicity have also been assessed in HIV-positive adults (well controlled with antiretroviral therapy), and adults and children with asymptomatic malaria infection, or with clinical malaria before or shortly

after vaccination [98, 102, 104]. As EVD mainly occurs in West and Central Africa and is thus the location and population where these vaccines will most often be administered, the functioning of these vaccines in individuals infected with the human immunodeficiency virus (HIV), acquired immunodeficiency syndrome (AIDS), or (asymptomatic) malaria, which most affect Sub-Saharan Africa [111, 112], was a crucial research objective. First, though vaccination led to an immune response in HIV-positive patients, this response was lower than in healthy adults vaccinated within the same study (geometric mean ratio (GMR) healthy versus HIV-positive adults of 1.4 (95%CI: 1.1-1.9)) [98]. However, of the 58 vaccinated HIV-positive adults with an evaluable serum sample 21 days after vaccination with the vaccine regimen, 100% were considered responders as per the responder rate definition and thus the regimen can be considered immunogenic in HIV-positive individuals [98]. Additionally, when taken up in a multi-study analysis, the lower response observed in HIV-positive individuals compared to healthy adults was no longer observed [109]. Second, in one study, children and adults with asymptomatic malaria (depicted by the presence of malaria parasitaemia without symptoms) prior to the administration of the first regimen dose (51.5% and 47.5%, respectively), had a lower, non-significant, EBOV GP-specific binding antibody response (GMR 0.82, 95%CI: 0.67-1.02) compared to individuals without malaria parasitaemia at the time of vaccination [102]. Finally, the regimen has shown to be immunogenic in individuals with previous exposure to malaria prior to vaccination and, likewise, in individuals who experience clinical malaria shortly after vaccination [104]. This indicates that the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen can be administered for prophylaxis against EVD in malaria-endemic regions (as is Boende).

Several trials have also assessed the long-term persistence of EBOV-specific binding antibodies after vaccination with the Ad26.ZEBOV, MVA-BN-Filo regimen at six months, eight months, one year, or two years in children, adolescents, and/or adults (Figure 5) [88, 90-92, 94, 97, 98, 100, 101, 103]. Throughout these studies, a decrease in binding antibodies was observed until 6 to 8 months after the first dose, with a stabilisation in EBOV-specific GMCs thereafter (Figure 5). In adults, this stabilisation has been assessed up to two years after the first dose [102]. An additional study, in children has shown that the

stabilisation lasted until more than three years after the first dose [113]. Likewise, in HIV-infected adults, persistent antibody levels have been observed until more than four years after the initial dose [114]. Whether this persistence in binding antibody response is equivalent to protection from EVD is unclear. As it has not been possible to conduct vaccine efficacy studies for the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen during an outbreak, it is unknown which level of binding antibodies in humans can be considered protective. To assess the likelihood of protection of the regimen in humans, immunobridging analysis between NHP models and human binding antibody levels after vaccination has been performed [73, 74]. While immunobridging shows that the regimen will likely provide protection against EBOV disease in humans [73, 74], the current immunobridging model unfortunately does not evaluate whether the persistence of the vaccine-induced immune response relates to the durability of protection in humans [115].

As previously stated, only for the single-dose rVSV△G-ZEBOV-GP vaccine a 97.5-100% clinical efficacy in humans has been established [64, 65]. For this vaccine, researchers have tried to identify a correlate of protection (CoP) and found that a EBOV-GP specific binding antibody seroresponse of ≥200 EU/mL post-vaccination, that was ≥2 times the pre-vaccination value, could be a possible CoP [116]. As the same assay (i.e., FANG ELISA) was used to measure the EBOV-specific GP binding antibody response after vaccination for both this vaccine and the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen, one could hypothesise that these results are extrapolatable and indicate that a persistence of EBOV-specific binding antibodies of ≥200 EU/mL one, two or more years after vaccination with the regimen still provides protection. However, this hypothesis has not been confirmed by NHP challenge models. Like in humans, a comparable pattern of 'waning followed by stabilisation' in EBOV-specific binding antibodies was noted among NHP following vaccination with the Ad26.ZEBOV, MVA-BN-Filo regimen [115]. In the absence of an Ad26.ZEBOV booster dose before an Ebola virus challenge 1.6 years after the first dose, NHP faced a fully lethal outcome [115]. Conversely, animals that received an Ad26.ZEBOV booster dose exhibited full protection, experiencing minimal morbidity and the absence of viremia, even when the Ebola virus challenge occurred as soon as 3 days after the booster

vaccination [115]. However, some researchers have hypothesised that the differences in EVD incubation time and progression between NHP and humans (i.e., longer and slower in humans, respectively) may provide humans with enough time to develop the necessary immune memory response compared to NHP based on persisting antibodies alone. Administering an Ad26.ZEBOV booster dose to previously vaccinated individuals at imminent risk of infection (e.g., HCP and frontliners in an outbreak) may thus not be required; however, as this is currently not supported by real-world evidence in humans or by NHP challenge models, it may be more prudent at the time of an outbreak.

While most studies have focussed on the binding antibody response, several phase 1 and 2 studies have also assessed the vaccine-induced neutralising antibody responses using an EBOV GP Pseudovirus Neutralizing Assay in adults, children (1-11 years old) and adolescents (12-17 years old) [90-92, 94, 97, 98, 100]. Overall, 94-100% of participants had a neutralising antibody response 21 days after the second dose of the vaccine regimen [90-92, 94, 97, 98, 100]. As for the binding antibody response, the magnitude of the neutralising antibody response declined by 6 months after the first dose and remained stable thereafter until one year after the first dose [90, 91]. Strong positive correlations, as measured by Spearman's correlation coefficients, between GP-binding and neutralising antibodies were observed both 21 days after vaccination with the heterologous 2-dose regimen (r>0.751) and when assessed, one year after the first dose ($r>0.631$) [92, 94, 97, 98, 100].

Next to the EBOV-neutralising antibody response, several studies have also looked into the effect of pre-existing Ad26-neutralising antibodies on the Ad26.ZEBOV vaccine response [94, 97, 98, 100, 101]. This was important because individuals with pre-existing immunity to Ad5, as indicated by the presence of neutralising antibodies, showed a reduced response to the target antigen of the Ad5-vectored Ebola vaccine [117]. Although this impact on vaccine immunity had not been noted for an Ad26-based HIV vaccine in rhesus monkeys [55], it was uncertain whether this was also the case for the Ad26.ZEBOV vaccine in humans. In studies conducted in several African study sites, where the proportion of participants considered positive for pre-existing Ad26-neutralising antibodies was

generally high in healthy adults (>91%), HIV infected adults (83%), children 1-3 years old (20%), children 4-11 years old (>71%), and adolescents 12-17 years old (>78%), no or negligible correlations, as measured using Spearman correlation coefficients, were observed between Ad26-specific neutralising antibodies and the vaccine-induced EBOV GP-specific binding antibodies 21 days after the second dose (*r*=-0.20-0.22) [94, 97, 98, 100]. A similar negligible correlation was found between Ad26-specific neutralising antibodies and EBOV GP-specific neutralising antibodies (*r*=-0.09-0.11) [97, 98]. Except for four infants <1 year old, pre-existing neutralising antibodies against the Ad26-vector were below the LLOQ and thus, a meaningful correlation analysis for this age group was not possible [101].

Only one trial previously looked at the effect of pre-existing MVA-neutralising antibodies on the regimen's immune response 21 days after vaccination with the MVA-BN-Filo vaccine in healthy vaccinated adults (≥18 years old), and healthy children and adolescents (1-17 years old) [94, 100]. However, only five out of 98 healthy adults (5%) and none of the children and adolescents had pre-existing neutralising antibodies against MVA and thus the influence of these antibodies on the EBOV GP-specific binding antibody response could not be assessed [94].

Several studies have also looked into the cellular response, and more specifically the $CD4^+$ and $CD8^+$ T-cell responses, of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen [88, 90-92, 97, 98]. Seven to 21 days after the second dose (depending on the assessment timepoint), $CD4^+$ and $CD8^+$ T-cell responder rates of healthy adults varied between 32-67% and 27-79%, respectively [88, 90-92, 98]. The $CD8^+$ T-cell response seemed to be relatively lower among African study populations (27-50%) compared to European study populations (55-79%) [88, 90-92, 98]. Depending on the study, T-cell responses were either sustained until 6-8 months after vaccination [88], or had declined [91]. Though a decline in T-cell response was observed at one year after the first dose, a response remained detectable among study participants [90, 91]. One study also assessed T-cell responses in HIV-infected adults and found that 40% of vaccinated individuals had a $CD4^+$ T-cell response, compared to 17% of participants having a $CD8^+$ T-cell response [98]. Another study looked at the T-cell

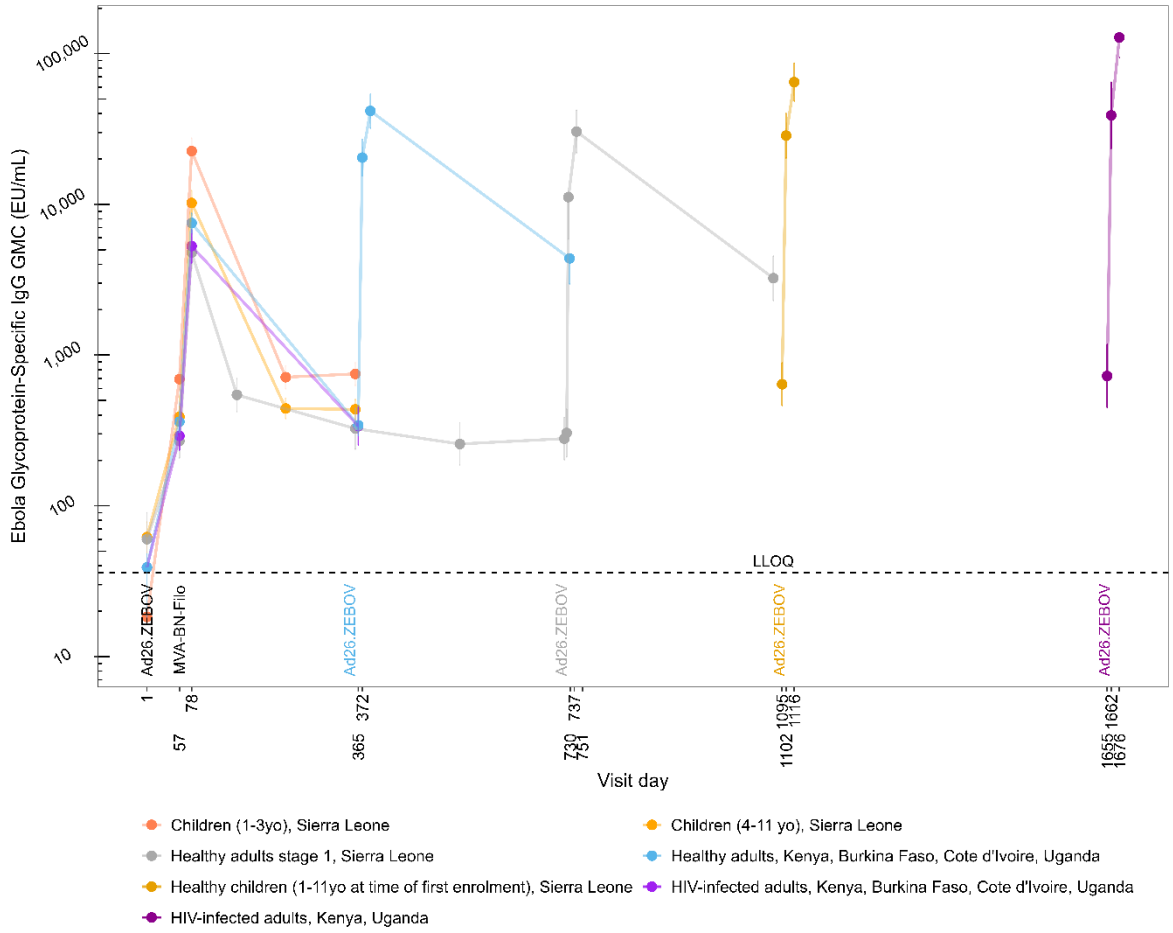
responses in children (4-11 years old) and adolescents (12-17 years old) and found no apparent differences in $CD4^+$ (33% vs 40% respectively) and $CD8^+$ T-cell responses (none were detectable) between both groups [97].

Two studies evaluated the response of natural killer (NK) cells post-regimen vaccination [118, 119]. This evaluation was conducted due to the established correlation between the initial innate cytokine response and the clinical outcomes of individuals infected with the Ebola virus [120]. However, the specific role of NK cells in vaccine-induced protection against EVD remains uncertain. The study findings demonstrated enhanced proliferation and activation of NK cells following vaccination with the vaccine regimen compared to baseline, suggesting that NK could potentially contribute to the early immune responses triggered by the regimen [118, 119]. Additionally, the observed NK cell responses were shown to be enduring (both ex vivo and in vitro) after vaccination with the Ad26.ZEBOV, MVA-BN-Filo regimen until 180 Days after vaccination with the first dose [119]. Following this, the authors suggest that this enhanced NK cell function after vaccination may contribute to both immediate and long-lasting immunity against EBOV infection.

Four studies have previously reported safety and humoral immunogenicity results of an Ad26.ZEBOV booster dose, administered one or more years after the initial dose of the Ad26.ZEBOV, MVA-BN-Filo regimen (56-day interval; Figure 6) [94, 98, 113, 114]. One study concentrated on children boosted approximately 3 years after the first dose (N=50), one on HIV-infected adults with a well-controlled infection and on highly active antiretroviral therapy boosted on average 4.5 years after the first dose (N=13), and two studies involved healthy adults; one (N=39) administered a booster dose one year after the first dose, while the other (N=29) administered a booster dose two years after the first dose. In all studies a fast (i.e., within seven days) and robust humoral immune memory response (i.e., >40-fold increase in binding antibodies compared to pre-booster vaccination) was observed. Importantly, the safety profile of the booster dose closely resembled that of the first Ad26.ZEBOV dose, and no vaccine-related serious adverse events were reported. Binding

antibody responder rates of ≥96% were reported and, when assessed, persisted in 100% of the participants one year after receiving the booster vaccination.

Figure 6. Ebola-specific geometric mean concentrations (GMCs) of all clinical trials where an Ad26.ZEBOV booster dose that was administered after vaccination with the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen at a 56-day interval (excluding our trial results).



The timing of the booster dose for each study is shown in colour at the bottom of the figure in the same colour as the portrayed immune memory response of that study. In children and HIV-infected individuals only a subset of participants was boosted. The original immunogenicity results of the Ad26.ZEBOV, MVA-BN-Filo regimen from which this selection of participants was boosted has been depicted in a slightly different but similar colour. In children 1-11 years old, no pooled GMC was available for 1-3 and 4-11 year olds. However, the booster results for these groups were pooled. This figure was developed in R v4.3.1 and EBOV-specific GP binding antibodies were obtained from [94, 98, 100, 113, 114].

1.5.1.3 *Rationale for the Ebola vaccine trial of this doctoral thesis*

In 2014, an EVD outbreak occurred (with an EBOV strain closely related to the 1995 Kikwit variant) in Inkanamongo, a small and remote village located in the health district of Boende, Tshuapa province, DRC [121]. Inkanamongo is not far from the province's capital city Boende (Figure 7), where some EVD cases were hospitalised and treated during this outbreak. This doctoral thesis describes the methodology, results and research challenges of an open-label, randomised, Ebola vaccine trial, conducted in Boende. The trial aimed to improve preparedness for future Ebola outbreaks by vaccinating a well-known population at risk, i.e., a cohort of approximately 700 HCP and frontliners who may be exposed to Ebola in the event of a future outbreak in the DRC. For this study, HCP were defined as professions working in a healthcare facility that could come into contact with infectious diseases in this facility (e.g., doctors, nurses, midwives, health facility cleaner, etc.) and frontliners as professions that could be exposed to infectious diseases in the community (e.g., community healthcare workers, first aid workers, pharmacists, etc.).

The trial was set up to assess the safety and (long-term; up to two years) humoral immune response (via the assessment of EBOV GP-specific binding antibodies) after vaccination with the two-dose heterologous Ad26.ZEBOV, MVA-BN-Filo vaccine regimen, administered at a 56 day interval. Additionally, to assess the immune memory response of participants vaccinated with this regimen, a booster vaccination with Ad26.ZEBOV was provided at one year or two years after the first dose (randomisation ratio 1:1). Next to the anamnestic response, the safety of the booster dose was assessed, and for participants boosted one year after the first dose the persistence of binding antibodies one year after booster vaccination was determined. The focus of this trial was on EBOV-specific GP binding antibodies and not on neutralising antibodies or cellular responses. This decision was based on NHP challenge models, which show a strong correlation between the amount of binding antibodies and survival [72].

Figure 7. Image of Boende taken during a mission in December 2021.

Finally, to circle back to what the trial discussed in this doctoral thesis will add to the already existing knowledge: our trial was (1) the first to vaccinate a large sample of at risk individuals (i.e., HCP and frontliners in an area previously affected by Ebola), follow them up to assess the safety and (persistence of) EBOV-specific binding antibodies produced after vaccination with the Ad26.ZEBOV, MVA-BN-Filo regimen up to one and two years after the first dose, and (2) the first to administer an Ad26.ZEBOV booster dose in a large cohort of participants one or two years after the first dose, making the booster dose responses (concerning both safety and immunogenicity) comparable within this population. The protocol of this trial was published and is described in Part I, Chapter 2. The results of the trial were published in two separate papers and presented in Part II, Chapters 3 and 4, of this doctoral thesis. Chapter 3 describes the safety and immunogenicity of the primary vaccine regimen [122], while chapter 4 addresses the long-

term humoral immune response after vaccination with the heterologous two-dose regimen, and the safety and immune memory response capabilities of two booster vaccination time points [123].

1.5.2 Vaccine research challenges in LMICs

While reporting the obtained scientific knowledge from this trial is highly relevant for further vaccine development and licensure, understanding the challenges, mitigations taken, and lessons learned from this trial, conducted in a remote and resource-poor area of the DRC, holds substantial importance. By also sharing these aspects, the focus of this vaccine trial moves from purely biomedical clinical research towards advancing global health and addressing health disparities.

When conducting trials in LMICs, researchers are often faced with unique challenges such as limited resources, poor infrastructures, challenging climate conditions, limited quality health care available in the area where research is being conducted, and diverse socio-cultural contexts [124-129]. Discussing these challenges, how they were mitigated, and which lessons were learned, provides valuable insights into the feasibility and effectiveness of vaccination strategies in real-world conditions of remote sections of the world. For example, for this trial, participants were required to come to one central vaccination point, i.e., the General Reference Hospital (GRH) of Boende. This is also the location where the routine vaccinations are stored by the Expanded Programme on Immunization (EPI) before being distributed to health facilities, where routine vaccinations are administered. Therefore, assessing for example vaccine storage challenges in this location as part of the vaccine trial, provides crucial information that can be used to tailor future vaccine programmes to the specific possibilities and constraints of these regions.

Secondly, sharing the challenges and lessons learned from this vaccine trial shows transparency and accountability towards the global health research community. It ensures that the scientific community (i.e., researchers, ethics committees, funders, etc.), policymakers, vaccine developers, and the general public have access to a comprehensive

overview of the challenges and successes encountered during the trial. By being transparent in all aspect of vaccine research – or clinical research in general for that matter – a better understanding and stronger collaboration can be forged between local, national and international stakeholders as these collaborations will more likely be based on realistic expectations and trust. Ultimately, the dissemination of these challenges, mitigations taken, and lessons learned is instrumental in creating a more inclusive, equitable, and effective approach to vaccine development, research and deployment worldwide.

Therefore Part III (Chapters 5-7) of this doctoral thesis addresses three papers (two published, one submitted). Chapter 5 and Chapter 6 present the published papers discussing the challenges, mitigations taken, and lessons learned from setting up and conducting our Ebola vaccine trial [130, 131]. Chapter 7 presents a recently submitted paper and is more focussed on the need to go beyond researchers' responsibilities when conducting research in a remote setting with limited available quality health care. This paper evaluates a study-specific policy and algorithm (developed by the sponsor and principal investigator (PI) teams of the trial [132]) and provides recommendations for researchers interested in applying a similar approach when conducting research in a remote and resource-constrained setting.

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Chapter 2 Ebola vaccine trial protocol

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

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2.1 Abstract

Introduction. This article describes the protocol of an Ebola vaccine clinical trial which investigates the safety and immunogenicity of a two-dose prophylactic Ebola vaccine regimen comprised of two Ebola vaccines (Ad26.ZEBOV and MVA-BN-Filo) administered 56 days apart, followed by a booster vaccination with Ad26.ZEBOV offered at either 1 year or 2 years (randomisation 1:1) after the first dose. This clinical trial is part of the EBOVAC3 project (an Innovative Medicines Initiative 2 Joint Undertaking), and is the first to evaluate the safety and immunogenicity of two different booster vaccination arms in a large cohort of adults.

Methods and analysis. This study is an open-label, monocentric, phase 2, randomised vaccine trial. A total of 700 healthcare providers and frontliners are planned to be recruited from the Tshuapa province in the Democratic Republic of the Congo (DRC). The primary and secondary objectives of the study assess the immunogenicity of the first (Ad26.ZEBOV), second (MVA-BN-Filo) and booster (Ad26.ZEBOV) dose. Immunogenicity is assessed through the evaluation of EBOV glycoprotein binding antibody responses after vaccination. Safety is assessed through the collection of serious adverse events from the first dose until 6 months post booster vaccination and the collection of solicited and unsolicited adverse events for 1 week after the booster dose.

Ethics and dissemination. The protocol was approved by the National Ethics Committee of the Ministry of Health of the DRC (n°121/CNES/BN/PMMF/2019). The clinical trial was registered on December 2019 on ClinicalTrials.gov. Trial activities are planned to finish in October 2022. All participants are required to provide written informed consent and no study-related procedures will be performed until consent is obtained. The results of the trial will be added on ClinicalTrials.gov, published in peer-reviewed journals and presented at international conferences.

Trial registration number. NCT04186000; Pre-results.

2.2 Strengths and limitations of this study

- With this randomised vaccine trial, being the first to evaluate the safety and immunogenicity in two different booster vaccine arms 1 or 2 years after the prime dose, new contributions will be added to already existing safety and immunogenicity data. Additionally, it is the first trial to assess the antibody response and (serious) adverse event occurrence of two different booster arms in a large adult cohort.
- Vaccination of healthcare providers and frontliners can potentially help protect a community which is at risk for future outbreaks.
- Innovative use of iris scanning biometric material to identify participants enrolled in the trial.
- This study takes place in a resource poor setting, impacting logistical set-up of the trial.
- Long duration of the trial (2.5 years) may lead to considerable loss to follow-up.

2.3 Introduction

Ebolaviruses (negative stranded RNA viruses) belong to the *Filoviridae* family and cause Ebola virus disease (EVD), which often leads to severe haemorrhagic fever in humans and non-human primates[1]. Contact with infected wild animals (such as fruit bats, gorillas, apes and monkeys) is often reported as the source of animal-to-human transmission[2-4] and once among humans, these public health pathogens spread via direct (body fluids) or indirect (contaminated surfaces) human-to-human contact[2, 3). While they do not spread via air or water[3], *Ebolaviruses* bring along a severe public health burden with case fatality rates that can range up to 90%[5]. Since the discovery of the *Ebolaviruses* in 1976[6], more than 20 outbreaks (most are endemic to regions in equatorial and western Africa) have taken place[7]. To date, the Democratic Republic of the Congo (DRC) has been the most affected country with its 12th outbreak taking place between February and May 2021[8]. However, it is only recently that the search for a safe and effective vaccine against Ebola was accelerated when the epidemic potential of *Ebolaviruses*, and more specifically the species *Zaïre Ebolavirus* (virus name: Ebola virus; abbreviation: EBOV[9]), became clear through the West African Ebola epidemic (2013-2016; 28,616 cases with 11,310 deaths[10]).

One of the initiatives to develop such a vaccine came from an international consortium, funded by the Innovative Medicines Initiative 2 (IMI2) Joint Undertaking, dedicated to evaluate a prophylactic Ebola vaccine regimen comprised of two candidate Ebola vaccines (Ad26.ZEBOV and MVA-BN-Filo) and aiming to bring this prophylactic Ebola vaccine to licensure[11]. Ad26.ZEBOV is a monovalent vaccine developed to provide active EBOV-specific immunity. MVA-BN-Filo, which is administered 56 days after the Ad26.ZEBOV vaccine, is a multivalent vaccine developed to establish active immunity to EBOV, *Sudan Ebolavirus*, *Taï Forest Ebolavirus* and the Marburg virus (also part of the *Filoviridae* family). In July 2020, the two-dose prophylactic vaccine regimen was granted market authorisation by the European Commission[12].

Several projects (PREVAC, EBOVAC1, EBOVAC2 and EBOVAC3) within the IMI2 Joint Undertaking, of which the first in-human clinical trials started in 2014, were at the basis of this successful authorisation[11]. Within these projects, multiple clinical trials assessed or are assessing the timing, tolerability, safety and immunogenicity of different Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in healthy adults (≥18 years old) and children/adolescents (1-17 years old) via phase 1, 2, 2B and 3 studies. Initial trials showed that healthy adult participants had higher geometric mean concentrations of binding antibodies for the regimen where Ad26.ZEBOV vaccination was followed by an MVA-BN-Filo vaccination 56 days later. Moreover, 100% of participants had detectable Ebola glycoprotein-specific Immunoglobulin G (IgG) antibodies up to at least 240 days after vaccination[13-15]. Even though some local (erythema, swelling and pain at injection site) and systemic (headache, nausea, fever, myalgia and fatigue) solicited adverse events were recorded, the vaccine regimen was generally well tolerated across studies[13-17].

While it is of utmost importance that the two-dose prophylactic vaccine regimen is safe and leads to an immune response, it is also crucial to find out whether or not this regimen can lead to induced immune memory at the time of imminent risk (ie, an outbreak) through a booster vaccination. To evaluate this induced immune memory response, three previous studies within EBOVAC projects have administered a booster vaccine with Ad26.ZEBOV at either 1 year (NCT02325050; NCT02564523) or 2 years (NCT02509494) post dose 1. Results from the NCT02325050 trial have already shown that an immunological memory was rapidly induced via booster vaccination with Ad26.ZEBOV, indicating that booster vaccination can be considered for at risk individuals (eg, when an outbreak occurs) that were previously vaccinated with the two-dose heterologous prophylactic regimen[18]. However, these trials only evaluated booster vaccination in a small amount of participants (n≤39) and it still has to be explored whether the induced immune memory response differs depending on the timing of the booster dose (ie, 1 or 2 years after dose 1).

Healthcare settings play an important role in the control of EVD and therefore healthcare providers (HCP) and frontliners, due to occupational exposure, are not only more at risk of

disease acquisition but also facilitate the spread of the virus[19-22]. Knowing that outbreaks of EVD often occur in regions where there is already a shortage of HCP and frontliners, this further depletes a weak healthcare system and the quality of care. Consequently, the Strategic Advisory Group of Experts stated in 2018 that for preventive strategies, vaccination of HCP as part of an emergency preparedness plan has significant potential of reducing the scale and duration of outbreaks[23].

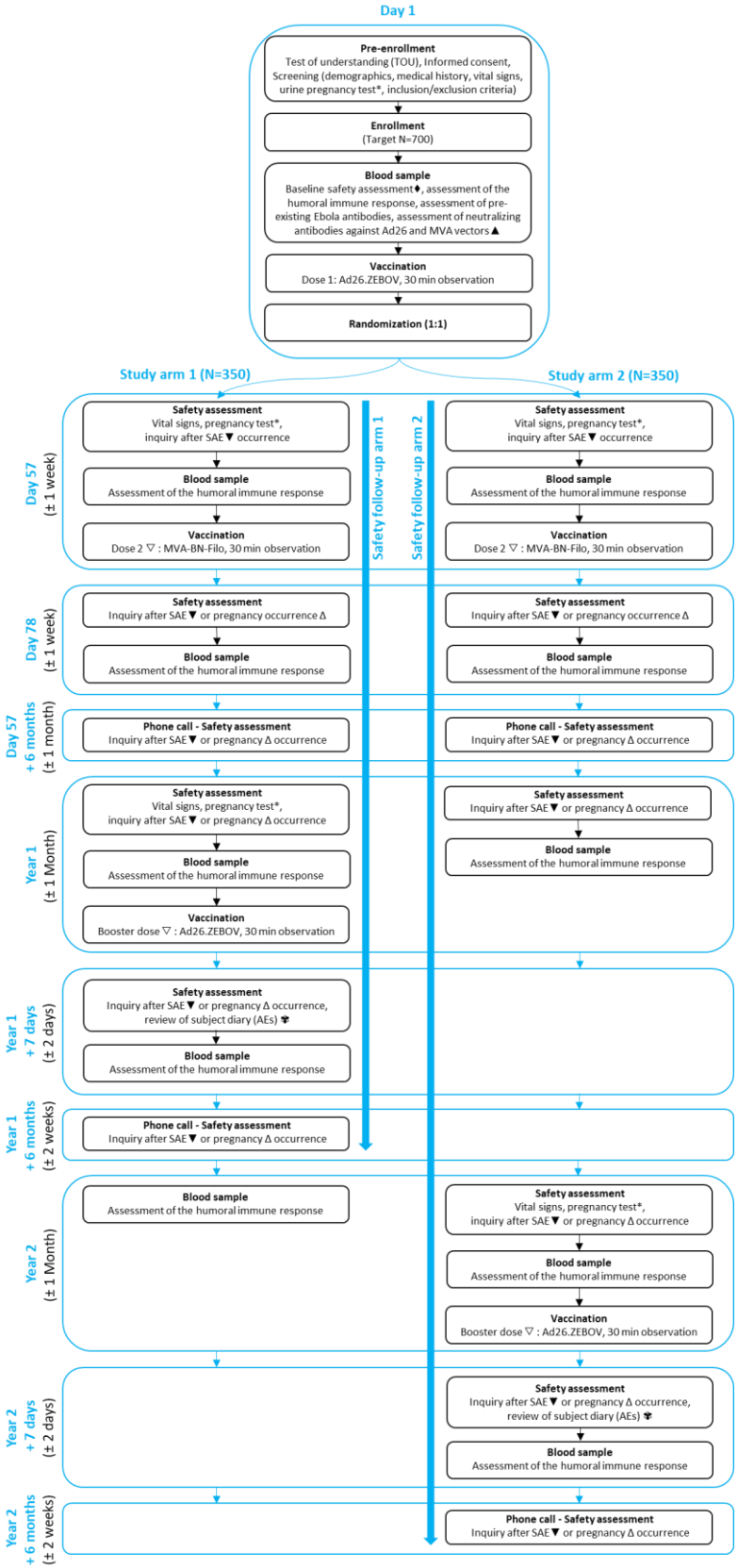
This phase 2 randomised clinical trial aims to determine the safety and immunogenicity of the two-dose heterologous vaccine regimen with Ad26.ZEBOV followed by MVA-BN-Filo 56 days later. Additionally, this trial aims to assess the safety and immunogenicity of a booster Ad26.ZEBOV vaccine administered either 1 or 2 years post first dose and to compare the induced immune memory response between both booster arms. The trial is conducted in a cohort of HCP and frontliners from the Boende health district in DRC, a well-known population at risk from clinical and epidemiological perspective.

2.4 Methods

2.4.1 Study design and setting

This study is an open-label, monocentric, phase 2, randomised trial to evaluate the immunogenicity and safety of Ad26.ZEBOV ($5x10^{10}$ viral particles) as first dose and MVA-BN-Filo ($1x10^{8}$ infectious units) as second dose vaccination at a 56-day interval in HCP and frontliners who may be exposed to Ebola in the event of a future Ebola outbreak in DRC. Additionally, after randomisation (1:1) a booster of Ad26.ZEBOV ($5x10^{10}$ viral particles) will be offered at either 1 year or 2 years after the first dose (figure 1).

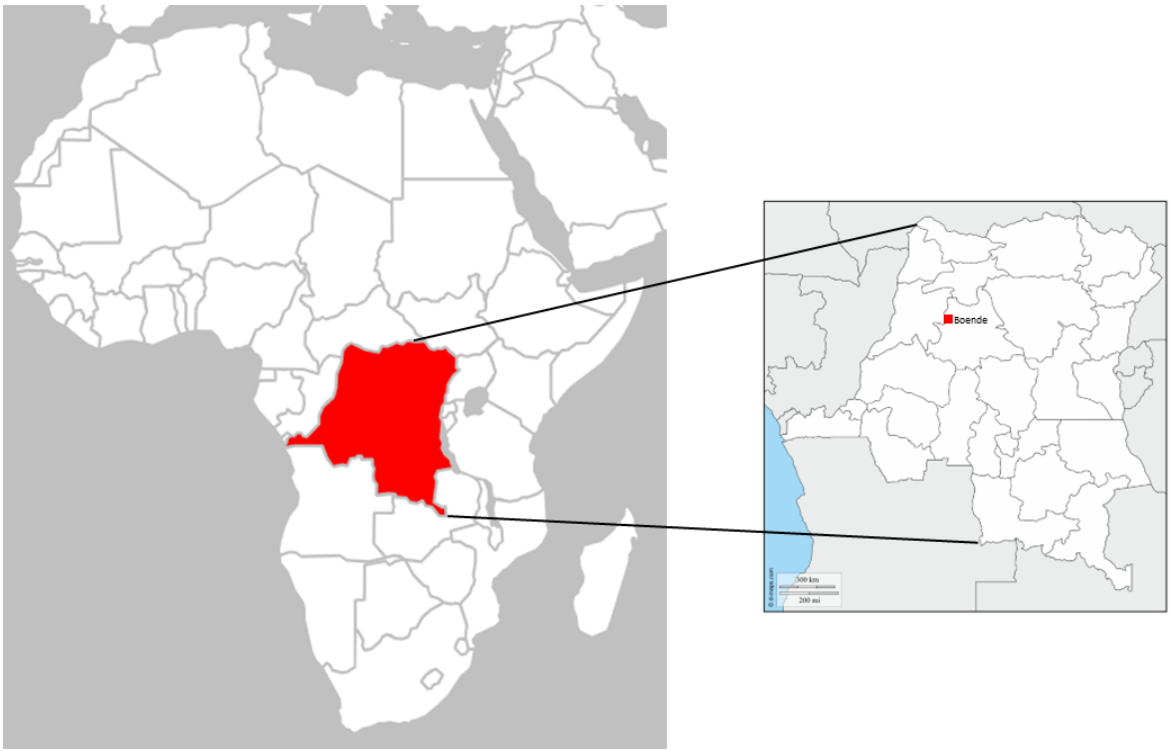
Figure 1. Study time and events overview.



*SAE: Serious Adverse Event; * Only for female participants of childbearing potential; ♦ Abnormal results will not exclude a participant, as results will not be reviewed prior to enrolment; ▲ Only the first 100 participants enrolled will be tested for neutralising antibody response against ad26 VNA and MVA vectors. Other blood analyses are for all 700 participants; ▼ Concomitant therapies given in conjunction with a serious adverse event (SAE) should be recorded from signing of the ICF onwards until 6 months post booster; ▽ The Investigator may withhold the second vaccine or booster dose if a participant's clinical status changes prior to vaccination. The participant should continue to be followed for safety and immunogenicity according to the protocol; Δ Only for female participants; ✾ Solicited and unsolicited Adverse Events (AEs) will be collected in a participant diary during 1 week post booster vaccination.*

The study site is located in Boende, Tshuapa province, DRC (figure 2), at approximately 750 km north-west of Kinshasa. Study participants will be enrolled at the General Reference Hospital in Boende.

Figure 2. Study site location.



On the left, the Democratic Republic of Congo (DRC) is highlighted on a map of the African continent. On the right, the study site location (Boende, Tshuapa province) is marked on a map of DRC indicating its provinces [35].

2.4.2 Objective

The primary, secondary and exploratory objectives and endpoints of this study are described in table 1.

Table 1. Objectives and endpoints.

Objectives	Endpoints
Primary	
• To assess binding antibody responses post-dose 2 vaccination with MVA-BN-Filo.	• Binding antibody levels against the EBOV GP using FANG ELISA at 21 days post-dose 2 (Day 78) vaccination with MVA-BN-Filo.
Secondary	
• To assess binding antibody responses after booster vaccination with Ad26.ZEBOV given at 1 or 2 years after first dose.	• Binding antibody levels against the EBOV GP using FANG ELISA at 7 days (excluding the day of vaccination) post booster.
• To assess the safety of a heterologous vaccine regimen utilizing Ad26.ZEBOV and MVA-BN-Filo administered at a 56-day interval and a booster vaccine with Ad26.ZEBOV at one or two years post first dose.	• Serious adverse events from first dose vaccination until 6 months post booster. • Solicited and unsolicited local and systemic adverse events until 7 days post booster vaccination (day of vaccination and subsequent 7 days) with Ad26.ZEBOV.
Exploratory	
• To assess binding antibody responses at different time points as indicated in the Study time and events overview (Figure 1).	• Binding antibody levels against the EBOV GP using FANG ELISA at different time points as indicated in the Study time and events overview (Figure 1).
• To assess neutralising antibody response directed against the Adenovirus vector prior to vaccination.	• Neutralising antibody levels against Ad26 using Ad26 VNA at the first visit.
• To assess neutralising antibody response directed against the MVA vector prior to vaccination.	• Neutralising antibody levels against MVA-BN-Filo using MVA PRNT assay at the first visit.
• To assess seroprevalence of Ebola virus disease prior to vaccination.	• Presence of pre-existing Human anti-EBOV GP IgG and anti-EBOV NP IgG using LUMINEX assay.

ELISA: enzyme-linked immunosorbent assay; EU/mL: ELISA units/mL; FANG: Filovirus Animal Nonclinical Group. VNA: Virus Neutralization Assay; PRNT: Plaque Reduction Neutralization Test

2.4.3 Participant population and sample size

A total of 700 Registered HCPs and frontliners in DRC (working in the Boende General Reference Hospital, Health Centres or Health Posts in the Boende health district) are planned to be recruited from the Tshuapa province. This assessment was based on information obtained from an ongoing (monkeypox) vaccine trial in the same area at the time the protocol was being written[24]. From discussions with the monkeypox research group, it became clear that a high enrolment rate and retention rate (>90% after 2 years) could be expected among HCPs and frontliners in the Boende health district. Based on this

ongoing monkeypox trial, it was estimated that enrolling approximately 50% of the HCP and frontliners working in the Boende health district would be feasible. The participant population is thus a convenience sample and the sample size is defined on the feasibility of recruitment of HCPs and frontliners in the region.

However, to determine whether it would be possible to compare the induced immune responses of the two booster arms, a power analysis was performed. A power of 0.99 was calculated based on the following parameters: two-sided t-test, equal samples of 350 participants, significance level of 0.05, an effect size of 0.49 in antibody response. The effect size was calculated based on trial data (NCT02564523 and NCT02509494) available in the first edition of the combined investigator's brochure of the vaccines with samples from 64 participants vaccinated either 1 year or 2 years after the first dose[25]. To obtain the effect size, the difference in geometrical mean concentrations (log scale) of the EBOV glycoprotein (GP)-specific antibody responses between the two groups was divided by the pooled standard deviations[26]. With a power of 0.99 it will thus be possible to perform a formal comparative analysis of the induced immune memory response of the two booster arms.

Unfortunately no power analysis could be performed to determine whether the sample size is sufficiently large to perform a formal statistical comparison of safety response (AEs and serious AEs (SAEs)) from both arms. In the current combined investigator's brochure of the vaccines[25], safety information is pooled for all booster doses independent of the timing of its administration (1 year or 2 years post dose 1) and thus no effect size can be calculated until the unpooled data from the different trials is obtained.

Inclusion and exclusion criteria that determine the eligibility of participants are reported in box 1.

Box 1. Inclusion and exclusion criteria

Inclusion criteria

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

1. The participant must pass the Test of Understanding.

   Note: If the participant fails the Test of Understanding 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 participants fail on the third attempt, they should not continue with screening or consenting procedures.
2. Each participant must sign an informed consent form indicating that he or she understands the purpose of, and procedures required for, the study and is willing to participate in the study. In case the participant cannot read or write, the procedures must be explained and informed consent must be witnessed by a trusted literate third party not involved with the conduct of the study.
3. The participant must be a man or women aged 18 years or older.
4. The participant must be a documented healthcare provider in the Democratic Republic of the Congo.
5. The participant must be healthy in the investigator's clinical judgement and on the basis of vital signs assessed at day 1 screening.

   Note: Subjects who are HIV-positive can be enrolled as long as their general condition is good, that is, they are on antiretroviral treatment or have no signs or symptoms of immunodepression, diagnosed on the basis of physical examination, medical history, and the investigator's clinical judgement.
6. Before vaccination, a woman must be either:
   - Of childbearing potential and practicing (or intending to practice) a highly effective method of birth control consistent with local regulations and/or local culture regarding the use of birth control methods for participants in clinical studies, beginning at least 28 days prior to vaccination and during the study up to at least 3 months after the first (or only) vaccination

(Ad26.ZEBOV) and 1 month after the MVA-BN-Filo vaccination (if applicable); and then starting again 14 days before the booster vaccination until 3 months after the booster vaccination. The sponsor considers the following methods of birth control to be highly effective: established use of oral, injected or implanted hormonal methods of contraception; placement of an intrauterine device or intrauterine system; barrier methods: condom or occlusive cap (diaphragm or cervical/vault caps) with or without spermicidal foam/gel/film/cream/suppository; male partner sterilisation (the vasectomised partner should be the sole partner for that participant); true abstinence (when this is in line with the preferred and usual lifestyle of the participant); OR

- Not of childbearing potential: postmenopausal (amenorrhoea for at least 12 months without alternative medical cause); permanently sterilised (eg, bilateral tubal occlusion (which includes tubal ligation procedures as consistent with local regulations), hysterectomy, bilateral salpingectomy, bilateral oophorectomy); or otherwise be incapable of pregnancy.

  Note: If the social situation of a woman of childbearing potential changes (eg, woman who is not heterosexually active becomes active), she must begin a highly effective method of birth control, as described above.

7. Woman of childbearing potential must have a negative urine β-human chorionic gonadotropin pregnancy test immediately prior to each study vaccine administration.
8. Participant must be available and willing to participate for the duration of the study.
9. Participant must be willing and able to comply with protocol requirements (including certain prohibitions and restrictions such as the use of anticonception and the discouragement of concomitant treatment that may alter the immune response).

10. Participant must be willing to provide verifiable identification.
11. Participant must have a means to be contacted.

Exclusion criteria

Participants will be excluded from study participation in case the following criteria apply:

1. The participant has a known history of Ebola virus disease.
2. The participant has received any experimental candidate Ebola vaccine less than 3 months prior to the first study visit.
3. The participant has received any experimental candidate Ad26-vaccine in the past.

   Note: Receipt of any approved or experimental vaccinia/smallpox vaccine or experimental Ad-vector vaccine other than Ad26 prior to study entry is allowed.
4. The participant has a known allergy or history of anaphylaxis or other serious adverse reactions to vaccines or vaccine products (including any of the constituents of the study vaccines (eg, polysorbate 80, ethylenediaminetetraacetic acid or L-histidine for Ad26.ZEBOV vaccine; and tris (hydroxymethyl)-amino methane for MVA BN-Filo vaccine), including known allergy to egg, egg products and aminoglycosides.
5. The participant has an acute illness (this does not include minor illnesses such as mild diarrhoea or mild upper respiratory tract infection) or temperature ≥38.0°C 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 if feasible.
6. The participant is a pregnant or breastfeeding women, or women planning to become pregnant while enrolled in this study until at least 3 months after the Ad26.ZEBOV vaccination or 1 month after MVA-BM-Filo.
7. The participant has significant conditions or clinically significant findings at screening or vital signs for which, in the opinion of the investigator, participation would not be in the best interest of the participant (eg, compromise the safety or well-being) or that could prevent, limit or confound the protocol-specified assessments.

Note: Participants who have recently received treatment for acute, uncomplicated malaria are eligible for participation if at least 3 days have elapsed from the conclusion of a standard, recommended course of therapy for malaria; participants who are acutely ill with malaria at the time of screening should complete therapy and wait an additional 3 days after completion before screening for the study.

Note: Participants with sickle cell trait can be included.

8. The participant had major surgery (per the investigator's judgement) within the 4 weeks prior to screening, or has planned major surgery during the study (from the start of screening onwards).
9. The participant had a post-organ and/or stem cell transplant whether or not with chronic immunosuppressive therapy.
10. The participant received an investigational drug or investigational vaccines or used an invasive investigational medical device within 3 months prior to screening, or current or planned participation in another clinical study during the study.

    Note: Participation in an observational clinical study is allowed.
11. The participant has a history of chronic urticaria (recurrent hives).

2.4.4 Randomisation procedure

The study randomisation list will be developed using an algorithm in the Statistical Analysis System software. This algorithm will randomly assign a treatment group (1:1) to a sequential randomisation number. Once established, the list will be shared with the principal investigator (University of Kinshasa), who is in charge of creating sealed envelopes under sponsor (University of Antwerp) supervision. A total of at least 700 randomisation envelopes will be created. Thirty envelopes will be grouped into one larger envelope, referred to as a "booklet". The booklets and envelopes will be numbered sequentially by a unique sequence of numbers. The booklets will be labelled in a sequential order (ie, 01-24) and the envelopes will be labelled with the study number "VAC52150-EBL-2007" and a

sequential randomisation number (ie, 001-700) to which a treatment group is linked via the algorithm. The staff delegated to make the envelopes will use the *Envelope Assembly Record Worksheet*, on which the randomisation number, initials of the assembler, date on which the assembly took place, and the initials of the staff member(s) that performed the quality control are collected. The randomisation booklets with envelopes will be stored and used in the study clinic.

Delegated site staff will assign and open booklets and envelopes in sequential order during study visits. Each envelope will contain two stickers. The first will contain space for writing the subject ID and participant's initials, the second will contain the randomisation number and treatment description (pre-printed based on the study randomisation list). Upon opening the sealed envelope, the subject ID and initials must be written in the space provided on the first sticker and the subject ID sticker must be placed on the outside of the envelope. To ensure proper source documentation, the sticker with the treatment information must be placed on the corresponding *Randomisation worksheet*. Thereafter, the empty envelope, with the subject ID sticker on the outside, must be placed back in the booklet. These booklets are to be stored by the principal investigator.

2.4.5 Study procedures (Figure 1)

At day 1, interested participants are informed about the study and are required to pass a test of understanding before providing written consent. No study activities are performed before the participant has signed the informed consent form. Afterwards, the study medical doctor evaluates his/her general health based on the inclusion criteria, vital signs (blood pressure, pulse/heart rate [both at rest] and body temperature) are collected and a urine pregnancy test for women of childbearing potential is performed. Further during this first visit, a blood sample is taken for baseline testing of binding antibody level (ie, humoral immune response) against EBOV glycoprotein (GP) using Filovirus Animal Non-Clinical Group (FANG) Ebola virus enzyme-linked immunosorbent assay (ELISA) and the presence of pre-existing Human anti-EBOV GP IgG and anti-EBOV nucleoprotein (NP) IgG using LUMINEX assay. For Day 1 samples, both FANG ELISA and LUMINEX assay will be carried

out. FANG ELISA is performed for all EBOVAC trials in the same laboratory (for consistency and comparability) and LUMINEX assay will provide a more detailed array of IgG antibodies that are not obtained via FANG ELISA. For the first 100 enrolled participants an additional test on the collected serum is performed to measure the neutralising antibody level against Ad26 and MVA vectors using the Ad26 virus neutralising assay (VNA) and MVA plaque reduction immunogenicity Test (PRNT), respectively. Subsequently, a blood sample for baseline safety assessment is collected to test haemoglobin, haematocrit, blood cell count (white and red), platelet count, urea, creatinine and transaminases. Then, participants are vaccinated with the first dose (Ad26.ZEBOV) and they are given instructions to contact the study team for any occurring SAEs, or in case of pregnancy of a participant during the study. After vaccination, participants remain at the study site for an observation period of 30 min to make sure no SAEs occur. SAEs are collected from first dose vaccination until 6 months post booster. Lastly on day 1, randomisation is performed to determine the timing of the booster vaccine at either 1 year or 2 years after the first dose. Contact information is verified, an appointment for the second dose on day 57 is arranged and a participant card is printed. Innovatively, next to a participant card, a biometric identification tool via iris scanning is foreseen to ensure correct identification of the participants during all study related visits.

At day 57, participants return to the study site for urine pregnancy testing (for women of childbearing potential), vital signs measurement, assessment of safety (SAEs), a blood sample for immunogenicity assessment (the binding antibody levels against EBOV GP using FANG ELISA) and afterwards administration of the second vaccine (MVA-BN-Filo). After an observation period of 30 min, participants are reminded to contact the study team for any SAEs that occurs, or in case of pregnancy of a participant during the study. Contact information is verified and an appointment for the 21-day post dose 2 visit (day 78) is arranged.

At 21 days post dose 2 (day 78), all participants return to the study site for a safety assessment (SAEs) and for the collection of a blood sample for immunogenicity assessment.

Contact information is re-verified and they are reminded to contact the study team in case of SAE occurrence, or in case of pregnancy of a participant.

To make sure no valuable information is missed, participants are contacted by phone to inquire about any occurrence of pregnancies (female participants) and SAEs at approximately 6 months post dose 2 vaccination.

At 1 year and 2 years after the first vaccine, when all participants return to the site, the clinical trial staff inquires after the occurrence of SAEs and a blood sample is collected for immunogenicity assessment of all participants (where applicable before administration of the booster dose). Depending on the study arm, a booster vaccination with Ad26.ZEBOV is given either 1 or 2 years after the first dose. Prior to vaccination, the general well-being of the participant is evaluated and urine pregnancy testing (for women of childbearing potential), as well as a vital signs measurement are performed. After vaccination, participants remain at the study site for a 30 min observation period. Participants are asked to collect solicited and unsolicited AEs in a participant diary starting on the day of the vaccination and continuing for the subsequent 7 days.

At day 8 post booster the safety data including solicited and unsolicited AEs is reviewed and a blood sample for immunogenicity assessment is taken to document the immune response. Should any solicited AEs persist at day 8 post booster, participants are asked to continue monitoring these in their participant diary. Once the solicited AEs have resolved, they are asked to make an unscheduled visit at the site so this information can be reported.

At 6 months post booster, all participants are contacted by phone and questioned about any SAEs or pregnancies (female participants) that have occurred since the last vaccination.

The total duration of the study is 2 years and 6 months post first dose. The study is considered completed when the last participant has been contacted for the 6 months post booster phone call or has left the study.

2.4.6 Study intervention

According to the predefined schedule (figure 1), participants receive a 0.5 mL intramuscular injection into the deltoid muscle of the upper arm with Ad26.ZEBOV or MVA-BN-Filo. The injection site should be free from any injury, local skin conditions or other issues that might interfere with the evaluation of local reactions. Every vaccination is given in the opposite arm of the previous vaccination (unless the opposite arm has a condition that prevents evaluating the arm after injection). No local or topical anaesthetic is used prior to the injection.

The second or booster vaccination is not administered if any of the following events occur at any time after the first dose vaccination:

- A participant experiences anaphylaxis clearly attributable to vaccination with the study vaccine; OR
- A participant experiences generalised urticaria within 72 hours of vaccination considered to be related to study vaccine; OR
- A participant experiences a SAE considered to be related to the study vaccine; OR
- A participant experiences injection site ulceration, abscess or necrosis considered to be related to the study vaccine; OR
- A participant has confirmed EVD; OR
- A female participant of childbearing potential has a positive urine β-human chorionic gonadotropin (β-HCG) pregnancy test before vaccination (on ay 57, Year 1 or Year 2 (depending on the randomisation group)); OR
- A female participant of childbearing potential has a positive urine β-HCG pregnancy test between dose 2 and the booster dose and is still pregnant or breast feeding at the time of the booster dose; OR
- A participant takes a concomitant treatment with drugs that may alter the immune response; OR
- The principal investigator believes that for safety reasons it is in the best interest of a participant to discontinue the study intervention.

Participants experiencing any of the events described above are still followed up for safety and immunogenicity according to the protocol. The decision to discontinue the study intervention is at the discretion of the principal investigator (University of Kinshasa) and after consultation with the sponsor (University of Antwerp) for any of the events described above.

2.4.7 Patient and public involvement

Difficulties were expected when setting up a clinical trial in Boende, a remote and resource-limited area of DRC. However, to avoid and anticipate some of these challenges and in order to support vaccination compliance, a collaboration is established between the study team and the provincial division of health. Throughout the trial, workshops are organised for HCP in the health district of Boende to sensitise and inform about EVD and other relevant medical topics. These gatherings should not only facilitate enrolment in the trial but also increase the engagement of participants by enhancing their understanding of the clinical trial and the importance of adherence. During these workshops time is available for questions and discussions. In addition to these gatherings for trial participants, community engagement activities and the training and capacity building of the local clinical trial team that is executing the trial (under supervision of the University of Kinshasa as principal investigator) are organised for the duration of the trial.

Each participant receives an individual visit schedule on enrolment in the trial and when participants miss a planned study visit, community health workers of the Ministry of Health trace the individual participant. Consent is asked in the informed consent form for this mode of contact.

Furthermore, prior to the start of the clinical trial, a pilot study was performed whereby potential participants were interviewed on the feasibility and acceptability of the use of a biometric iris scanning tool for participant identification during the trial and the use of telephone messaging with visit reminders for participant adherence[27].

2.4.8 Data management

All information is collected during study visits on source documents by study staff. These source documents with confidential information are transcribed into the electronic clinical database by site data managers. To make sure that all entered data (collected in DFexplore V.5.2.1) is correct, the principal investigator reviews each source document and confirms its correct transcription in the database. Additionally, the sponsor performs quality checks of the entered data in the database and, during monitoring visits, source data verification is performed.

2.4.9 Statistical analysis

A differentiation in analysis will be made according to: (1) the *full analysis set* (FAS; all participants who received at least one dose, regardless of the occurrence of protocol deviations), (2) *per protocol set for primary vaccination series* (all vaccinated participants, who received both dose 1 and dose 2 (administered within the protocol-defined visit window) vaccinations, have at least one post-vaccination (ie, after the date of dose 1) evaluable immunogenicity sample and have no major protocol deviations influencing the immune response) and (3) *per protocol set for the booster vaccination* (includes all participants in the per-protocol set for the primary vaccination series who received a booster dose and have at least one post booster vaccination evaluable immunogenicity sample, and have no major protocol deviations influencing the immune response).

Participant information (ie, demographics and baseline characteristics, disposition information, treatment compliance, extent of exposure, protocol deviations and concomitant medications) is planned to be tabulated and summarised with descriptive statistics for all participants. For continuous data, such as age, the mean and SD will be provided if applicable, otherwise the geometric means, related SD or median and IQRs will be used.

For the immunogenicity analysis, two *per-protocol sets* will be used, that is, the p*er-protocol set for primary vaccination series* and the *per-protocol set for the booster*. If more

than 10% of participants from the FAS are excluded from the per-protocol immunogenicity set, the immunogenicity analysis will be repeated on the FAS to evaluate the robustness of the analysis results. A subgroup analysis of the immune response at different time points will be performed stratified by age (18-40, 40-60 and >60), gender, prior vaccinia/smallpox vaccination, pre-existing Ebola antibodies (positive or negative for pre-existing human anti-EBOV GP IgG and anti-EBOV NP IgG and for both), baseline immunogenicity (positivity vs negativity for antibody levels against EBOV GP using FANG ELISA) and the presence of neutralising antibody levels against Ad26 and MVA vectors using Ad26 VNA and MVA PRNT assays (only the first 100 enrolled participants), respectively. For these planned subgroup analyses, N (%), geometric mean concentrations and 95% CI will be provided as appropriate. Finally, a formal comparative analysis of the induced immune memory response between the two booster arms will be performed.

Safety analyses include SAEs collected during the whole study and solicited and unsolicited AEs for 1 week post booster vaccination. The analysis of SAEs will be performed using the FAS and the solicited and unsolicited AEs will be analysed for the participants who received the booster vaccination. Continuous variables will be summarised using the following statistics: number of observations; arithmetic or geometric mean/median (if applicable) with their related measures of dispersion (95% CI for the mean, SD or IQR (Q1-Q3)). Minimum and maximum frequencies and percentages (one decimal place) will be generated for categorical variables. If the unpooled safety data from the NCT02564523 and NCT02509494 studies can be obtained, a power analysis will be performed to assess whether the safety data of the two booster arms can potentially be compared through formal statistical analysis.

The primary endpoint analysis is planned to be performed when all participants have completed the 21-day post dose 2 visit (day 78) or discontinued earlier. This analysis includes all available immunogenicity and safety data up to this point (date cut-off). Additional interim analyses may be performed during the study for the purpose of informing future vaccine-related decisions in a timely manner.

The final analysis will be performed when all participants have completed the last study-related phone call (6 months post booster) or left the study.

2.5 Discussion

The aim of this phase 2 trial is to obtain further safety and immunogenicity data on the two-dose prophylactic heterologous Ebola vaccine regimen and to assess the safety and immunogenicity of a booster dose with Ad26.ZEBOV administered either 1 or 2 years post first dose in a large cohort of HCPs and frontliners. By doing so, this study will feed the immunogenicity and safety databases of the Ad26.ZEBOV and MVA-BN-Filo vaccines. It will also be the first study to compare the induced immune memory response between two different booster arms in a large cohort of adults.

Boende (the capital city of Tshuapa province) was selected as the trial site for several reasons. First, the Tshuapa province recovered from an EBOV outbreak that occurred in the Boende Health District in 2014[21]. Following this outbreak, a study (n=565) conducted in the Tshuapa region in 2015 found that 41.4% of the tested HCPs were seroreactive to at least one EBOV protein and 2.8% of the HCPs showed a neutralising capacity while never having developed EVD symptoms[20]. This observation suggests a possible endemic EBOV exposure in the Tshuapa province of DRC. These are interesting observations for future ecological research as the ecology and reservoir(s) of EBOV and other filoviruses remain largely unknown[28, 29]. Second, in addition to the previous outbreak of EVD, Boende was chosen to perform the current clinical trial as there was expertise available after carrying out a phase 3 monkeypox vaccine trial that took place in 2017[24].

Some limitations are present in the current set-up of the trial. First, by focussing on occupation (registered HCPs and frontliners) rather than age and gender, in the inclusion and exclusion criteria, the aim is to easily reach the target of 700 participants. However, a recent review by Flanagan *et al* has shown that immune responses to vaccination can differ based on gender and age[30]. To take this limitation into account, stratification for age and gender has been foreseen during statistical analysis. Second, while HIV-positive

participants can participate in this trial if their general condition is good, it is not possible to be certain of the HIV-status of all participants as no routine checks prior to enrolment or during the course of the trial are foreseen. It is possible that some participants either are unwilling to share their HIV-positive status as a consequence of the stigma that is often linked to it[31] or are simply unaware of their positive status (eg, during an asymptomatic phase of the disease[32]). However, due to the low prevalence (0.6%) of HIV-positive people in the province of the trial[31], it was chosen not to perform routine checks and to trust the willingness of a participant to share his/her status as it is not considered an exclusion criterium for the trial. Finally, at the start of the project the protocol initially only included a vaccination strategy with the two-dose heterologous vaccine regimen (Ad26.ZEBOV followed by MVA-BN-Filo 56 days later) and was later adapted to include a booster vaccination at the request of the vaccine producer. The purpose of the initial observational trial was, next to obtaining additional immunogenicity data, a way to see if performing a vaccination trial in a remote area of DRC was feasible and accepted by the population. While writing the protocol however, administering a booster dose in this large cohort was added as a novel aspect and thus this was entered as a secondary objective/endpoint. Currently it is unknown whether this booster dose will be required or not at the moment of an outbreak and what its protective effect would be. However, to explore its safety and immunogenicity, this study protocol was transformed and became a randomised clinical trial. Unfortunately, as the comparison of the two booster arm induced immune responses is not required for approval of the licensure of the two-dose heterologous vaccine regimen and the booster dose was added as a second stage to the study design, no sample size calculations were initially performed for this trial and sample size was selected based on available information from a previous monkeypox vaccine trial in the same area. While this trial thus mainly has a descriptive set-up, scientifically it is interesting to learn if there is a significant difference in the induced immune memory response of the two booster arms. For this reason, a power analysis was retrospectively performed to determine whether it would be possible to compare the induced immune memory response of the two arms. Fortunately, this will be possible as a power of 0.99 was

calculated and a formal statistical comparison induced immune memory responses of the two booster arms has now been foreseen in the statistical analysis plan. It is however important to take into account that a varying antibody response after booster vaccination is not necessarily directly correlated with protective vaccine efficacy[33] and that a high power (99% for this study) can lead to significant differences, even if the difference between both groups is small. Prudent and careful interpretation of the results will thus be crucial[34].

In conclusion, because EVD remains a very deadly disease, effective and safe preventive measures will play a crucial role to protect vulnerable communities. While the prophylactic heterologous two-dose regimen was recently granted market authorisation by the European Commission, further research into the safety and immunogenicity of the two-dose regimen is still required to obtain worldwide licensure of the regimen. Furthermore, limited data has previously been collected on the safety and immunogenicity of a booster dose with Ad26.ZEBOV. This is the first large, randomised vaccine trial that assesses the safety and compares the immunogenicity of two different booster arms in a large cohort.

2.6 Ethics and dissemination

This protocol was submitted and approved by the National Ethics Committee of the Ministry of Health of the Democratic Republic of Congo (approval number: n°121/CNES/BN/PMMF/2019). Prior to being enrolled in the trial, all participants are required to provide written informed consent by singing the informed consent form after having performed a test of understanding. If the participant 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 should personally date and sign the informed consent form after the oral consent of the participant is obtained. No study-related procedures are performed until the participant has signed the informed consent form.

The trial was registered on Clinicaltrial.gov on 4 December 2019 and recruitment started on 18 December 2019. All participants were recruited by the 8 February 2020 and the study is planned to finish in October, 2022. Results of the trial will be entered on Clinicaltrial.gov, published in peer-reviewed journals and presented at international conferences.

2.7 Declarations

Author contributions. YL wrote the manuscript. TZ, ES, YL, VM, JM, PM, HM-M, J-PVG and PVD wrote the initial English protocol on which this manuscript is based. TZ, VM, PM, JM and HM-M translated the English protocol into French for submission to the National Ethics Committee and the "Direction de la Pharmacie et des Médicaments" of the Ministry of Health of the Democratic Republic of Congo as well as the National Scientific committee against Ebola. All authors (YL, TZ, ES, JDB, VM, JM, PM, HM-M, J-PVG and PVD) reviewed and contributed to the final manuscript.

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Competing interests. The authors declare that they have no competing interests.

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Part II – Vaccine trial results

Chapter 3. Safety and immunogenicity of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen

Chapter 4. Long-term antibody persistence of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen, and safety and (long-term) immunogenicity of an Ad26.ZEBOV booster dose

Chapter 3 Safety and immunogenicity of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen

Safety and immunogenicity of the heterologous two-dose Ad26.ZEBOV, MVA-BN-Filo vaccine regimen in healthcare providers and frontliners of the Democratic Republic of the Congo

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3.1 Abstract

Background. In response to recent Ebola epidemics, vaccine development against the *Zaire ebolavirus* (EBOV) has been fasttracked in the past decade. Health care providers and frontliners working in Ebola-endemic areas are at high risk of contracting and spreading the virus.

Methods. This study assessed the safety and immunogenicity of the 2-dose heterologous Ad26.ZEBOV, MVA-BN-Filo vaccine regimen (administered at a 56-day interval) among 699 health care providers and frontliners taking part in a phase 2, monocentric, randomized vaccine trial in Boende, the Democratic Republic of Congo. The first participant was enrolled and vaccinated on 18 December 2019. Serious adverse events were collected up to 6 months after the last received dose. The EBOV glycoprotein FANG ELISA (Filovirus Animal Nonclinical Group enzyme-linked immunosorbent assay) was used to measure the immunoglobulin G-binding antibody response to the EBOV glycoprotein.

Results. The vaccine regimen was well tolerated with no vaccine-related serious adverse events reported. Twenty-one days after the second dose, an EBOV glycoprotein-specific binding antibody response was observed in 95.2% of participants.

Conclusions. The 2-dose vaccine regimen was well tolerated and led to a high antibody response among fully vaccinated health care providers and frontliners in Boende.

Key words. Ad26.ZEBOV; MVA-BN-Filo; health care providers and frontliners; safety and immunogenicity; ebola vaccine trial.

3.2 Background

Ebola virus disease (EVD) was discovered in 1976 and became known worldwide between 2013 and 2016 during the devastating West African epidemic. During this epidemic, EVD spread across multiple countries and infected >28 600 people with a 40% case fatality rate (CFR) [1]. The Democratic Republic of Congo (DRC) is the most afflicted country, with at least 15 outbreaks (CFRs ranging from 42% to 100%); 1 of which was an epidemic that led to 3470 cases and 2287 deaths (CFR, 66%) in the North Kivu, South Kivu, and Ituri provinces between 2018 and 2020 [1, 2]. When EVD epidemics occurred in unexpected locations (ie, 2013-2016 West Africa epidemic) or in politically unstable locations (ie, 2018-2020 Kivu and Ituri Ebola epidemic in the DRC), they have had a greater impact than previously expected possible [3, 4]. In response to these epidemics and the global health threat that EVD continues to pose, vaccine development against this deadly disease has been fast-tracked in the past decade [5].

Because of the unpredictability of when and where the next Ebola outbreak will occur [6] and considering the potential of vaccinating high-risk exposure groups such as health care providers and frontliners (hereafter, HCPs) [7, 8], the use of a vaccine that induces a durable and protective immune response is crucial. Janssen Vaccines & Prevention B.V., together with Bavarian Nordic, developed the 2-dose heterologous Ad26.ZEBOV (Zabdeno®) and MVA-BN-Filo (Mvabea®) vaccine regimen. The Ad26.ZEBOV vaccine is a monovalent replication-incompetent adenoviral vector serotype 26 (Ad26) vaccine, encoding the full-length glycoprotein (GP) of the *Zaire ebolavirus* (EBOV) Mayinga variant [9]. The MVA-BN-Filo vaccine is a nonreplicating multivalent modified vaccinia Ankara (MVA) vaccine, encoding the EBOV Mayinga GP, the *Tai Forest ebolavirus* nucleoprotein, the *Sudan ebolavirus* Gulu GP, and the *Marburg virus* Musoke GP [9]. While it has not been possible to measure clinical efficacy with a classical clinical study, immunobridging analysis from nonhuman primates to humans supports the likelihood of protection [10], and the regimen was therefore granted marketing authorization by the European Medicines Agency in 2020 for use under “exceptional circumstances” as prophylactic vaccination in

children and adults [11]. Preliminary studies for the Ad26.ZEBOV, MVA-BN-Filo heterologous vaccine regimen have shown that it is generally well tolerated and safe and leads to a durable immune response up to at least to 2 years after the initial vaccination [12-16].

The DRC's seventh Ebola outbreak took place in the Boende health district in 2014 [17]. Therefore, to protect HCPs in this Ebola-endemic region of the DRC, we performed a randomized vaccine trial whereby HCPs were first vaccinated with the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen and then boosted with Ad26.ZEBOV either 1 or 2 years after the first dose (1:1 randomization) [18]. This article presents the safety and immunogenicity of the Ad26.ZEBOV vaccine as the first dose, followed by the MVA-BN-Filo vaccine as the second dose at a 56-day interval, in HCPs of the Boende health district of the Tshuapa province in the DRC.

3.3 Methods

3.3.1 Study Participants

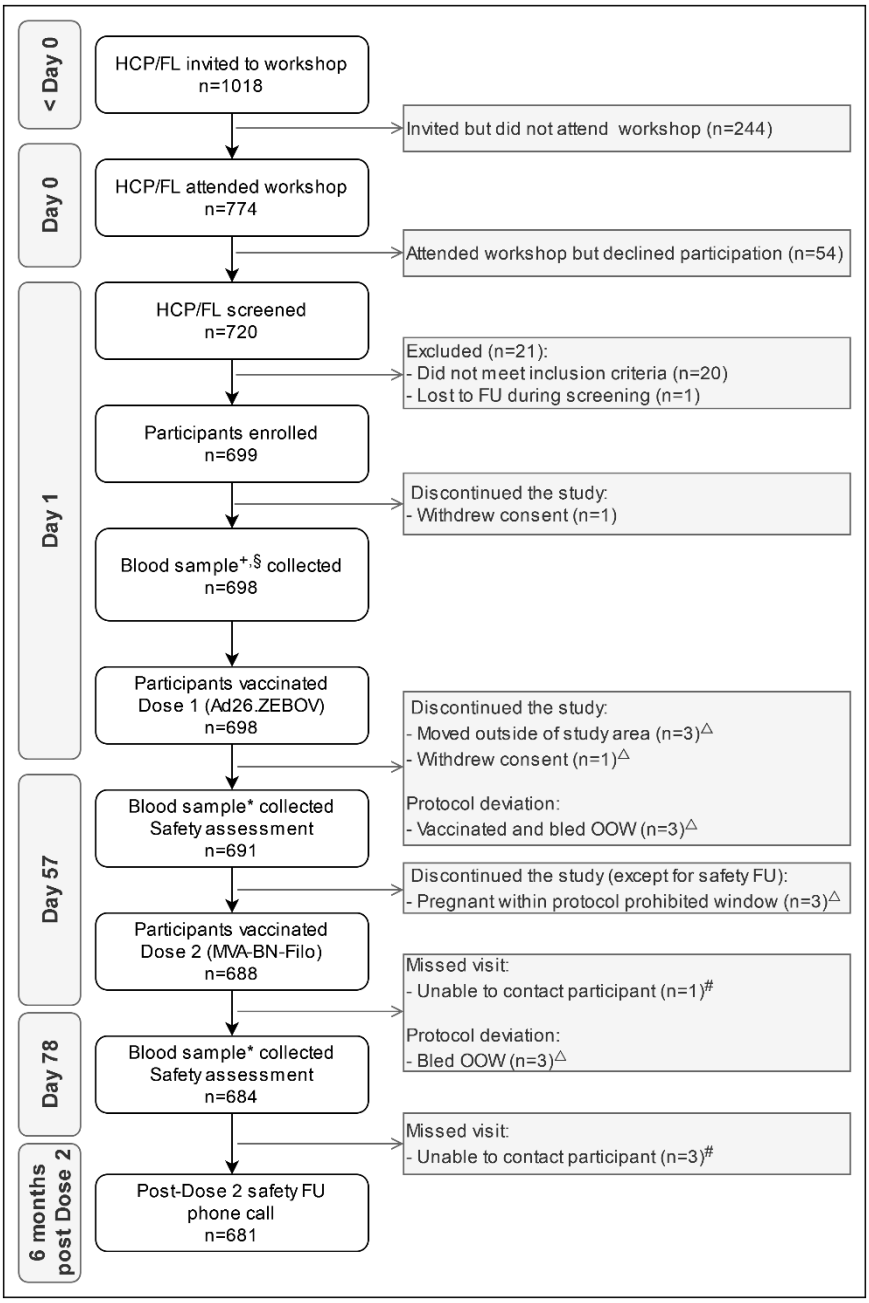
Participants had to be at least 18 years old and apparently healthy, pass a test of understanding (ie, a 10-question true/false questionnaire, for which 3 attempts were allowed to obtain a score of 9 or 10/10, assessing the participant's understanding of the trial and their consent), and have the means to be contacted. HCPs who were pregnant, breastfeeding, or planning to become pregnant within 3 months after the initial vaccination were excluded from enrollment. Further details on inclusion and exclusion criteria are provided by Larivière et al [18].

3.3.2 Study Design and Procedures

Based on convenience sampling, enrollment targeted 700 participants starting in December 2019 for a vaccine trial with an open-label, monocentric, randomized design (Figure 1). On day 0, in maximum groups of 40 individuals, registered HCPs working in the Boende health district were invited to attend a workshop where the informed consent form

was explained. If they were willing to participate in the study after attending the workshop, they were asked to return the next day for screening and consent (day 1). In case a HCP was illiterate, a literate third party not involved in the conduct of the study served as a witness to the consenting procedure and was asked to sign the informed consent form if the HCP agreed to participate. This article addresses the primary and one of the secondary objectives of the trial, which assessed the safety and immunogenicity of the heterologous 2-dose vaccine regimen in HCPs working in the Boende health district of the Tshuapa province in the DRC. Additionally, in a subset of participants, the exploratory objectives assessed the impact of the presence of baseline neutralizing antibodies against Ad26 and MVA vectors on the EBOV-specific immune response. Information on the clinical trial itself is available on www.clinicaltrials.gov (NCT04186000), and study procedures are explained in detail by Larivière et al [18]. The trial was approved by the National Ethics Committee of the Ministry of Health of the DRC (121/CNES/BN/PMMF/2019) and the Ethics Committee of the University Hospital of Antwerp/University of Antwerp (19/14/177).

Figure 1. Study flowchart.



*HCP = Healthcare providers; FL = frontliners; FU = Follow-up; OOW = Out of window; + For baseline hematology, biochemistry and immunogenicity assessment; * For immunogenicity assessment; § On Day 1, five samples were unable to be analyzed: four samples failed to meet acceptance criteria during multiple independent runs and one sample exceeded stability before a final result could be obtained; △ Part of full analysis set: all participants that received at least one dose of the heterologous Ad26.ZEBOV, MVA-BN-Filo vaccine regimen, irrespective of protocol deviations that occurred; # Part of per protocol set: Ad26.ZEBOV, MVA-BN-Filo regimen received and at least one immunogenicity sample post vaccination and no protocol deviations with impact on immunogenicity.*

3.3.2.1 *Safety Assessment*

Participants remained in observation for 30 minutes after the Ad26.ZEBOV vaccination on day 1 and the MVA-BN-Filo vaccination on day 57. The presence of any serious adverse events (SAEs) – related or unrelated to the investigational product (IP; Ad26.ZEBOV or MVA-BN-Filo) and as defined by the E2A clinical safety data management scientific guideline of the International Conference on Harmonisation [19] – was assessed up to 6 months after the last received dose.

3.3.2.2 *Immunogenicity Assessment*

Blood samples were collected from all participants to identify human anti-EBOV GP immunoglobulin G (IgG) antibody levels on day 1 (pre-vaccination, baseline immunogenicity assessment), day 57 (pre-vaccination, dose 1 immunogenicity assessment), and day 78 (dose 2 immunogenicity assessment).

All samples were analyzed at Q² Solutions Vaccine Testing Laboratory with the EBOV GP FANG ELISA (Filovirus Animal Nonclinical Group enzyme-linked immunosorbent assay) [20]. This validated assay was used to measure the IgG antibody concentrations against EBOV surface GP in the collected serum.

From the first 98 participants enrolled in the trial, additional serum was collected at baseline to assess the presence of neutralizing antibodies against the Ad26 and MVA vector backbone by using Ad26 and MVA virus backbone neutralization assays. The Ad26 virus neutralization assay (Ad26 VNA) was developed, qualified, and performed by Janssen Vaccines & Prevention BV, and the MVA virus neutralization assay (ie, human vaccinia plaque reduction neutralization assay) was developed, validated, and performed by Bavarian Nordic.

3.3.3 Data Management and Analysis

3.3.3.1 *Data Management*

Data were collected in French on paper source documents and then transcribed into the electronic database (DFdiscover version 5.2.0). All data were reviewed by the principal investigator or delegated staff. Monitors of a clinical research organization performed source data verification. All open-field translations from French to English were certified.

3.3.3.2 *Demographics and Safety Data Analysis*

The full analysis set was used to analyze demographics, baseline characteristics, and safety data. This included all participants who received at least 1 dose of the heterologous vaccine regimen, irrespective of the protocol deviations that occurred. Descriptive statistics were used to present these data in number (%), mean (SD), or median (range). All safety data were coded with MedDRA coding (version 22.1) and presented with the MedDRA Preferred Term.

3.3.3.3 *Immunogenicity Analysis*

The immunogenicity analysis was conducted with the per-protocol set (PPS). This consisted of all participants who received both vaccinations and had at least 1 postvaccination immunogenicity result and no major protocol deviations with a consequence on immunogenicity. Anti–EBOV GP IgG geometric mean concentrations with 95% CIs were calculated for all available time points (days 1, 57, and 78). Participants were considered responders when they tested below or equal to the lower limit of quantification (LLOQ; ≤36.11 ELISA units [EU]/mL) at baseline and >2.5× LLOQ after baseline or had at least a 2.5-fold increase in antibodies after vaccination if they were already above the LLOQ at baseline. Except for calculation of the response rates, all values below or equal to the LLOQ (≤36.11 EU/mL) were imputed with half the value (18.06 EU/mL) to account for censoring in the parameter estimation. On a subset of participants, the Spearman correlation was assessed between preexisting neutralizing antibodies against the Ad26 and MVA vector and the anti−EBOV GP IgG antibody response before and after vaccination.

Statistical modeling was performed to investigate whether and how the following relate to differences in the mean antibody response (μ) and variability (σ): time in days between 2 collected blood samples, sex (male or female), age, previous vaccination with a third-generation smallpox vaccine (IMVAMUNE [also known as MVA-BN, JYNNEOS, and IMVANEX]; Bavarian Nordic A/S) against mpox (formerly monkeypox), and profession. Details on the methodology of this statistical model are available in the supplementary material.

All statistical analyses were performed in R (version 4.2.2); for statistical modeling, the *gamlss* package (version 5.4.3) was used.

3.4 Results

3.4.1 Full Analysis Set

3.4.1.1 *Demographic Characteristics*

Enrollment began on 18 December 2019, and the post–dose 2 safety follow-up phone call visits ended on 23 October 2020. Data collected up to 23 October 2020 were used for analyses. Overall, 699 participants were enrolled (Figure 1). One participant withdrew consent before any study-related activity could be performed. Thus, the full analysis set consisted of 698 participants. All enrolled participants were Black and of African descent, and 76.5% were male (Table 1). The study population had a median age of 46.0 years, and the majority of the participants were community health workers (33.8%). Nurses and first aid workers were the second- and third-largest HCP groups, representing 25.9% and 25.4% of the participants, respectively. The majority of the participants worked in health centers (53.2%), for the Red Cross (25.4%), or in hospitals (12.0%). Out of 698 participants, 129 (18.5%) were vaccinated with a third-generation smallpox vaccine (IMVAMUNE) against mpox during a vaccine trial conducted in Boende in 2017 [21].

Table 1. Baseline Sociodemographic Characteristics: Full Analysis Set

Characteristic	No (%)
Sex	
Male	534 (76.5)
Female	164 (23.5)
Black	698 (100.0)
Age, years	
Mean (SD)	45.0 (12.0)
Median (range)	46.0 (19.0-75.0)
Profession	
Community health worker	236 (33.8)
Nurse	181 (25.9)
First aid worker	177 (25.4)
Hygienist	37 (5.3)
Midwife	30 (4.3)
Doctor	13 (1.9)
Health facility cleaner	10 (1.4)
Care giver	7 (1.0)
Lab technician	2 (0.3)
Pharmacist aid	2 (0.3)
Other	3 (0.4)
Work establishment	
Health centre	371 (53.2)
Red cross	177 (25.4)
Hospital	84 (12.0)
Health post	37 (5.3)
Health area	10 (1.4)
Provincial health department	9 (1.3)
Health zone	8 (1.2)
Health inspection	1 (0.1)
Staff member of the expanded programme on immunisation	1 (0.1)
Smallpox vaccination against mpox	
Yes	129 (18.5)
No	569 (81.5)

Health care providers and frontliners vaccinated with Ad26.ZEBOV, MVA-BN-Filo vaccine regimen in Boende, the Democratic Republic of the Congo, December 2019 (participants who received at least 1 study vaccine dose, N = 698).

[a]Data are presented as No. (%) unless indicated otherwise.

3.4.1.2 *Safety Assessment*

For the 698 participants who received at least 1 vaccination, no SAEs related to the IP were reported up to 6 months post–dose 2. In total, 31 SAEs unrelated to the IP were recorded

among 20 participants. Of the 31 SAEs, 58.1% were considered severe, 35.5% moderate, and 6.5% mild (numbers add to 100.1% due to rounding). Overall, 77.4% of participants with (a) SAE(s) recovered or their SAE(s) resolved; 9.7% recovered/resolved with sequelae; and 3 died during the study—1 from HIV infection (diagnosis unknown at recruitment), 1 from dermohypodermitis, and 1 from ureterolithiasis and calculus bladder—accounting for 12.9% of SAEs. Further details on all reported SAEs that started between enrollment and 23 October 2020 are presented in Supplementary Table 1.

3.4.2 Immunogenicity Assessment

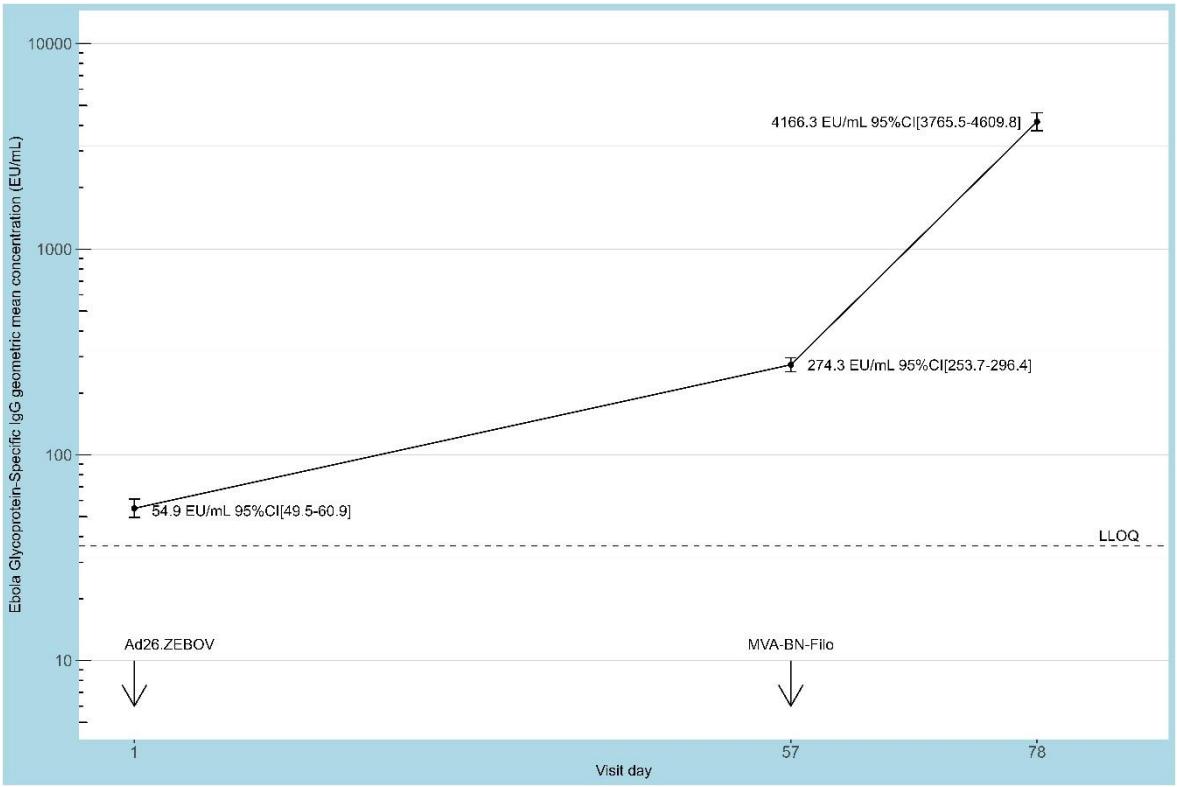
3.4.2.1 *Per-Protocol Analysis: PPS*

Of the 688 participants who received 2 doses, 3 were excluded from PPS due to a protocol deviation with impact on immunogenicity. Therefore, the immunogenicity analyses consisted of 685 PPS participants. Five serum samples (0.7%), collected on day 1, were unable to be analyzed: 4 failed to meet acceptance criteria during multiple independent runs, and 1 exceeded stability before a final result could be obtained. For participants with missing baseline results, it was not possible to determine if they were responders.

At baseline, participants had an anti-EBOV GP IgG geometric mean concentration of 54.8 EU/mL (95% CI, 49.4–60.8) (Figure 2), with 340 (49.6%) having antibody responses below or equal to the LLOQ. After dose 1, the geometric mean concentration increased to 274.3 EU/mL (95% CI, 253.8–296.4) at day 57 and 4166.3 EU/mL (95% CI, 3765.5–4609.8) 21 days after dose 2. This indicates a 5-fold increase in antibodies 56 days after administration of the first dose and a further 15-fold increase 21 days after administration of the second dose. After a single Ad26.ZEBOV vaccination (day 57 immune response), 431 participants (62.9%) were considered responders. Of the 679 participants for whom the immune response could be assessed at approximately 21 days after vaccination with the Ad26.ZEBOV, MVA-BN-Filo heterologous vaccine regimen (1 participant did not attend the day 78 visit), 652 (95.2%) were considered responders.

Finally, for a subset of 95 PPS participants, baseline Ad26- and MVA-specific seroprevalence rates of 93.7% and 70.5% were calculated, respectively. Negligible correlations were observed between Ad26-specific neutralizing antibodies at baseline and EBOV GP–specific binding antibodies 56 days post–dose 1 and 21 days post–dose 2 (Spearman correlation coefficients, -0.21 and -0.14), as well as between MVA-specific neutralizing antibodies at baseline and EBOV GP–specific binding antibodies 56 days post–dose 1 and 21 days post–dose 2 (Spearman correlation coefficients, -0.08 and -0.31). Based on these weak correlations (Supplementary Figure 1), there is no indication that the presence of the Ad26- and MVA-specific neutralizing antibodies had an impact on the vaccine-induced immune responses after vaccination.

Figure 2.Geometric mean concentrations with 95% CIs of EBOV-specific binding antibodies.



The lower limit of quantification is indicated by a dashed line (36.11 EU/mL). The Ad26.ZEBOV vaccine was administered at day 1 as the first dose, followed by the MVA-BN-Filo vaccine on day 57 (±7 days) as second dose at a 56-day interval. Blood samples were collected prior to the first dose as baseline, prior to the second dose on day 57 (±7 days), and at 21 days (day 78 ± 7 days) after the second dose to assess the humoral immune response after vaccination. EBOV, Zaire ebolavirus; EU, enzyme-linked immunosorbent assay unit; LLOQ, lower limit of quantification.

3.4.2.2 *Statistical modeling*

Statistical modeling indicates that there is a significant increase in the mean antibody response after each vaccination, with variability in the response declining by 59.8% and 71.3% for the day 57 and 78 visits, respectively, as compared with baseline (Table 2). This indicates an increase in homogeneity of the antibody response at each blood collection time point. While men started with a higher antibody response at baseline than women, a clear boost in antibody response was observed in men and women from day 57 until day 70, with women reaching a higher antibody response than men from day 70 onward (Supplementary Figure 2). For participants vaccinated against mpox, the EBOV GP–specific binding antibody response increased by 34% between the second and third visits. In contrast, for participants not vaccinated against mpox, a lower increase of 26% was observed between days 57 and 78. When assessing the profession, the estimated mean antibody response for first aid workers was 8% higher on average than for community health care workers (95% CI, 5%–20%). Finally, at baseline, younger participants (quartile 1, age 36 years) had a 43% higher mean antibody response than older participants (quartile 3, age 54 years), and this difference persisted after vaccination (no significant change in variability over time for age).

Table 2. The mean response and variability coefficients as determined by the GAMLSS model

Coefficient	Estimate	(SE)	P-value
μ: mean antibody response			
(Intercept)	4.363	(0.095)	<0.001
Age	-0.025	(0.001)	<0.001
Mpox received	0.494	(0.169)	0.003
Profession – First aid worker	0.089	(0.039)	0.024
Profession – Nurse	-0.072	(0.049)	0.143
Profession – Other	-0.075	(0.055)	0.171
Sex – Male	0.104	(0.037)	0.005
Time in days	0.062	(0.001)	<0.001
Mpox received:Time in Days (interaction)	-0.014	(0.003)	<0.001
σ: variability			
(Intercept)	0.339	(0.060)	<0.001
Time in days	-0.017	(0.001)	<0.001
Sex – Male	0.110	(0.045)	0.014
Profession – First aid worker	0.078	(0.045)	0.081
Profession – Nurse	0.217	(0.046)	<0.001
Profession – Other	0.205	(0.059)	<0.001

Abbreviation: GAMLSS, generalized additive model for location, scale and shape.

3.5 Discussion

Overall, the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen (administered 56 days apart) was safe and led to a clear humoral immune response among study participants. In this study, no IP-related SAEs were observed up to 6 months after vaccination with the heterologous 2-dose vaccination series. One study previously paused vaccination in adults with the Ad26.ZEBOV, MVA-BN-Filo regimen after 2 neurologic SAEs (1 possibly related to the IP) were reported within a short interval at different stages of the vaccine regimen [22]. However, the study resumed when an external expert panel of neurologists did not raise any specific safety concerns [22]. Of the per-protocol vaccinated HCPs in our study, 95.2% were considered responders roughly 21 days after the second dose. This is a high response rate and similar to what was observed in previous studies assessing the EBOV GP–specific binding antibody response to this heterologous regimen among adult participants (ie, 98.0%–100.0% responder rates) [13, 15, 16, 22–26].

Evaluating vaccine efficacy against EBOV infection is extremely challenging due to the sporadic nature and unpredictable location of the next Ebola outbreak. The World Health Organization's Strategic Advisory Group of Experts on Immunization currently recommends the single-dose rVSV-ZEBOV-GP (Ervebo) vaccine for use in high-risk populations during Ebola outbreaks [27]. This vaccine has shown 97.5%–100.0% clinical efficacy from day 10 after vaccination through ring vaccination in the DRC and Guinea [28, 29]. While ring vaccination during EVD outbreaks is recommended with the rVSV-ZEBOV-GP vaccine, in June 2021 the strategic advisory group's recommendations were amended to include the vaccination of populations at lower risk of contracting EVD (eg, HCPs in neighboring regions to an outbreak) with the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen [27]. Using immunobridging of EBOV GP–binding antibody responses between nonhuman primates and humans, Bockstal et al calculated a mean predicted survival probability of 53.4% (95% CI, 36.7%–67.4%) among humans vaccinated with the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen [30]. Due to the strictness of the parameters under which the model was built, this is expected to be an underestimation of the actual vaccine efficacy in humans.

Responses to vaccination can vary for individuals depending on factors such as age and sex [31, 32]. Even though no formal statistical modeling of the immune response was initially foreseen for the current study, post hoc statistical modeling was able to provide new insights. For example, the model indicated that female and younger participants had a higher mean EBOV-specific antibody response after full vaccination, as compared with their male and older counterparts, respectively. Researchers have attributed differences in vaccine responses to (1) hormonal changes among male and female aging and their influence on the immune system and (2) the deterioration of adaptive immune responses with age [31–33]. However, interpretations of this model should be handled with caution, as a correlate of protection for EBOV-specific antibodies remains unknown [34]. Despite the observed differences in the model, 95.2% of participants were considered vaccine responders. Therefore, differences in EBOV-specific antibody response based on certain variables (eg, sex, profession, age) may be clinically irrelevant.

While the preexisting Ad26-specific neutralizing antibody response observed in this study was similar to responses observed by Ishola et al (93.7% vs 93.0%–94.0%, respectively), the preexisting MVA-specific neutralizing antibody response was considerably higher (70.5% vs 5.0%–17.0%) [15]. There are several possible explanations for the high MVA-specific reactivity at baseline. First, this could be attributed to the high number of participants (50 of 95) who were vaccinated against mpox with IMVAMUNE, a live modified vaccinia virus Ankara vaccine, in a vaccine trial conducted in the same study area in 2017 [21]. Second, smallpox vaccination, a live virus vaccinia vaccine, was part of the routine vaccination in the DRC until 1977 (when the DRC was officially declared smallpox-free), and sporadic vaccination continued until 1984 [35]. As the median age of the study population was 46.0 years, several participants would have been vaccinated against smallpox. Finally, the Tshuapa province, of which Boende is the capital, is considered a mpox-endemic area with an elevated incidence among HCPs as compared with the general public [21]. Cross-reactivity between local exposure to the monkeypox virus with the vaccinia virus could have occurred [36]. However, while the MVA-specific neutralizing antibody titer was considerably higher, only weak correlations were observed between the EBOV-specific binding antibodies and the MVA-specific neutralizing antibodies, indicating no apparent impact on the EBOV-specific binding antibody response.

This study has some limitations, such as the imbalance of male and female participants (76.5% and 23.5%, respectively) most likely due to socioeconomic and cultural factors within the local health care system. This imbalance was potentially enhanced through the exclusion of pregnant and breastfeeding women at enrollment, as is often the case during trials assessing a candidate IP [37]. As a second limitation, the HIV status of participants was mostly unknown, as this was not an exclusion criterion for the trial if the general condition of the participant was good and he or she was taking suppressive therapy. Only if participants disclosed their HIV status at screening or during the course of the trial was this information recorded. However, a previous study assessed the antibody response of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen in healthy adults and those who were HIV infected (well controlled by highly active antiretroviral therapy), and the authors found that

the HIV status of participants did not have an apparent influence on the immune response as compared with healthy adults [24]. Finally, the data presented in this study were limited to a short immunogenicity assessment period after vaccination (up to 21 days after the second dose). Nevertheless, results show that the EBOV-specific immune response approximately 21 days after Ad26.ZEBOV and MVA-BN-Filo vaccination is high (ie, 95.2%) among vaccinated HCPs working in this Ebola-endemic area. Ultimately, the vaccination of this population therefore contributes to the epidemic preparedness within the Boende health district.

This study also has several strengths, including the high retention rate of participants and the vaccination of an at-risk population in an EBOV-endemic location. Epidemiologic modeling provides evidence that prophylactic vaccination of a small proportion of HCPs in an endemic at-risk location could significantly reduce Ebola incidence and associated mortality [38, 39]. As the 698 HCPs vaccinated in this study are a risk group working in an Ebola-endemic area, they may function as a sentinel demonstrating clinical efficacy if a new outbreak would occur in the region. Also, to the best of our knowledge, the current study is the first to assess the correlation between MVA-specific neutralizing antibodies at baseline and EBOV GP–specific binding antibodies after dose 1 and dose 2. Finally, by analyzing the data through a statistical model, more insights into variables affecting the immune response were achieved.

By recognizing the unpredictability of the next outbreak location and the potential of the prophylactic use of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen, preventative vaccination of HCPs working in Ebola-endemic areas could help prevent drastic consequences of the next Ebola outbreak. To ensure that prophylactic vaccination is useful, a durable immune response is crucial after vaccination. The next step within our study is to determine the durability of these vaccines among the HCP population, as well as their potential to induce an immune memory response through the administration of an Ad26.ZEBOV booster dose 1 or 2 years after vaccination.

3.6 Supplementary data

Supplementary materials are available at The Journal of Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

3.7 Notes

Author Contributions. Y. L. performed all analyses included in the manuscript (except statistical modeling) and wrote the manuscript. I. G.-F. performed analyses for the statistical model under the guidance of N. H. B. O. performed data management for the trial at the sponsor level. P. K. performed data management for the trial at the principal investigator level. H. M.-M. was principal investigator of the trial, with P. M. and J. M. as co–principal investigators. The University of Antwerp (P. V. D., J.-P. V. G.) was the sponsor of the trial. Y. L. was project manager at the sponsor level. V. M. was project manager at the investigator level. T. M. Z. was the study site coordinator. S. M. and R. M. were assistant site coordinators. S. M. was in charge of safety and cold chain and investigational product management. C. R., M. K., and C. M. were involved in trial discussions at the investigational product level. All authors reviewed and contributed to the final manuscript.

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Potential Conflict of Interests. C. R., M. K., and C. M. were full-time employees of Janssen, Companies of Johnson & Johnson, at the time of the study and report stock or stock options in Janssen, Pharmaceutical Companies of Johnson & Johnson. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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3.9 Supplementary material

3.9.1 EBL2007 study group

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3.9.2 Supplementary methods

3.9.2.1 *Methodology statistical model*

To achieve symmetry in the antibody response, values were log-transformed. To further account for the high number of participants with values below or equal to the LLOQ at baseline, a normal left-censored distribution was applied in the model. Due to the presence of censoring, non-linearity and individual variability in the data, several statistical models were explored, concluding that the assumption of a constant variability was not realistic. Therefore, a statistical Generalized Additive Model for Location, Scale and Shape (GAMLSS) was used. Variables that were assessed were: time in days between two collected blood samples, sex (male or female), age, previous vaccination with a third-generation smallpox vaccine (IMVAMUNE® (also known as MVA-BN®, JYNNEOS®, and IMVANEX®), Bavarian Nordic A/S, Kvistgaard, Denmark) against MPOX (formerly monkeypox), and profession. To obtain large enough categories per variable for the model, participants not working as

community health workers, nurses or first aid workers, were grouped under the profession category “other”. In the mean response (μ) of the model, varying coefficient smoothing terms against time between blood sample collection for each level of the factor sex were fitted. In addition, random effects were used to account for the individual variability in the mean response. Parameter estimation in GAMLSS is performed using the method of maximum likelihood.

3.9.2.2 *The GAMLSS model*

The full GAMLSS model can be defined as follows:

$$Y_i \sim D(\mu_i, \sigma_i, \nu_i, \tau_i) \qquad \text{independently for } i=1, \ldots, n$$

where $D(\cdot)$ is a parametric distribution, having up to four parameters μ, σ, ν, and τ representing the mean, variance, skewness, and kurtosis respectively. We define below how we modeled our parameters (μ, σ), where the function s() represents a variety of different effects: smooth terms and random effects. The functions g(k), k=1, …, 4 for the different parameters are of the following (linear) form:

$$g(\mu) = \beta_0 + \beta_i * X_i + s_p(X_i)$$

$$g(\sigma) = \alpha_0 + \alpha_i * X_i$$

$$g(\nu) = \lambda_0$$

$$g(\tau) = \theta_0$$

3.9.3 Supplementary results

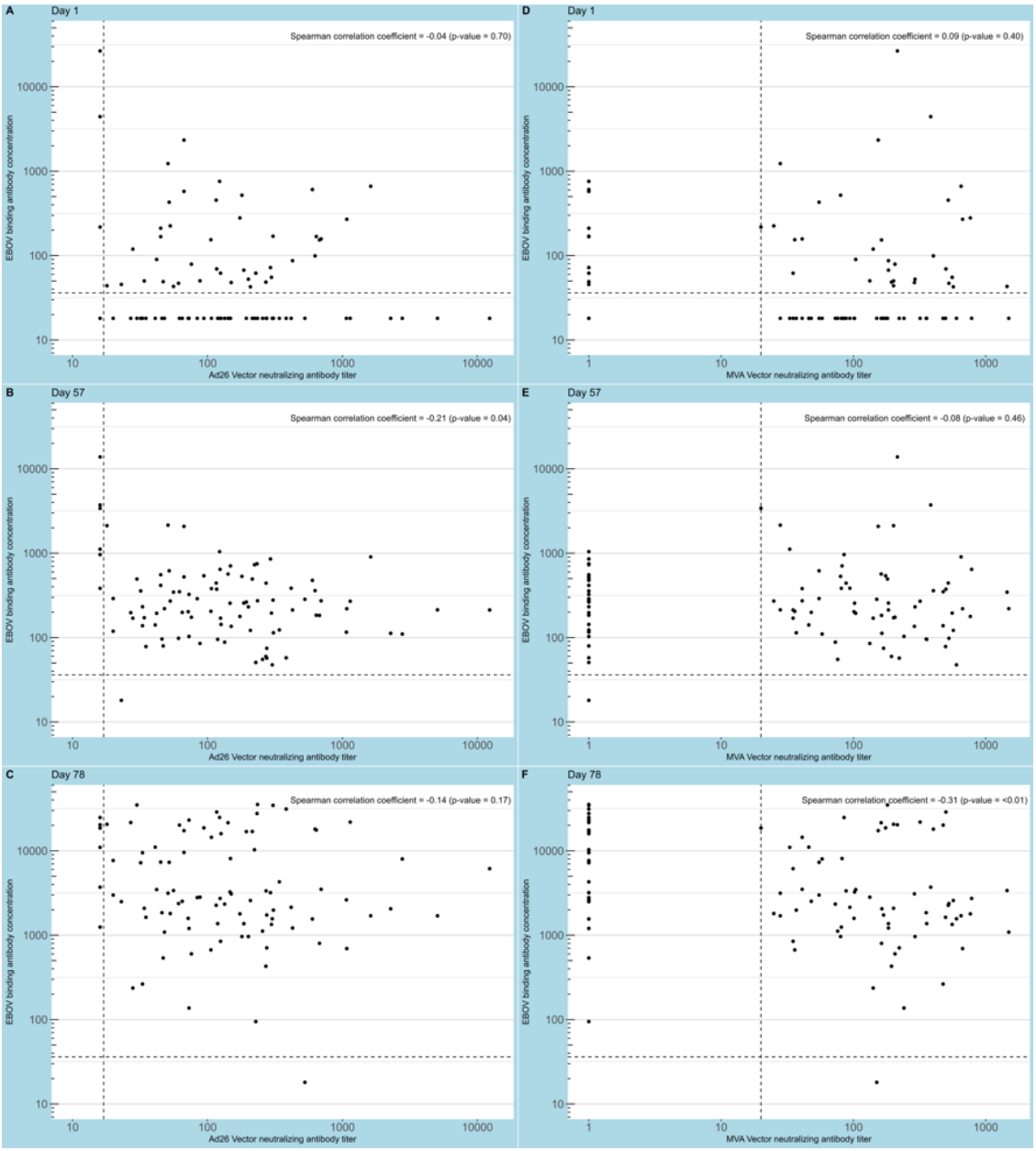
Table 1. Overview of SAEs starting before 23 October 2020 (FAS) of HCP and FL vaccinated with Ad26.ZEBOV, MVA-BN-Filo vaccine regimen in Boende, the Democratic Republic of the Congo.

SAE	MeDra Preferred Term	Start date	End date	Toxicity grade	Relatedness to IP	Outcome	Congenital anomaly	Persistent disability	Hospitalization	Life-threatening	Other medically important event
1	Ovarian cyst	5-Feb-20	15-Feb-20	Moderate	Not related to IP	Recovered/ resolved	No	No	Yes	No	No
2	Uterine leiomyoma	5-Feb-20	15-Feb-20	Moderate	Not related to IP	Recovered/ resolved	No	No	Yes	No	No
3	Enteritis	11-Feb-20	15-Feb-20	Moderate	Not related to IP	Recovered/ resolved	No	No	Yes	No	No
4	Lower limb fracture	12-Feb-20	18-Feb-20	Moderate	Not related to IP	Recovered/ resolved with sequelae	No	No	Yes	No	No
5	Skin ulcer	18-Feb-20	18-Apr-20	Severe	Not related to IP	Recovered/ resolved	No	No	Yes	No	No
6	Cerebro-vascular accident	15-Mar-20	20-Mar-20	Severe	Not related to IP	Recovered/ resolved with sequelae	No	No	Yes	No	No
7	Abortion spontaneous	30-Mar-20	31-Mar-20	Moderate	Not related to IP	Recovered/ resolved	No	No	No	No	Yes
8	Malaria	30-Mar-20	2-Apr-20	Severe	Not related to IP	Recovered/ resolved	No	No	Yes	No	No
9	Typhoid fever	30-Mar-20	2-Apr-20	Moderate	Not related to IP	Recovered/ resolved	No	No	Yes	No	No
10	HIV infection	15-Apr-20	21-May-21*	Severe	Not related to IP	Fatal	No	No	No	No	Yes
11	Dyspepsia	15-Jun-20	20-Jun-20	Mild	Not related to IP	Recovered/ resolved	No	No	Yes	No	No
12	Asthenia	16-Jun-20	21-Jun-20	Severe	Not related to IP	Recovered/ resolved	No	No	Yes	No	No
13	Pyrexia	16-Jun-20	21-Jun-20	Mild	Not related to IP	Recovered/ resolved	No	No	Yes	No	No
14	Cerebro-vascular accident	25-Jun-20	20-Jul-20	Severe	Not related to IP	Recovered/ resolved with sequelae	No	No	Yes	No	No
15	Malaria	1-Jul-20	6-Jul-20	Severe	Not related to IP	Recovered/ resolved	No	No	Yes	No	No
16	Pneumonia	1-Jul-20	6-Jul-20	Moderate	Not related to IP	Recovered/ resolved	No	No	Yes	No	No

SAE	MeDra Preferred Term	Start date	End date	Toxicity grade	Relatedness to IP	Outcome	Congenital anomaly	Persistent disability	Hospitalization	Life-threatening	Other medically important event
17	Abdominal strangulated hernia	14-Jul-20	19-Jul-20	Moderate	Not related to IP	Recovered/ resolved	No	No	Yes	No	No
18	Malaria	23-Jul-20	26-Jul-20	Severe	Not related to IP	Recovered/ resolved	No	No	Yes	No	No
19	Typhoid fever	23-Jul-20	26-Jul-20	Moderate	Not related to IP	Recovered/ resolved	No	No	Yes	No	No
20	Calculus bladder	27-Jul-20	21-Aug-20	Severe	Not related to IP	Recovered/ resolved	No	No	Yes	No	No
21	Malaria	2-Aug-20	6-Aug-20	Severe	Not related to IP	Recovered/ resolved	No	No	Yes	No	No
22	Dehydration	2-Aug-20	6-Aug-20	Severe	Not related to IP	Recovered/ resolved	No	No	Yes	No	No
23	Dermo-hypodermitis	13-Aug-20	20-Jan-21*	Severe	Not related to IP	Fatal	No	Yes	No	No	No
24	Abdominal adhesions	22-Aug-20	31-Aug-20	Severe	Not related to IP	Recovered/ resolved	No	No	Yes	No	No
25	Malaria	29-Aug-20	11-Sep-20	Severe	Not related to IP	Recovered/ resolved	No	No	Yes	No	No
26	Typhoid fever	29-Aug-20	11-Sep-20	Severe	Not related to IP	Recovered/ resolved	No	No	Yes	No	No
27	Dehydration	29-Aug-20	11-Sep-20	Severe	Not related to IP	Recovered/ resolved	No	No	Yes	No	No
28	Abortion spontaneous	7-Oct-20	7-Oct-20	Moderate	Not related to IP	Recovered/ resolved	No	No	No	No	Yes
29	Malaria	7-Oct-20	12-Oct-20	Moderate	Not related to IP	Recovered/ resolved	No	No	No	No	Yes
30	Uretero-lithiasis	15-Oct-20	10-Nov-20*	Severe	Not related to IP	Fatal	No	No	Yes	No	No
31	Calculus bladder	15-Oct-20	10-Nov-20*	Severe	Not related to IP	Fatal	No	No	Yes	No	No

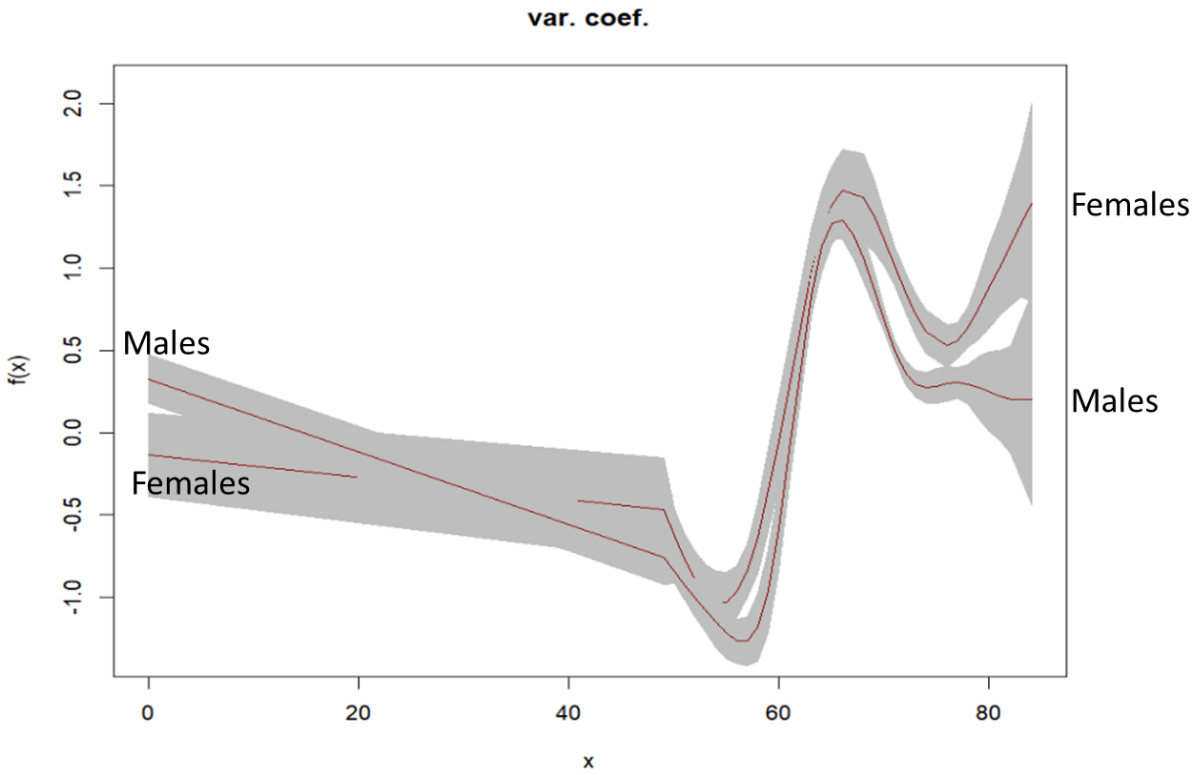
** On October 23rd, 2020, four SAEs were still ongoing. However, end dates and outcomes for these SAEs are reported as they were available at the time of analysis.*

Figure 1. Scatterplots and spearman correlations of pre-existing neutralizing antibody titres against the Ad26- and MVA-vector versus the anti-EBOV GP IgG antibody response before and after vaccination (Per protocol set 1)



On the left, scatterplots between Ebola virus binding antibody concentrations and Ad26 neutralizing antibody titres are depicted with spearman correlation coefficients; on the right, scatterplots between Ebola virus binding antibody concentrations and MVA neutralizing antibody titres are depicted with spearman correlation coefficients; Panels A and D show the relation before vaccination (Day 1/baseline), panels B and E show the relation 56 days (±7 days) after vaccination with Ad26.ZEBOV (Day 57) and panels C and F present the relation 21 days (±7 days) after MVA-BN-Filo vaccination (Day 78); Horizontal dashed lines indicate the lower limit of quantification of the Ebola virus binding antibody concentrations and were set at 36.11 ELISA units/mL; Vertical dashed lines indicate the lower limit of quantification of Ad26- and MVA-vector antibody titres and were set at 17 and 20, respectively.

Figure 2. varying coefficient spline of time between blood samples for each level of the variable sex



Men started with higher Ebola virus binding antibody geometric concentrations at baseline than women, a boost in antibody response was observed in both men and women from Day 57 until Day 70, with women reaching a higher antibody response than men from Day 70 onwards.

Chapter 4 Long-term antibody persistence of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen, and safety and (long-term) immunogenicity of an Ad26.ZEBOV booster dose

Ad26.ZEBOV, MVA-BN-Filo Ebola virus disease vaccine regimen plus Ad26.ZEBOV booster at 1 year versus 2 years in health-care and front-line workers in the Democratic Republic of the Congo: secondary and exploratory outcomes of an open-label, randomised, phase 2 trial

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4.1 Summary

Background. Health-care providers and front-line workers are at risk of contracting Ebola virus disease during an Ebola virus outbreak and consequently of becoming drivers of the disease. We aimed to assess the long-term immunogenicity of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen and the safety of and immune memory response to an Ad26.ZEBOV booster vaccination at 1 year or 2 years after the first dose in this at-risk population.

Methods. This open-label, single-centre, randomised, phase 2 trial was conducted at one study site within a hospital in Boende, Democratic Republic of the Congo. Adult health-care providers and front-line workers, excluding those with a known history of Ebola virus disease, were vaccinated with a two-dose heterologous regimen administered at a 56-day interval via a 0·5 mL intramuscular injection in the deltoid muscle, comprising Ad26.ZEBOV as the first dose and MVA-BN-Filo as the second dose. After the initial vaccination on day 1, participants were randomly assigned (1:1) via randomisation envelopes, opened in a sequential order, to receive an Ad26.ZEBOV booster vaccination at 1 year (group 1) or 2 years (group 2) after the first dose. We present the secondary and exploratory objectives of the trial—results of the primary objective have been published elsewhere. We measured immunogenicity at six timepoints per group as geometric mean concentrations (GMCs) of Ebola virus glycoprotein-specific IgG binding antibodies, using the Filovirus Animal Non-Clinical Group ELISA. We assessed serious adverse events occurring up to 6 months after the last dose and local and systemic solicited and unsolicited adverse events reported for 7 days after the booster vaccination. Antibody responses were analysed per protocol, serious adverse events per full analysis set (FAS), and adverse events for all boosted FAS participants. This trial is registered as completed on ClinicalTrials.gov (NCT04186000).

Findings. Between Dec 18, 2019, and Feb 8, 2020, 699 health-care providers and front-line workers were enrolled and 698 were randomly assigned (350 to group 1 and 348 to group 2 [FAS]); 534 (77%) participants were male and 164 (23%) were female. 319 in group 1 and 317 in group 2 received the booster. 29 (8%) in group 1 and 26 (7%) in group 2 did not

complete the study, mostly due to loss to follow-up or moving out of the study area. In both groups, injection-site pain or tenderness (87 [27%] of 319 group 1 participants vs 90 [28%] of 317 group 2 participants) and headache (91 [29%] vs 93 [29%]) were the most common solicited adverse events related to the investigational product. One participant (in group 2) had a related serious adverse event after booster vaccination (fever of ≥40·0°C). Before booster vaccination, Ebola virus glycoprotein-specific IgG binding antibody GMCs were 279·9 ELISA units (EU) per mL (95% CI 250·6–312·7) in 314 group 1 participants (1 year after first dose) and 274·6 EU/mL (242·1–311·5) in 310 group 2 participants (2 years after first dose). These values were 5·2 times higher in group 1 and 4·9 times higher in group 2 than before vaccination on day 1. 7 days after booster vaccination, these values increased to 10 781·6 EU/mL (9354·4–12 426·4) for group 1 and 10 746·9 EU/mL (9208·7–12 542·0) for group 2, which were approximately 39 times higher than before booster vaccination in both groups. 1 year after booster vaccination in 299 group 1 participants, a GMC that was 7·6-times higher than before booster vaccination was still observed (2133·1 EU/mL [1827·7–2489·7]).

Interpretation. Overall, the vaccine regimen and booster dose were well tolerated. A similar and robust humoral immune response was observed for participants boosted 1 year and 2 years after the first dose, supporting the use of the regimen and flexibility of booster dose administration for prophylactic vaccination in at-risk populations.

Funding. Innovative Medicines Initiative 2 Joint Undertaking and Coalition for Epidemic Preparedness Innovations.

4.2 Research in context

4.2.1 Evidence before this study

The Ad26.ZEBOV and MVA-BN-Filo heterologous, two-dose Ebola virus vaccine regimen, administered at a 56-day interval, was granted marketing authorisation for use under exceptional circumstances by the European Medicines Agency in 2020 as prophylactic vaccination against Ebola virus disease caused by Zaire Ebola virus in children and adults. To assess evidence on the safety, immunogenicity, and durability of this regimen, we searched for the terms “Ad26.ZEBOV” AND “MVA-BN-Filo” AND (“safety” OR “immunogenicity” OR “durability”) in PubMed on Jan 3, 2024. No restrictions were placed on the type of article, publication timeframe, or language. In total, 29 articles were identified. Additionally, to identify existing research on Ad26.ZEBOV booster vaccination, the search string was amended to include AND “Boost*” in PubMed on Jan 3, 2024. 13 articles were identified, all of which were also part of the original search output.

Vaccine trials assessing the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen have been conducted in infants (aged <1 year), children (aged 1–11 years), adolescents (aged 12–17 years), healthy adults (aged ≥18 years), HIV-infected adults with well controlled infection and on highly active antiretroviral therapy (ART), and children and adults with malaria exposure before and after vaccination. All reported that the vaccine regimen was generally well tolerated with mostly mild to moderate adverse events reported. The humoral immune responder rate (defined as having a binding antibody concentration that was at least 2·5 times greater than at baseline) 21 days after the heterologous, two-dose vaccine regimen, administered at a 56-day interval, ranged between 95% and 100%, depending on the study and population. In adults, immune persistence after primary vaccination has been assessed at 6 months, 8 months, 1 year, 2 years, and 4·5 years. Throughout the articles, a decrease in antibodies is described until 6 months after the first dose, with a stabilisation in geometric mean concentrations (GMCs) thereafter. Four studies reported an Ad26.ZEBOV booster administration 1 or more years after the initial dose of the

Ad26.ZEBOV and MVA-BN-Filo regimen administered at a 56-day interval. One study in children and adolescents (n=50) administered a booster dose more than 3 years after the first dose of the regimen; the responder rate was 100% among boosted children and adolescents. Two studies were in healthy adults; one (n=39) administered a booster dose 1 year after the first dose and the other (n=29) administered a booster dose 2 years after the first dose. Overall, the safety profile of the booster dose did not differ notably from the first Ad26.ZEBOV dose and no vaccine-related serious adverse events were reported. 7 days after booster vaccination a 100% responder rate was observed among participants boosted after 1 year and a 96% responder rate was observed in the participant group boosted after 2 years. In both studies, responses persisted in 100% of participants 1 year after booster vaccination. Finally, one study was in HIV-positive adults (n=13) with well controlled infection and on highly active ART and administered a booster dose 4·5 years after the first dose. 7 days after booster vaccination, a 100% responder rate was observed.

4.2.2 Added value of this study

This study reports the secondary and exploratory outcomes of a trial for which the primary endpoint has been reported elsewhere. To the best of our knowledge, this study is, to date, the largest published adult (ie, health-care providers and frontline workers) study assessing the safety and long-term immunogenicity (up to 2 years after the first dose) of the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen administered at a 56-day interval. Additionally, this study is, to date, the largest trial assessing the safety of and humoral immune memory to an Ad26.ZEBOV booster vaccination, and the first trial to compare, in an exploratory analysis, the humoral immune memory response of an Ad26.ZEBOV booster dose 1 year or 2 years after the first dose in the same study population.

4.2.3 Implications of all available evidence

Previous studies combined with our study findings show that vaccination with the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen at a 56-day interval is generally well tolerated, leads to a persistent immune response, and a humoral immune memory

response can be elicited with an Ad26.ZEBOV booster vaccination in adults for at least 2 years after initial vaccination. These findings support the strategy of a prophylactic vaccination regimen in health-care providers and front-line worker populations, who are at greater risk of contracting and spreading Ebola virus disease than the general population, to minimise the impact of the next outbreak in Ebola-endemic locations. Booster vaccinations provide a similar and robust humoral immune memory response at 1 year and 2 years after the first dose, indicating flexibility in booster administration timing (eg, when an outbreak occurs).

4.3 Introduction

Health-care providers are at higher risk of contracting and spreading Ebola virus disease (EVD) than the general population during an Ebola outbreak or epidemic. During the west African Ebola virus epidemic (2013–16), a disproportionate amount of EVD deaths was observed in doctors, nurses, and midwives (1·5–8·1%) compared with the general population (<0·2%) in Guinea, Sierra Leone, and Liberia.[1] Such a decrease in the population of health-care providers can have a considerable impact on health-care provision and consequently on population health in low-income and middle-income countries (LMICs) already struggling with shortages in skilled health-care providers.[1,2] Preventing devastating consequences of future EVD outbreaks and preparing areas endemic for Ebola virus against the next outbreak are therefore crucial.

A two-dose, heterologous Ebola virus vaccine regimen was developed that consists of a monovalent, recombinant, replication-incompetent adenovirus type 26 (Ad26) vector-based vaccine, which encodes the glycoprotein of the Ebola virus Mayinga strain (Ad26.ZEBOV), and a multivalent, recombinant, replication-incompetent modified vaccinia Ankara (MVA) vector-based vaccine, which encodes glycoproteins from the Ebola virus Mayinga, Sudan virus Gulu, and Marburg Musoke strains and the nucleoprotein from the Taï Forest virus (MVA-BN-Filo). The regimen has been shown to be safe and immunogenic in clinical trials.[3–8]

Although determining its efficacy has not been possible through trials in humans, the heterologous vaccine regimen was protective in challenged non-human primates.[9] Through immunobridging, researchers inferred the likelihood of the vaccine regimen's protective effect in humans from its protective effect in animals, concluding that the vaccine regimen is likely to provide protection against EVD in humans.10,11 Modelling studies have shown that prophylactic vaccination of health-care providers and front-line workers would be the most effective way to reduce EVD and its related morbidity and mortality, even at 30% vaccine coverage and 50–60% vaccine efficacy.[12,13]

At the time of writing this Article, the Democratic Republic of the Congo has had 15 EVD outbreaks since discovery of the disease in 1976.[14] We previously reported results on the safety and immunogenicity of the primary vaccine regimen in health-care providers and front-line workers living and working in the Boende health zone in the Democratic Republic of the Congo.[8] However, little information has been published on the persistence of antibodies after vaccination with the primary regimen and whether the timing of the booster dose has an effect on the immune memory response.[4,5] Here, we present the secondary and exploratory objectives of this trial, which consist of the long-term persistence of vaccine-induced antibodies after the primary regimen, the safety of an Ad26.ZEBOV booster dose at 1 year or 2 years after the first dose in the same population, and long-term immunogenicity of the booster dose (when given at 1 year after the primary vaccine regimen).

4.4 Methods

4.4.1 Study design and participants

This open-label, randomised, phase 2 trial was performed at one study site in Boende, the Democratic Republic of the Congo, and recruited registered health-care providers and front-line workers as participants. Health-care providers were defined as professionals who work in a health-care facility and are potentially exposed to Ebola virus within this facility (doctors, nurses, midwives, laboratory technicians, health-facility cleaners, etc). People

with professions that mean they are potentially exposed to Ebola virus in the community were considered to be front-line workers (community healthcare workers, first aid workers, stretcher bearers, etc). In this Article, we refer to health-care providers and frontline workers collectively as health-care providers.

During the recruitment period, all known health-care providers living and working in the Boende health zone were invited to attend a workshop explaining the objectives of the trial, the intended trial procedures, and the informed consent procedure. Health-care providers who were willing to participate in the trial after the workshop were asked to return to the trial site on the next day for a screening visit. The trial site consisted of a wing of the general reference hospital of Boende that was refurbished (in the context of capacity building) and rented by the study team for the duration of the trial.[15] The trial site was only accessible for study staff and participants.

Participants were eligible if they were aged 18 years and older; did not have a known history of EVD; had a good understanding of the trial and its consenting process, as established by a test of understanding (ten true or false questions requiring a score of ≥9 to pass; three attempts were allowed); were apparently healthy as judged by the study physician (on the basis of vital signs and physical examination); were not pregnant (negative pregnancy test required), breastfeeding, or planning to become pregnant within 3 months after the first vaccine dose; were available for the entire study duration; and were willing to provide contact information and have means to be contacted. Additionally, participants were not allowed to have had organ or stem-cell transplantation, a history of chronic urticaria, or been vaccinated with any experimental Ebola vaccines within 3 months before enrolment or any Ad26-based vaccine in the past. Sex assigned at birth was self-reported by participants with the options of male and female.

The trial was conducted according to the most recent Declaration of Helsinki[16] and Good Clinical Practice guidelines[17] and approved by the National Ethics Committee of the Ministry of Health of the Democratic Republic of the Congo (121/CNES/BN/PMMF/2019) and the ethics committee of the University Hospital of Antwerp–University of Antwerp,

Belgium (19/14/177). All participants provided written informed consent before enrolment. The study protocol is available online.[18]

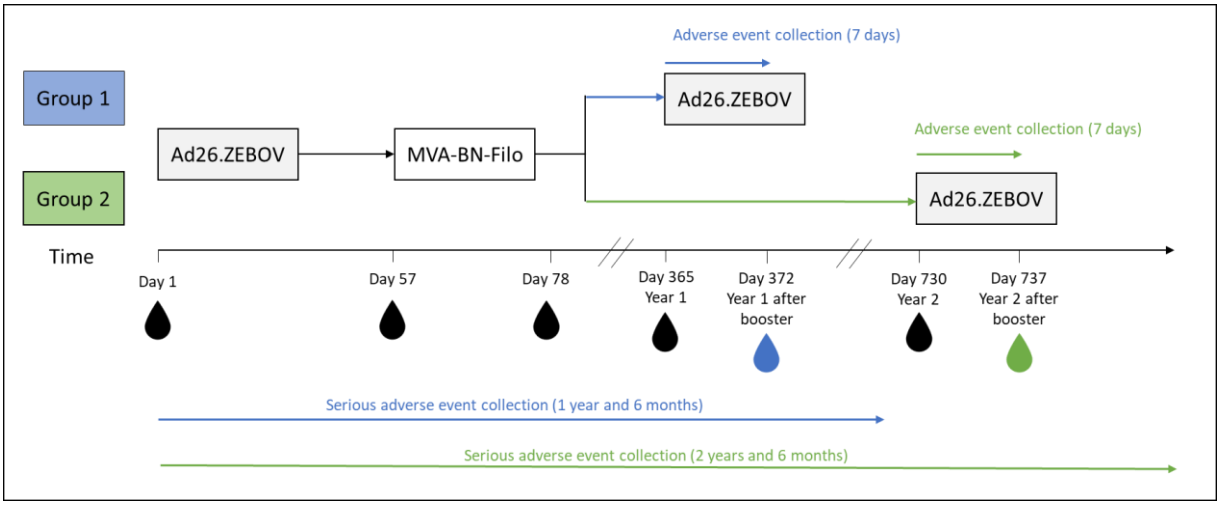
4.4.2 Randomisation and masking

Randomisation was performed by the data management team using a software algorithm that randomly assigned a booster timepoint (1:1) to a sequential number. These sequential numbers were compiled in a randomisation list that was used to create sealed envelopes, which were assembled and checked by delegated principal investigator staff. On day 1, after vaccination with the first dose, delegated site staff opened the envelopes in a sequential order, randomly assigning participants to receive an Ad26.ZEBOV booster vaccination either 1 year (group 1) or 2 years (group 2) after the initial dose. The opening of the envelopes in sequential order was monitored by external clinical research associates. No masking of participants, study staff, or laboratory staff took place.

4.4.3 Procedures

The timing and duration of study procedures are shown (figure 1). Participants were vaccinated with the heterologous, two-dose Ad26.ZEBOV (5×10^{10} viral particles) and MVA-BN-Filo (1×10^{8} infectious units) vaccine regimen at a 56-day interval, followed by an Ad26.ZEBOV (5×10^{10} viral particles) booster dose either 1 year (group 1) or 2 years (group 2) after the first dose. Vaccinations were administered via a 0·5 mL intramuscular injection in the deltoid muscle, changing arms for each vaccination. If participants presented with an acute illness, such as fever above 38·0°C, on the day of vaccine administration, vaccination was postponed until the illness was resolved.

Figure 1. Study design.



Black drops represent the collection of blood samples from group 1 and group 2, blue drops represent the collection of blood samples from group 1 only, and green drops represent the collection of blood samples from group 2 only. Adverse events were collected with a participant diary.

Participants remained in observation for 30 min after vaccination to record any immediate serious adverse events. For 7 days after booster vaccination, participants recorded local and systemic solicited and unsolicited adverse events in a participant journal. On the eighth day after booster vaccination, these recorded symptoms were discussed with a study physician during a reactogenicity assessment and relatedness and severity were assessed and recorded. For this trial, relatedness to the investigational product was reported by the physician on a binary scale as either related (ie, definitely, probably, and possibly) or not related (ie, unlikely, unrelated). Severity was assessed with the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials.[19] Grade 3 adverse events were events that led to the inability to work or perform usual activities or the need for narcotic pain relief.[19] Grade 4 adverse events were events that led to an emergency room visit or hospitalisation.[19] If any adverse event was still ongoing on the eighth day after booster vaccination, participants were asked to return to the site when the symptom had resolved to report an end date. Serious adverse events, as defined by the International Conference on Harmonisation's E2A clinical safety data management scientific guideline,[20] were collected from enrolment until 6 months after the last received dose. Therefore, the recording of serious adverse events was generally longer after the primary vaccine regimen than after the booster dose, and overall, the recording of serious

adverse event was longer for participants in group 2 than in group 1. For serious adverse event reporting, toll-free telephone numbers were made available to participants, and at each scheduled visit participants were asked if they had had any medical events that could be considered a serious adverse event. Finally, 6 months after the second dose and 6 months after the booster dose, participants were contacted via telephone, or visited at home by study staff if living outside the mobile phone network range around Boende,[21] to ask whether any serious adverse event had occurred since last contact.

For immunogenicity assessments, blood samples were collected before each primary regimen vaccination, at 21 days after the second dose, at 1 year after the first dose for group 1, at 1 year and 2 years after the first dose for group 2, at 7 days after the booster vaccination for both groups, and at 1 year after booster vaccination for group 1. Ebola virus glycoprotein-specific IgG binding antibodies were analysed with the validated Ebola virus glycoprotein Filovirus Animal Non-clinical Group (FANG) ELISA.[22] The laboratory analysis was conducted by Q² Solutions, who changed laboratory location from San Juan Capistrano, CA, USA (vaccine regimen immunogenicity analysis) to Durham, NC, USA (booster immunogenicity analysis) within the course of the trial. As endorsed by the US Food and Drug Administration, the assay was shown to be equivalent between the two laboratory locations, across the entire assay range (data not shown).

4.4.4 Outcomes

In this Article we describe the results of the secondary objectives of the trial: to assess the safety and immunogenicity of an Ad26.ZEBOV booster vaccination administered 1 years or 2 years after the initial dose. We also report findings of the exploratory objectives to assess the long-term persistence of vaccine-induced antibodies after the primary vaccine regimen and booster vaccination (for group 1 only), and to compare the binding antibody responses after booster vaccination given 1 year or 2 years after the first dose between the two randomised groups. The primary endpoint—binding antibody concentrations against Ebola virus glycoprotein at 21 days after the second dose—was reported elsewhere along with the other endpoints of the trial.[8,23]

Immunogenicity was assessed with Ebola virus glycoprotein-specific IgG antibody geometric mean concentrations (GMCs) and responder rates. A responder was defined as a participant with a binding antibody concentration that was at least 2·5 times higher than the lower limit of quantification (LLOQ) after vaccination when the participant had antibodies below or equal to the LLOQ (≤36·11 ELISA units [EU] per mL) at baseline on day 1, or an antibody value that was at least 2·5 times higher after vaccination when the participant already had a value above the LLOQ at baseline on day 1. The safety of the regimen and booster vaccination was measured as the proportion of participants with serious adverse events related to the vaccine occurring up to 6 months after the last received vaccination and the proportion of participants with local and systemic solicited and unsolicited adverse events related to the vaccine reported on the day of vaccination and within the consecutive 7 days.

4.4.5 Statistical analysis

The sample size was a convenience sample based on the number of health-care providers living and working in the Boende health zone and was not based on formal hypothesis-testing considerations. To assess whether the comparison of the humoral immune memory response between those boosted at 1 year or 2 years was possible with this convenience sample, a power calculation was performed after the study had started, which showed a power of 99% to detect a difference between the two groups.[18]

Demographics, baseline characteristics, and serious adverse events are summarised for the full analysis set (FAS), which included all enrolled participants who received at least one dose of the Ebola vaccine regimen. Adverse events are summarised for all participants in the FAS who received a booster dose. Immunogenicity analysis was performed in per-protocol set 1 (PPS1) to assess the humoral immune response after vaccination with the primary Ebola vaccine regimen and in per-protocol set 2 (PPS2) to assess the humoral immune response after booster vaccination. Per-protocol set analyses included all participants for whom blood sample collection and vaccination occurred per protocol, who had at least one evaluable immunogenicity serum sample after vaccination (after the first

or second dose for PPS1 and after the booster for PPS2), and who had no major protocol deviations that had an effect on the immune response.

As a post-hoc analysis, and to identify differences in the number of group 1 and group 2 participants reporting adverse events 7 days after booster vaccination, risk ratios with 95% CIs were calculated. Ebola virus glycoprotein-specific IgG antibody responses are reported as GMCs with 95% CIs. All values below or equal to the LLOQ were imputed with half the LLOQ (18·055 EU/mL), and values above the upper limit of quantification (ULOQ; 194 938·88 EU/mL) were imputed with the ULOQ. For calculation of the responder rate, values below or equal to the LLOQ were imputed to the LLOQ (36·11 EU/mL). Cohen's d statistics were used to compare antibody concentrations after the booster between group 1 and group 2. Finally, a post-hoc analysis was performed to assess whether the GMCs after vaccination differed for participants with or without baseline binding antibody concentrations above the LLOQ.

R version 4.3.1 was used to perform all statistical analysis. The trial was registered at ClinicalTrials.gov (NCT04186000).

4.4.6 Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, writing of the report, or the decision to submit the report for publication.

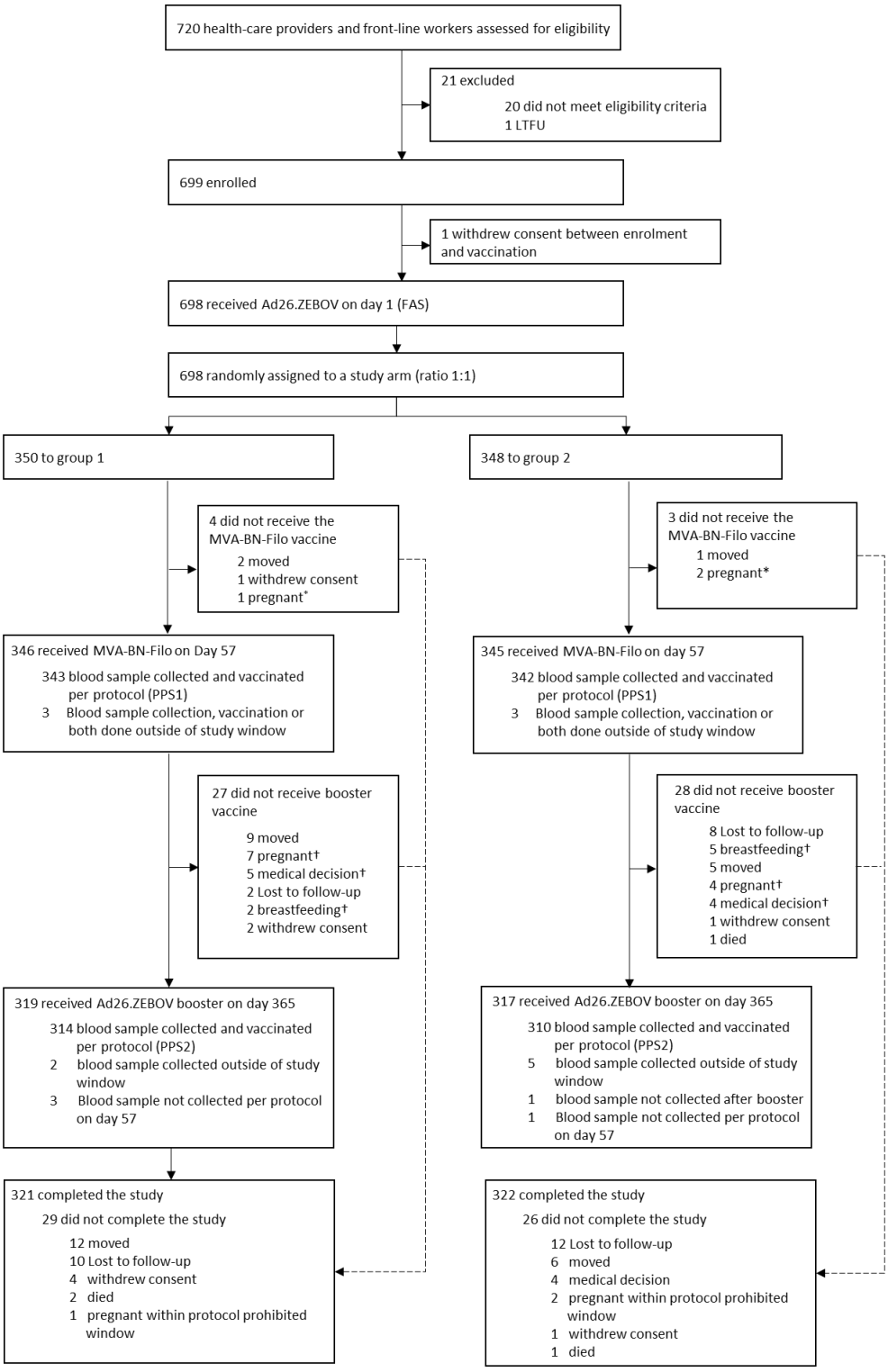
4.5 Results

Health-care providers were recruited between Dec 18, 2019, and Feb 8, 2020. After 720 participants were assessed for eligibility, 699 participants were enrolled, one of whom withdrew consent before any study activity was performed. The FAS therefore consisted of 698 participants, 350 randomly assigned to group 1 and 348 to group 2 (figure 2). Demographic and baseline characteristics for these participants are presented (table 1). 534 (77%) participants were male and most worked in direct contact with patients. Overall, 691 participants (346 in group 1 and 345 in group 2) were vaccinated with both doses of

the primary vaccine regimen and 636 participants (319 in group 1 and 317 in group 2) received the booster dose. Of these participants, 685 were included in PPS1 and 624 in PPS2. Overall, 643 participants (92%) completed the study, with the last participant visit taking place on Oct 12, 2022.

Local and systemic solicited adverse events after the booster vaccination in group 1 and group 2 were mostly mild (table 2). At least one local solicited adverse event was reported by 95 (30%) of 319 group 1 participants and 95 (30%) of 317 group 2 participants. The most commonly reported local solicited adverse event in both groups was pain or tenderness at the injection site (87 [27%] of 319 group 1 participants and 90 [28%] of 317 group 2 participants). One participant in group 2 reported a severe pain or tenderness event. All local adverse events were considered related to the investigational product.

Figure 2. Study trial profile.



*FAS=full analysis set. PPS1=per-protocol set 1. PPS2=per-protocol set 2. *These participants became pregnant within the protocol-prohibited window and were discontinued from the trial because they did not receive both doses of the two-dose heterologous regimen. †Participants for whom treatment was stopped but other trial activities (eg, safety follow-up) continued.*

Table 1. Baseline sociodemographic characteristics of enrolled health-care providers and front-line workers (full analysis set).

Characteristic	Group 1 (N = 350)	Group 2 (N = 348)	Overall (N = 698)
Sex as assigned at birth			
Male	260 (74%)	274 (79%)	534 (77%)
Female	90 (26%)	74 (21%)	164 (23%)
Race			
Black	350 (100%)	348 (100%)	698 (100%)
Age, years			
Mean (SD)	45·4 (11·5)	44·6 (12·5)	45·0 (12·0)
Median (range)	46 (20-75)	46 (19-74)	46 (19-75)
Profession			
Community health worker	117 (33%)	119 (34%)	236 (34%)
Nurse	87 (25%)	94 (27%)	181 (26%)
First aid worker	91 (26%)	86 (25%)	177 (25%)
Hygienist	22 (6%)	15 (4%)	37 (5%)
Midwife	19 (5%)	11 (3%)	30 (4%)
Doctor	6 (2%)	7 (2%)	13 (2%)
Health facility cleaner	2 (1%)	8 (2%)	10 (1%)
Care giver	2 (1%)	5 (1%)	7 (1%)
Lab technician	2 (1%)	0	2 (<1%)
Pharmacist aid	0	2 (1%)	2 (<1%)
Other	2 (1%)	1 (<1%)	3 (<1%)
Work establishment			
Health centre	182 (52%)	189 (54%)	371 (53%)
Red cross	91 (26%)	86 (25%)	177 (25%)
Hospital	44 (13%)	40 (12%)	84 (12%)
Health post	21 (6%)	16 (5%)	37 (5%)
Health area	4 (1%)	6 (2%)	10 (1%)
Provincial health department	6 (2%)	3 (1%)	9 (1%)
Health zone	1 (<1%)	7 (2%)	8 (1%)
Health inspection	0	1 (<1%)	1 (<1%)
Staff member of the expanded programme on immunisation	1 (<1%)	0	1 (<1%)
Medical history*			
Yes	71 (20%)	64 (18%)	135 (19%)
No	279 (80%)	284 (82%)	563 (81%)
Smallpox vaccination against mpox†			
Yes	61 (17%)	68 (20%)	129 (19%)
No	289 (83%)	280 (80%)	569 (81%)

*N represents all participants who received at least one study vaccine dose. Both groups received the Ad26.ZEBOV, MVA-BN-Filo primary vaccination regimen, but group 1 participants received an Ad26.ZEBOV booster dose at 1 year after the first dose and group 2 participants received it at 2 years after the first dose. Democratic Republic of Congo is divided into different health zones, each of which has a general referral hospital where hospitalisation is possible and doctors are present. Health zones are further split into health areas, which can have several health centres where patients can be hospitalised and only nurses are available. Health areas can also, but not always, contain health posts in which hospitalisation is not possible and community health workers assist nurses. A health post is an optional health-care delivery structure created to meet the accessibility needs for addressing specific problems of a particular population within a health area. EPI=Expanded Program on Immunization. *Yes and No indicate whether the participant reported current or past medical issues during the medical history inquiry. †Formerly known as monkeypox*

At least one systemic solicited adverse event was reported by 133 (42%) of 319 group 1 participants and 127 (40%) of 317 group 2 participants. Of these, 128 (40%) group 1 participants and 123 (39%) group 2 participants were considered to have had systemic adverse events related to the investigational product. Headache was the most commonly reported related solicited systemic adverse event in both groups, followed by myalgia, fatigue, and nausea. Severe vaccine-related headache (three [1%] in group 1 vs one [<1%] in group 2), fatigue (none vs two [1%]), and myalgia (two [1%] vs one [<1%]) were infrequently reported by participants in both groups. The median duration of local adverse events was 2 days (IQR 1–4 days; 2 days [1–3 days] for group 1 and 2 days [1–4 days] for group 2) and systemic solicited adverse events was 2 days (1–6 days; 2 days [1–6 days] for group 1 and 2 days [1–5 days] for group 2).

Fever related to vaccination was reported by 21 participants (nine [3%] in group 1 and 12 [4%] in group 2) within 7 days after booster vaccination. One group 2 participant had fever above 40·0°C at 3 days after booster vaccination, which was reported as a related serious adverse event by the principal investigator and categorised as an “other medically important event”. No hospitalisation was required to treat this serious adverse event, and it resolved without sequelae the day after onset. No other vaccine-related serious adverse events were reported during the trial. Of the 698 participants, 47 (7%) had one or more serious adverse events: 27 (4%) after the primary vaccination regimen; 19 (3%) after the booster vaccination (six [2%] of 319 in group 1 vs 13 [4%] of 317 in group 2); and one (<1%) participant in group 2 had two simultaneous serious adverse events after the primary vaccination and one serious adverse event after booster vaccination. 64 serious adverse events were reported in total (42 occurring between the primary regimen and the booster vaccination and 22 events after booster vaccination [seven in group 1 vs 15 in group 2]; appendix pp 4–6). Most serious adverse events were considered to have resolved without sequelae. For five participants (none in group 1 vs five [1%] in group 2), serious adverse events were considered resolved with sequelae and three participants (one [<1%] vs two [1%]) had fatal serious adverse events (appendix pp 4–6).

Table 2. Solicited and unsolicited adverse events in the 7 days after booster vaccination in participants in the FAS who received the booster dose and serious adverse events up to 6 months after the last received dose in the FAS

	Overall (N=636)		**Group 1 (N=319)**		**Group 2 (N=317)**		**RR (95% CI)[Δ]**
	Participants, n (%)	Events, n	Participants, n (%)	Events, n	Participants, n (%)	Events, n	
Solicited adverse events[#]							
Any local adverse event[@]	190 (30%)	230	95 (30%)	104	95 (30%)	126	0·997 (0·901-1·104)
Mild	169 (27%)	200	85 (27%)	92	84 (26%)	108	1·002 (0·913-1·100)
Moderate	28 (4%)	29	12 (4%)	12	16 (5%)	17	0·987 (0·954-1·020)
Severe	1 (<1%)	1	0	0	1 (<1%)	1	0·997 (0·991-1·003)
Potentially life-threatening	0	0	0	0	0	0	1·000 (1·000-1·000)
Erythema							
Any	24 (4%)	24	10 (3%)	10	14 (4%)	14	0·987 (0·957-1·018)
Severe	0	0	0	0	0	0	1·000 (1·000-1·000)
Swelling							
Any	15 (2%)	15	7 (2%)	7	8 (3%)	8	0·997 (0·973-1·021)
Severe	0	0	0	0	0	0	1·000 (1·000-1·000)
Pain/Tenderness							
Any	177 (28%)	191	87 (27%)	87	90 (28%)	104	0·985 (0·894-1·084)
Severe	1 (<1%)	1	0	0	1 (<1%)	1	0·997 (0·991-1·003)
Any systemic adverse events	260 (41%)	565	133 (42%)	252	127 (40%)	313	1·028 (0·903-1·170)
Mild	232 (36%)	424	115 (36%)	186	117 (37%)	238	0·987 (0·877-1·110)
Moderate	77 (12%)	125	38 (12%)	57	39 (12%)	68	0·996 (0·940-1·055)
Severe	12 (2%)	15	7 (2%)	9	5 (2%)	6	1·006 (0·985-1·028)
Potentially life-threatening	1 (<1%)	1	0	0	1 (<1%)	1	0·997 (0·991-1·003)
Any related systemic adverse events	251 (39%)	543	128 (40%)	242	123 (39%)	301	1·022 (0·916-1·159)
Mild	225 (35%)	411	111 (35%)	179	114 (36%)	232	0·982 (0·875-1·102)
Moderate	75 (12%)	118	37 (12%)	55	38 (12%)	63	0·996 (0·941-1·054)
Severe	10 (2%)	13	6 (2%)	8	4 (1%)	5	1·006 (0·987-1·026)
Potentially life-threatening	1 (<1%)	1	0	0	1 (<1%)	1	0·997 (0·991-1·003)
Fatigue							
Any	121 (19%)	137	60 (19%)	60	61 (19%)	77	0·995 (0·922-1·072)
Severe	2 (<1%)	2	0	0	2 (1%)	2	0·994 (0·985-1·002)
Any related	120 (19%)	136	59 (18%)	59	61 (19%)	77	0·991 (0·920-1·068)
Severe related	2 (<1%)	2	0	0	2 (1%)	2	0·994 (0·985-1·002)
Headache							
Any	194 (31%)	224	97 (30%)	101	97 (30%)	123	0·997 (0·900-1·105)
Severe	5 (1%)	5	4 (1%)	4	1 (<1%)	1	1·010 (0·996-1·024)
Any related	184 (29%)	211	91 (29%)	94	93 (29%)	117	0·989 (0·895-1·092)
Severe related	4 (1%)	4	3 (1%)	3	1 (<1%)	1	1·006 (0·994-1·019)
Nausea							
Any	39 (6%)	44	17 (5%)	18	22 (7%)	26	0·983 (0·945-1·023)
Severe	0	0	0	0	0	0	1·000 (1·000-1·000)
Any related	38 (6%)	42	16 (5%)	16	22 (7%)	26	0·980 (0·942-1·019)
Severe related	0	0	0	0	0	0	1·000 (1·000-1·000)

	Overall (N=636)		**Group 1 (N=319)**		**Group 2 (N=317)**		**RR (95% CI)[Δ]**
	Participants, n (%)	Events, n	Participants, n (%)	Events, n	Participants, n (%)	Events, n	
Myalgia							
Any	124 (19%)	133	63 (20%)	64	61 (19%)	69	1·006 (0·932-1·086)
Severe	3 (<1%)	3	2 (1%)	2	1 (<1%)	1	1·003 (0·992-1·014)
Any related	122 (19%)	131	63 (20%)	64	59 (18%)	67	1·014 (0·940-1·094)
Severe related	3 (<1%)	3	2 (1%)	2	1 (<1%)	1	1·003 (0·992-1·014)
Fever							
Any	24 (4%)	27	9 (3%)	9	15 (5%)	18	0·980 (0·951-1·011)
≥ 38.0°C	19 (3%)	21	6 (2%)	6	13 (4%)	15	0·977 (0·951-1·004)
≥ 39.0°C	5 (1%)	5	3 (1%)	3	2 (1%)	2	1·003 (0·989-1·017)
≥ 40.0°C	1 (<1%)	1	0	0	1 (<1%)	1	0·997 (0·991-1·003)
Any related	21 (3%)	23	9 (3%)	9	12 (4%)	14	0·990 (0·962-1·019)
≥ 38.0°C related	16 (3%)	18	6 (2%)	6	10 (3%)	12	0·987 (0·963-1·012)
≥ 39.0°C related	4 (1%)	4	3 (1%)	3	1 (<1%)	1	1·006 (0·994-1·019)
≥ 40.0°C related	1 (<1%)	1	0	0	1 (<1%)	1	0·997 (0·991-1·003)
Unsolicited adverse events[#]							
Any unsolicited adverse event	143 (22%)	226	64 (20%)	94	79 (25%)	132	0·939 (0·864-1·021)
Mild	113 (18%)	162	53 (17%)	71	60 (19%)	91	0·972 (0·904-1·045)
Moderate	44 (7%)	60	17 (5%)	21	27 (8%)	39	0·966 (0·926-1·008)
Severe	3 (<1%)	3	2 (1%)	2	1 (<1%)	1	1·003 (0·992-1·014)
Potentially life-threatening	1 (<1%)	1	0	0	1 (<1%)	1	0·997 (0·991-1·003)
Any related unsolicited adverse event	59 (9%)	73	31 (10%)	38	28 (9%)	35	1·010 (0·961-1·061)
Mild	43 (7%)	52	22 (7%)	26	21 (7%)	26	1·003 (0·962-1·046)
Moderate	16 (3%)	18	8 (3%)	10	8 (3%)	8	1·000 (0·975-1·025)
Severe	3 (<1%)	3	2 (1%)	2	1 (<1%)	1	1·003 (0·992-1·014)
Potentially life-threatening	0	0	0	0	0	0	1·000 (1·000-1·000)
Serious adverse events[§]							
Any reported serious adverse event	47/698 (7%)	64	15/350 (4%)	22	32/348 (9%)	42	-
Serious adverse event related to vaccination	1/698 (<1%)	1	0/350	0	1/348 (<1%)	1	-
SAE Outcome							-
Fatal	3/698 (<1%)	4	1/350 (<1%)	1	2/348 (1%)	3	-
Recovered/ resolved	40/698 (6%)	55	15/350 (4%)	21	25/348 (7%)	34	-
Recovered/ resolved with sequelae	5/698 (1%)	5	0/350	0	5/348 (1%)	5	-

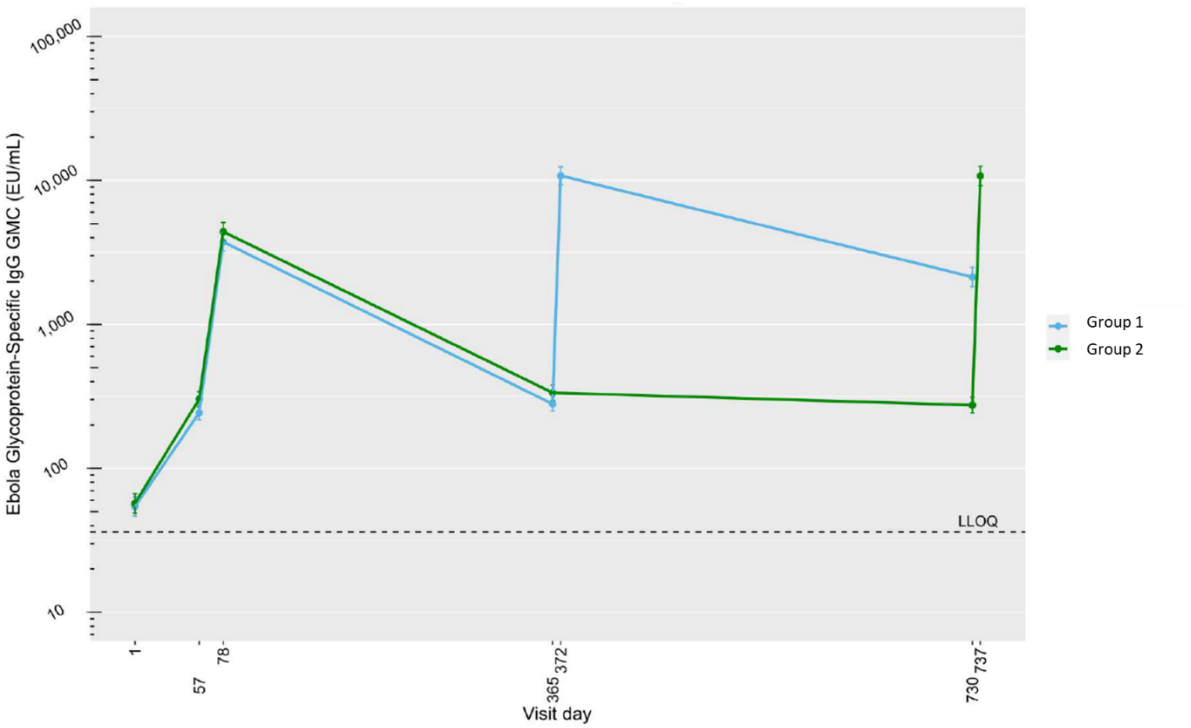
*Some participants had more than one event. RRs were calculated to determine whether there was a difference in the number of participants reporting adverse events between group 1 and group 2. A value of 1 within the confidence interval indicates no significant difference. Both groups received the Ad26.ZEBOV, MVA-BN-Filo primary vaccination regimen, but group 1 participants received an Ad26.ZEBOV booster dose at 1 year after the first dose and group 2 participants received it at 2 years after the first dose. FAS=full analysis set. RR=risk ratio. *All local solicited adverse events were considered related to the investigational product.*

Unsolicited adverse events after the booster vaccination were reported in 143 (22%) of 636 participants (64 [20%] of 319 group 1 participants vs 79 [25%] of 317 group 2 participants; table 2). For 59 (9%) participants (31 [10%] vs 28 [9%]), these were considered related to the investigational product. One (<1%) unsolicited grade 4 adverse event (ie, potentially life threatening), considered unrelated to the investigational product, was reported for a participant in group 2—abdominal pain with onset 1 day after the booster vaccination that resolved after 5 days. This grade 4 unsolicited adverse event was associated with an adverse event of typhoid fever, which was considered and reported as a serious adverse event by the investigator. The most frequent unsolicited adverse events related to the investigational product when classified per System Organ Class were gastrointestinal disorders (diarrhoea and abdominal pain; eight [3%] of 319 participants in group 1, ten [3%] of 317 participants in group 2), nervous system disorders (dizziness and drowsiness; seven [2%] in group 1, seven [2%] in group 2), musculoskeletal and connective tissue disorders (muscle pain or weakness and joint pain; six [2%] in group 1, two [1%] in group 2), and general disorders and administration site conditions (chills and itchiness at the injection site; six [2%] in group 1, one [<1%] in group 2). In a post-hoc analysis, no statistically significant differences were observed in the number of group 1 and group 2 participants reporting solicited and unsolicited adverse events after booster vaccination (table 2).

At baseline, before the first dose of the primary regimen, group 1 participants had a GMC of 53·7 EU/mL (95% CI 46·5–62·1) and group 2 participants of 56·2 EU/mL (48·4–65·2; table 3; figure 3). Ad26.ZEBOV vaccination on day 1 resulted in 204 (60%) of 342 group 1 participants and 231 (68%) of 342 group 2 participants being classified as responders on day 57 (table 3). On day 78, 21 days after MVA-BN-Filo vaccination, 328 (96%) of 342 group 1 participants and 327 (96%) of 341 group 2 participants were classified as responders (table 3). 1 year after the first dose for group 1 and group 2, and before booster vaccination for group 1, the GMC was 305·7 EU/mL (281·5–332·1) overall (279·9 EU/mL [250·6–312·7] for group 1 participants and 334·8 EU/mL [296·0–378·7] for group 2 participants; table 3; figure 3). When compared with the binding antibody GMC against Ebola virus glycoprotein at baseline on day 1 before vaccination, a 5·6-fold increase was observed 1 year later (5·2-

fold increase for group 1 and 6·0-fold increase for group 2; table 3). 2 years after the first dose and before booster vaccination, group 2 participants had a GMC of 274·6 EU/mL (242·1–311·5), which was 4·9 times higher than at baseline on day 1 before vaccination (table 3; figure 3).

Figure 3. GMCs with 95% CIs of Ebola virus-specific binding antibodies for participants of per-protocol set 2.



LLOQ was 36·11 EU/mL. Ad26.ZEBOV vaccination was administered at day 1 and MVA-BN-Filo at day 57 in both groups; Ad26.ZEBOV booster was administered on day 365 in group 1 and on day 730 in group 2. EU=ELISA units. GMC=geometric mean concentration. LLOQ=lower limit of quantification.

Within 7 days after booster vaccination, group 1 and group 2 participants' GMCs were approximately 39 times higher than before the booster vaccination, and both groups had a 98% response rate (table 3). The difference in mean antibody concentrations after booster vaccination between group 1 and group 2 was negligible (Cohen's d –0·065 [95% CI –0·222 to 0·092]). For group 1 participants, 1 year after booster vaccination, GMC of Ebola virus glycoprotein binding antibody was 39·7 times higher than at baseline (day 1) and 7·6 times higher than before booster vaccination (table 3).

Table 3. GMCs of Ebola virus glycoprotein binding antibodies induced by the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen and by an Ad26.ZEBOV booster vaccination after 1 year (group 1) or 2 years (group 2) and the number of responders at each timepoint

	Responders	GMC EU/mL (95%CI)	Fold increase in GMC vs baseline on day 1
Baseline day 1: before Ad26.ZEBOV vaccination*			
Arm 1	166/342 (49%)†	53·7 (46·5-62·1); 342	NA
Arm 2	178/342 (52%)†	56·2 (48·4-65·2); 342	NA
Overall	344/684 (50%)†	54·9 (49·5-60·9); 684	NA
Day 57: before MVA-BN-Filo vaccination *			
Arm 1	204/342 (60%)	248·2 (223·4-275·8); 343	4·6
Arm 2	231/342 (68%)	303·1 (270·5-339·7); 342	5·4
Overall	435/684 (64%)	274·3 (253·7-296·4); 685	5·0
Day 78*			
Arm 1	328/342 (96%)	3,854·6 (3,340·7-4,447·5); 343	71·8
Arm 2	327/341 (96%)	4,505·2 (3,903·8-5,199·3); 341	80·2
Overall	655/683 (96%)	4,166·3 (3,765·5-4,609·8); 684	75·9
Day 365: before Ad26.ZEBOV vaccination to group 1‡			
Arm 1	188/314 (60%)	279·9 (250·6-312·7); 314	5·2
Arm 2	208/305 (68%)§	334·8 (296·0-378·7); 305§	6·0
Overall	396/619 (64%)	305·7 (281·5-332·1); 619	5·6
Day 372: 7 days after booster in group 1‡			
Arm 1	307/314 (98%)	10,781·6 (9,354·4-12,426·4); 314	200·8
Arm 2	NA	NA	NA
Overall	NA	NA	NA
Day 730: before Ad26.ZEBOV vaccination to group 2‡			
Arm 1	269/299 (90%)¶	2,133·1 (1,827·7-2,489·7); 299¶	39·7
Arm 2	185/310 (60%)	274·6 (242·1-311·5); 310	4·9
Overall	NA	NA	NA
Day 737: 7 days after booster in group 2‡			
Arm 1	NA	NA	NA
Arm 2	303 (98%)	10,746·9 (9,208·7-12,542·0); 310	191·2
Overall	NA	NA	NA

*Data are n/N (%) or GMC (95% CI); number of blood samples, unless otherwise specified. One participant in group 2 missed the day 78 visit. EU=ELISA units. GMC=geometric mean concentration. LLOQ=lower limit of quantification. NA=not applicable. *Analyses were conducted in per protocol set 1, which included 343 participants in group 1 and 342 participants in group 2. For one participant in group 1, results from the day 1 sample could not be obtained (the sample exceeded the refrigerator storage stability of 30 days in the analysing laboratory before a result could be obtained), and so the participant could at no timepoint be included in the responder rate calculation. Blood samples collected for the day 57 and day 78 assessments from this participant are included to determine GMCs with 95% CIs, but a protocol deviation meant that this participant was excluded from all assessments at later timepoints. †Corresponds to number of participants with GMCs above the LLOQ at baseline. ‡Analyses were conducted on per protocol set 2, which included 314 participants in group 1 and 310 participants in group 2. §Five group 2 participants missed the year 1 visit but returned for their year 2 booster vaccination and had blood samples taken and were vaccinated per protocol. ¶For 15 group 1 participants vaccinated and with blood samples taken per protocol at year 1, a blood sample could not be collected at the year 2 visit for various reasons.*

At baseline on day 1, binding antibody concentrations above the LLOQ were observed in 344 (50%) of 684 participants (table 3). After the first Ad26.ZEBOV vaccination, participants with antibody concentrations equal to or below the LLOQ at baseline had a steeper increase in antibody GMCs than participants with baseline antibody concentrations above the LLOQ (appendix p 3). Before the second dose (MVA-BN-Filo), participants with antibody concentrations equal to or below the LLOQ at baseline had a numerically lower binding antibody GMC than participants with antibody concentrations above the LLOQ at baseline (appendix p 3). Nevertheless, this difference in antibody concentrations was no longer present 21 days after full vaccination with the heterologous, two-dose vaccine regimen, and a similar peak in antibody response after vaccination with the two-dose regimen was observed in both groups (appendix p 3). Among participants with antibody concentrations equal to or below the LLOQ at baseline, more waning of antibodies was observed 1 year (in both group 1 and group 2) and 2 years (in group 2 but not in group 1 after booster vaccination) after the initial dose. Even so, the humoral immune response 7 days after booster vaccination led to similar GMC values in participants with antibody concentrations below and above LLOQ at baseline on day 1, independent of the timing of the booster dose.

Seven (1%) of 636 participants (two [1%] from 319 in group 1, five [2%] from 317 in group 2) had antibody values below the LLOQ before booster vaccination. Of these, six (86%; two [100%] from group 1, four [80%] from group 2) had a rapid (ie, within 7 days after vaccination) and strong immune memory response (>15 times higher than the LLOQ) after booster vaccination. For one participant in group 2, no antibody response was observed after booster vaccination. However, for this participant no response was observed after either of the Ad26.ZEBOV vaccinations; a response was observed only after MVA-BN-Filo vaccination.

4.6 Discussion

To our knowledge, this study is the largest trial in adults to assess the persistence of binding antibodies after the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen and a booster dose, and the safety of and immune memory response to an Ad26.ZEBOV booster dose administered 1 year or 2 years after the first dose. Overall, the vaccine regimen and booster dose were well tolerated, which corresponds to findings from previous trials assessing the safety of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen, followed by an Ad26.ZEBOV booster vaccination, in a healthy adult population.[4,5] One serious adverse event (ie, fever >40·0°C) was considered related to booster vaccination by the principal investigator in this study. No other serious adverse events were considered related to vaccination.

The adverse event profile after the Ad26.ZEBOV booster in our population of health-care providers was similar to the safety profiles reported after Ad26.ZEBOV vaccination as a first dose of the primary vaccine regimen in adults from several African countries and the UK.[3–7] In these studies, as in our participants, adverse events after Ad26.ZEBOV vaccination were mostly mild to moderate in severity and transient, with injection-site pain the most frequently reported local solicited adverse event.[3–7] Likewise, for systemic adverse events, headache, fatigue, and myalgia were most commonly reported.[3–7] Unfortunately, the adverse events that occurred after the initial Ad26.ZEBOV dose could not be compared with those that occurred after the booster Ad26.ZEBOV dose within this study. Solicited and unsolicited adverse events were collected only after booster vaccination and not after vaccination with the primary vaccine regimen. There were two reasons for this decision. First, many study participants had to travel long distances using modes of transport such as walking, cycling, motorbikes, or dugout canoes to reach the trial site. Therefore, scheduled study visits were limited to a minimum during protocol development. Second, the safety profile of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen had been studied in several phase 1 and 2 trials before the start of this trial and was considered safe.[3–7]

As previously reported, we observed a strong immune response 21 days after the primary vaccination with the heterologous, two-dose vaccine regimen, at which point 652 (95%) of 679 participants—updated in this Article to 655 (96%) of 683 with the inclusion of the analysis from four participants whose samples did not meet acceptance criteria during initial analysis but for whom backup samples were taken on day 1 that could be analysed—could be considered responders.[8] Although this percentage is slightly lower than the 97–100% responder rates observed in previous trials assessing the immunogenicity of the Ad26.ZEBOV, MVA-BN-Filo regimen administered at a 56-day interval,[3–7,24] this slight difference could be due to the high number of participants in our study who already had Ebola virus-specific binding antibodies above the LLOQ at baseline (344 [50%] of 684 participants). As a 2·5-fold increase in GMC of Ebola virus glycoprotein binding antibody from baseline was required for participants with antibodies already above the LLOQ at baseline to be considered as responders (instead of 2·5 times >LLOQ for participants with antibodies ≤LLOQ at baseline), a higher number of participants with detectable antibodies at baseline could have led to a slightly lower percentage being considered responders. Additionally, we found that participants with antibodies above the LLOQ at baseline on day 1 had a smaller fold-increase in binding antibodies at 56 days after the first dose than participants with antibodies equal to or below the LLOQ. However, this difference was no longer observed after the full regimen was administered. Furthermore, although the waning of antibodies after vaccination seemed steeper for the group that did not have detectable antibodies at baseline, the GMC after booster vaccination was similar for both groups, indicating no notable effect on immune memory response.

We observed a decrease in the presence of vaccine-induced antibodies between day 78 and day 365 for group 1, and between day 78 and day 730 for group 2 but with a stabilisation between day 365 and 730 at a GMC similar to that of the day 57 visit. This finding was consistent with studies of the same vaccine regimen that observed a decline between day 78 and 6 months after the first dose (a timepoint not assessed in our trial), after which the circulating antibody concentration stabilised at a concentration similar to day 57 values.[3,25] When assessed, and as observed in our trial in group 2, the stabilisation

persisted up to 2 years after the first dose.[5,25] However, as no human vaccine efficacy data are available for the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen, the antibody concentration associated with protection is not known. For this reason, immunobridging has been used to infer the likelihood of protection in humans from nonhuman primate models.[10,11] Unfortunately, the current immunobridging model does not provide information on how the persistence of the vaccine-induced immune response relates to the durability of protection in humans, nor has a new model been developed to assess the likelihood of booster doses providing protection in humans.[25] Only one approved Ebola vaccine (recombinant vesicular stomatitis virus-based vaccine [rVSV] in which the VSV envelope glycoprotein is replaced with the glycoprotein of ZEBOV [rVSV△G-ZEBOV-GP]) has been able to show 97·5–100% clinical efficacy in humans through ring vaccination in the Democratic Republic of the Congo and Guinea.[26,27] For this vaccine, a seroresponse of 200 EU/mL or higher after vaccination that is at least twice the baseline value has been described as a possible correlate of protection.[28] Because the same FANG ELISA was used to measure the Ebola virus glycoprotein-binding antibody response after vaccination for both the rVSV△G-ZEBOV-GP vaccine and the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen, these results might be extrapolatable to the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen. If this assumption is correct, then at each timepoint the GMC in our trial was above this threshold, including before booster vaccination at the year 1 and year 2 visits. However, although a similar pattern of antibody waning followed by stabilisation has been observed in non-human primates after vaccination with the regimen, an Ebola virus challenge administered 1·6 years after initial vaccination with the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen was fully lethal in nonhuman primates that did not receive a booster dose before the challenge but was fully protective (with minimal morbidity and absence of viraemia) in boosted animals,[25] even when the Ebola virus challenge occurred as soon as 3 days after booster vaccination.[25] Therefore, booster doses might also be indicated in humans before exposure to Ebola virus in case of an outbreak.

Independent of the antibody persistence before the booster vaccination and the timing of the booster, a humoral immune memory response was observed 7 days after Ad26.ZEBOV

booster vaccination in both groups, leading to a 98% responder rate in both group 1 and group 2. Additionally, in all but one of seven participants whose antibody concentrations had declined below the LLOQ before booster vaccination, a rapid (ie, within 7 days) and strong (ie, antibody GMC >15 times higher than LLOQ) immune memory response was observed. This responder rate at 7 days after booster vaccination (98%) was similar to the responder rate reported in two smaller studies of the same vaccine regimen administering a booster dose after 1 year (100%) or after 2 years (96%) in healthy adults.[4,5]

This trial has some limitations. First, considering the time needed to recruit 700 participants, the trial was planned to last approximately 3 years within a 4-year project. For this reason, a blood collection timepoint 1 year after booster vaccination in group 2 was not planned because this would have prolonged the trial by an additional 6 months and not provided enough time to analyse the samples, clean the database, and analyse the data. Therefore, a comparison of the long-term persistence of antibodies after booster vaccination between both study groups was not possible. Second, we did not measure neutralising anti-Ebola virus glycoprotein antibodies. Assessing both binding and neutralising antibodies was not within the financial possibilities of this project in a large cohort of participants. The focus on binding antibodies was supported by non-human primate challenge models that had shown a strong correlation between binding antibody responses and survival.[9]

A great accomplishment was the retention of 643 (92%) of 699 participants in Boende, a remote area of the Democratic Republic of the Congo, over the more than 2·5-year trial duration. In 2014, 69 suspected, probable, or confirmed EVD cases were reported in the vicinity of Boende.[29] By performing this trial and vaccinating approximately 20% of the health-care providers living and working in the Boende health zone who might be exposed to, and therefore become drivers of, EVD in the event of a future outbreak, we aimed to improve readiness for future Ebola outbreaks in this Ebola-endemic area of the Democratic Republic of the Congo. Unfortunately, the trial exhibited a notable disparity in the gender distribution of participants, with men amounting to 77% and women 23% of the study

participants. This imbalance is likely to be attributable to socioeconomic and cultural factors inherent in the local health-care system, as well as the exclusion of pregnant women during enrolment.

In conclusion, the vaccine regimen and booster dose were well tolerated and the primary vaccine regimen led to persisting Ebola virus glycoprotein binding antibodies up to 2 years after the first dose. Additionally, a rapid and similar immune memory response was recalled by an Ad26.ZEBOV booster vaccination at 1 year or 2 years after the first dose of the primary vaccine regimen, illustrating flexibility in booster administration timing. Combined with modelling research that has estimated a considerable decrease in EVD cases, hospitalisations, and deaths when preventive vaccination strategies target a small percentage of health-care providers in Ebola-endemic areas,[13] our data suggest that an Ad26.ZEBOV booster vaccination could be considered for previously vaccinated individuals at risk of Ebola virus infection (eg, health-care providers who are at risk, during emergency situations, or during an outbreak) at least up to 2 years after vaccination with the primary vaccine regimen.

4.7 Declarations

Author contributions. YL performed all analyses included in the article and wrote the article. BIO performed data management and data analysis for the trial at sponsor level and verified analyses in the Article with those in the clinical study report. PK performed data management for the trial at principal investigator level. HM-M was principal investigator of the trial, with PM and JM as co-principal investigators. The University of Antwerp (PVD, JPVG) was sponsor of the trial. YL and GL were project managers at sponsor level. VM was project manager at investigator level. TZM was the study site coordinator. SM and RM were assistant site coordinators. SM was in charge of safety and cold chain and investigational product management. CR, MK, CM were involved in trial discussions at investigational product level. All authors have access to the raw study data if required. The data was queried by BIO, YL, and an external data manager from DFNet (in charge of database build

and maintenance). The data was verified by monitors from an external clinical research organization. All authors reviewed and contributed to the final manuscript. All authors were responsible for the decision to submit the manuscript for publication.

Declaration of interests. CR, MK and CM were full-time employees of Janssen, Companies of Johnson & Johnson, at the time of the study and report stock or stock options in Janssen, Pharmaceutical Companies of Johnson & Johnson. HM-M was appointed member of the Advisory Board of Janssen Global Services during the COVID-19 pandemic. All other authors declare that they no competing interests.

Data sharing. Individual, de-identified participants' data and a data dictionary will be made available to others in the scientific community upon request after publication of this study. Standard criteria for making data available for valid research projects will be used following application by suitably qualified researchers and upon presentation of a defined analysis plan. For data access requests, please contact cev@uantwerpen.be. The clinical study protocol has been published.

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4.9 Supplementary material

4.9.1 EBL2007 study group

List of contributors to the study over the four-year study duration period (2018-2022):

Local study team in Boende

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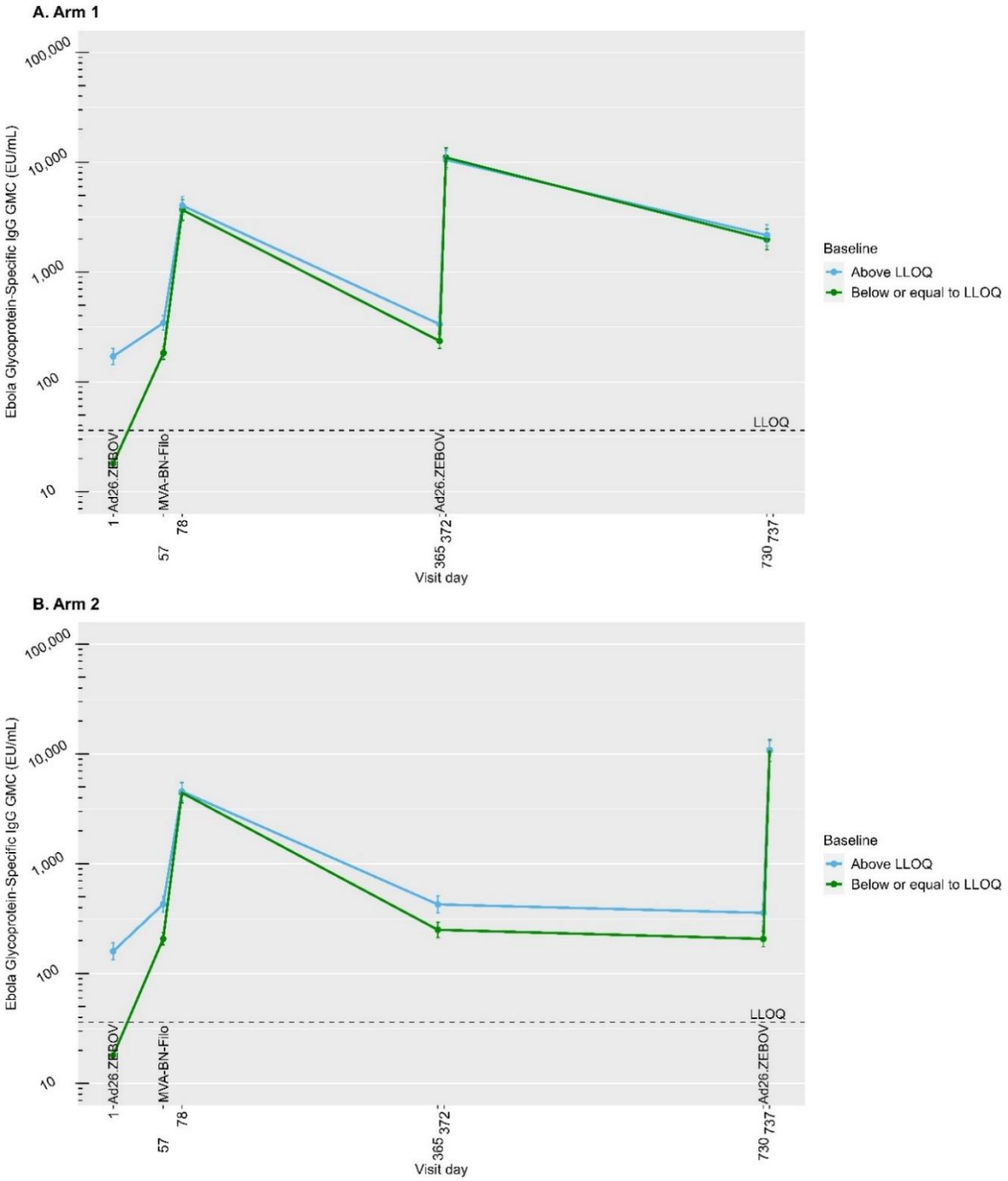
4.9.2 Supplementary Figures

Supplementary Figure 1. Geometric mean concentration among both study arms according to the baseline antibody detectability



Binding antibody geometric mean concentrations (GMC) for participants with a baseline GMC below or equal to the lower limit of quantification (LLOQ = 36.11 ELISA Units/mL) in green versus for participants with a baseline GMC above the LLOQ in blue among (A) Arm 1 participants and (B) Arm 2 participants.

4.9.3 Supplementary tables

Supplementary Table 1. Overview of serious adverse events.

SAE nr.	Timing	System Organ Class (SOC)	MeDra Preferred Term	Toxicity grade	Relatedness	Outcome	Arm
1	Vaccine regimen	Congenital, familial and genetic disorders	Hydrocele	Moderate	Not related to IP	Recovered/ resolved	Arm 2
2	Vaccine regimen	Gastrointestinal disorders	Abdominal adhesions	Severe	Not related to IP	Recovered/ resolved	Arm 2
3	Vaccine regimen	Gastrointestinal disorders	Inguinal hernia	Moderate	Not related to IP	Recovered/ resolved	Arm 2
4	Vaccine regimen	Gastrointestinal disorders	Inguinal hernia	Moderate	Not related to IP	Recovered/ resolved	Arm 2
5	Vaccine regimen	Gastrointestinal disorders	Dyspepsia	Mild	Not related to IP	Recovered/ resolved	Arm 1
6	Vaccine regimen	Gastrointestinal disorders	Enteritis	Moderate	Not related to IP	Recovered/ resolved	Arm 2
7	Vaccine regimen	Gastrointestinal disorders	Inguinal hernia	Severe	Not related to IP	Recovered/ resolved	Arm 2
8	Vaccine regimen	Gastrointestinal disorders	Abdominal strangulated hernia	Moderate	Not related to IP	Recovered/ resolved	Arm 1
9	Vaccine regimen	General disorders and administration site conditions	Asthenia	Severe	Not related to IP	Recovered/ resolved	Arm 2
10	Vaccine regimen	General disorders and administration site conditions	Pyrexia	Mild	Not related to IP	Recovered/ resolved	Arm 2
11	Vaccine regimen	Infections and infestations	Malaria	Severe	Not related to IP	Recovered/ resolved	Arm 1
12	Vaccine regimen	Infections and infestations	Typhoid fever	Moderate	Not related to IP	Recovered/ resolved	Arm 1
13	Vaccine regimen	Infections and infestations	Malaria	Moderate	Not related to IP	Recovered/ resolved	Arm 1
14	Vaccine regimen	Infections and infestations	Malaria	Severe	Not related to IP	Recovered/ resolved	Arm 2
15	Vaccine regimen	Infections and infestations	Typhoid fever	Severe	Not related to IP	Recovered/ resolved	Arm 2
16	Vaccine regimen	Infections and infestations	Dermo-hypodermitis	Severe	Not related to IP	Fatal	Arm 2
17	Vaccine regimen	Infections and infestations	Malaria	Severe	Not related to IP	Recovered/ resolved	Arm 1
18	Vaccine regimen	Infections and infestations	Malaria	Severe	Not related to IP	Recovered/ resolved	Arm 2
19	Vaccine regimen	Infections and infestations	Typhoid fever	Moderate	Not related to IP	Recovered/ resolved	Arm 2
20	Vaccine regimen	Infections and infestations	HIV infection	Severe	Not related to IP	Fatal	Arm 1
21	Vaccine regimen	Infections and infestations	Malaria	Severe	Not related to IP	Recovered/ resolved	Arm 2
22	Vaccine regimen	Infections and infestations	Malaria	Severe	Not related to IP	Recovered/ resolved	Arm 1
23	Vaccine regimen	Infections and infestations	Pneumonia	Moderate	Not related to IP	Recovered/ resolved	Arm 1

24	Vaccine regimen	Injury, poisoning and procedural complications	Lower limb fracture	Moderate	Not related to IP	Recovered/ resolved with sequelae	Arm 2
25	Vaccine regimen	Injury, poisoning and procedural complications	Wound haemorrhage	Moderate	Not related to IP	Recovered/ resolved	Arm 2
26	Vaccine regimen	Metabolism and nutrition disorders	Dehydration	Severe	Not related to IP	Recovered/ resolved	Arm 2
27	Vaccine regimen	Metabolism and nutrition disorders	Dehydration	Moderate	Not related to IP	Recovered/ resolved	Arm 1
28	Vaccine regimen	Metabolism and nutrition disorders	Dehydration	Severe	Not related to IP	Recovered/ resolved	Arm 2
29	Vaccine regimen	Neoplasms benign, malignant and unspecified (incl cysts and polyps)	Uterine leiomyoma	Moderate	Not related to IP	Recovered/ resolved	Arm 2
30	Vaccine regimen	Nervous system disorders	Cerebrovascular accident	Severe	Not related to IP	Recovered/ resolved with sequelae	Arm 2
31	Vaccine regimen	Nervous system disorders	Cerebrovascular accident	Severe	Not related to IP	Recovered/ resolved with sequelae	Arm 2
32	Vaccine regimen	Pregnancy, puerperium and perinatal conditions	Foetal distress syndrome	Severe	Not related to IP	Recovered/ resolved	Arm 1
33	Vaccine regimen	Pregnancy, puerperium and perinatal conditions	Abortion spontaneous	Moderate	Not related to IP	Recovered/ resolved	Arm 1
34	Vaccine regimen	Pregnancy, puerperium and perinatal conditions	Placenta praevia haemorrhage	Severe	Not related to IP	Recovered/ resolved	Arm 1
35	Vaccine regimen	Pregnancy, puerperium and perinatal conditions	Foetal distress syndrome	Severe	Not related to IP	Recovered/ resolved	Arm 1
36	Vaccine regimen	Pregnancy, puerperium and perinatal conditions	Abortion spontaneous	Moderate	Not related to IP	Recovered/ resolved	Arm 2
37	Vaccine regimen	Renal and urinary disorders	Ureterolithiasis	Severe	Not related to IP	Fatal*	Arm 2
38	Vaccine regimen	Renal and urinary disorders	Calculus bladder	Severe	Not related to IP	Fatal*	Arm 2
39	Vaccine regimen	Renal and urinary disorders	Urinary retention	Moderate	Not related to IP	Recovered/ resolved	Arm 2
40	Vaccine regimen	Renal and urinary disorders	Calculus bladder	Severe	Not related to IP	Recovered/ resolved	Arm 1

41	Vaccine regimen	Reproductive system and breast disorders	Ovarian cyst	Moderate	Not related to IP	Recovered/ resolved	Arm 2
42	Vaccine regimen	Skin and subcutaneous tissue disorders	Skin ulcer	Severe	Not related to IP	Recovered/ resolved	Arm 2
43	After booster dose	Gastrointestinal disorders	Inguinal hernia	Moderate	Not related to IP	Recovered/ resolved	Arm 2
44	After booster dose	Gastrointestinal disorders	Umbilical hernia	Severe	Not related to IP	Recovered/ resolved	Arm 1
45	After booster dose	Gastrointestinal disorders	Inguinal hernia	Moderate	Not related to IP	Recovered/ resolved	Arm 2
46	After booster dose	Gastrointestinal disorders	Inguinal hernia	Moderate	Not related to IP	Recovered/ resolved	Arm 2
47	After booster dose	Gastrointestinal disorders	Strangulated umbilical hernia	Severe	Not related to IP	Recovered/ resolved	Arm 1
48	After booster dose	Gastrointestinal disorders	Inguinal hernia	Moderate	Not related to IP	Recovered/ resolved	Arm 2
49	After booster dose	General disorders and administration site conditions	Pyrexia	Severe	Related to IP	Recovered/ resolved	Arm 2
50	After booster dose	Infections and infestations	Typhoid fever	Moderate	Not related to IP	Recovered/ resolved	Arm 2
51	After booster dose	Infections and infestations	Appendicitis	Moderate	Not related to IP	Recovered/ resolved	Arm 2
52	After booster dose	Infections and infestations	Postoperative wound infection	Moderate	Not related to IP	Recovered/ resolved	Arm 2
53	After booster dose	Infections and infestations	Appendicitis	Moderate	Not related to IP	Recovered/ resolved	Arm 2
54	After booster dose	Infections and infestations	Appendicitis	Moderate	Not related to IP	Recovered/ resolved	Arm 2
55	After booster dose	Infections and infestations	Typhoid fever	Moderate	Not related to IP	Recovered/ resolved	Arm 2
56	After booster dose	Infections and infestations	Appendicitis	Moderate	Not related to IP	Recovered/ resolved	Arm 2
57	After booster dose	Infections and infestations	Appendicitis	Severe	Not related to IP	Recovered/ resolved	Arm 1
58	After booster dose	Injury, poisoning and procedural complications	Clavicle fracture	Moderate	Not related to IP	Recovered/ resolved with sequelae	Arm 2

59	After booster dose	Injury, poisoning and procedural complications	Head injury	Moderate	Not related to IP	Recovered/ resolved	Arm 1
60	After booster dose	Injury, poisoning and procedural complications	Uterine rupture	Potentially life threatening	Not related to IP	Recovered/ resolved	Arm 1
61	After booster dose	Nervous system disorders	Ischaemic stroke	Moderate	Not related to IP	Recovered/ resolved with sequelae	Arm 2
62	After booster dose	Pregnancy, puerperium and perinatal conditions	Stillbirth	Severe	Not related to IP	Recovered/ resolved	Arm 1
63	After booster dose	Renal and urinary disorders	Urinary retention	Severe	Not related to IP	Recovered/ resolved	Arm 1
64	After booster dose	Renal and urinary disorders	Calculus bladder	Moderate	Not related to IP	Recovered/ resolved	Arm 2

*IP = investigational product; * These are two events reported simultaneously for one participant, leading to a fatal outcome in the participant.*

Part III – Research challenges in LMICs

Chapter 5 Setting-up the Ebola vaccine trial

Setting-up an Ebola vaccine trial in a remote area of the Democratic Republic of the Congo: Challenges, mitigations, and lessons learned

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5.1 Abstract

Since the largest Ebola outbreak in West Africa (2013–2016) highlighted the potential threat of the Ebola virus to the world, several vaccines have been under development by different pharmaceutical companies. To obtain vaccine licensure, vaccine trials assessing the safety, immunogenicity and efficacy of new vaccines among different populations (e.g. different in age, gender, race, and ethnicity) play a crucial role. However, while this deadly disease mainly affects Central and West Africa, clinical trial regulations are becoming increasingly complex and consequently more expensive, influencing the affected low- and middle-income countries (LMICs) in performing high quality clinical trials. Consequently, the completion of such trials in LMICs takes more time and vaccines and drugs take longer to be licensed. To overcome some of the obstacles faced, the EBOVAC3 consortium, funded by the European Union's Innovative Medicines Initiative and the Coalition for Epidemic Preparedness Innovations, enabled high quality vaccine trials in Central and West Africa through extensive North-South collaborations. In this article, the encountered challenges, mitigations, recommendations and lessons learned from setting-up an Ebola vaccine trial in a remote area of the Democratic Republic of Congo are presented. These challenges are grouped into eight categories: (1) Regulatory, political and ethical, (2) Trial documents, (3) International collaborations, (4) Local trial staff, (5) Community engagement and sensitization, (6) Logistics, (7) Remoteness and climate conditions, (8) Financial. By sharing the encountered challenges, implemented mitigations and lessons learned for each of these categories, we hope to prepare and inform other researchers aspiring a well-functioning clinical trial unit in similar remote settings in LMICs. ClinicalTrials.gov identifier: NCT04186000.

Key words. Ebola Virus Disease, Endemic, Health care providers, Democratic Republic of the Congo, Lessons learned, Challenges, Mitigations, Vaccine trial, Experiences, Past activities

5.2 Background

Despite major health care improvements in the past decades, the global burden of disease remains high [1] with sub-Saharan Africa continuously most affected by premature mortality and morbidity (Fig. 1) [2]. While non-communicable diseases are increasing worldwide [1], the recent COVID-19 pandemic has proven once more that infectious diseases remain a serious threat to the world and that vaccine development is essential to prevent them and/or limit their burden. Vaccine trials, assessing the safety and efficacy of new vaccines, play a crucial role in obtaining vaccine licensure [3]. However, despite the highest burden of diseases (Fig. 1) [2], a minority of clinical trials are performed in low- and middle-income countries (LMICs) (Fig. 2) [4].

Ebola Virus Disease (EVD) is responsible for outbreaks characterized by deadly hemorrhagic fevers that have primarily occurred in Central and West Africa [5]. Depending on the quality and access of care, available resources, outbreak management, and virulence of the circulating Ebolavirus, the case-fatality rate can range from 36 to 90% [5,6]. The natural reservoir host(s) has (have) yet to be identified, which implies that the virus may continue to resurface anywhere and unexpectedly throughout Sub-Saharan Africa [6]. Furthermore, recent outbreaks in Guinea (February-June 2021) and the Democratic Republic of the Congo (DRC) (February-May 2021 and October-December 2021) have shown that a resurgence of a persistent (latent) infection in a survivor is possible up to several years after contracting the disease [7–9]. Since the discovery of the Ebola virus in 1976 in Zaire (now known as DRC), the country has recorded the highest number of all EVD outbreaks, [10]. However, only 48% and less than 10% of the 107 clinical trials targeting EVD, registered on ClinicalTrials.gov on December 13th 2021, take place in Africa and the DRC, respectively [11].

Figure 1. Age-standardized DALY (Disability-Adjusted Life Year) rates per 100,000 individuals from all causes [2]. DALYs measure the total burden of disease – both from years.

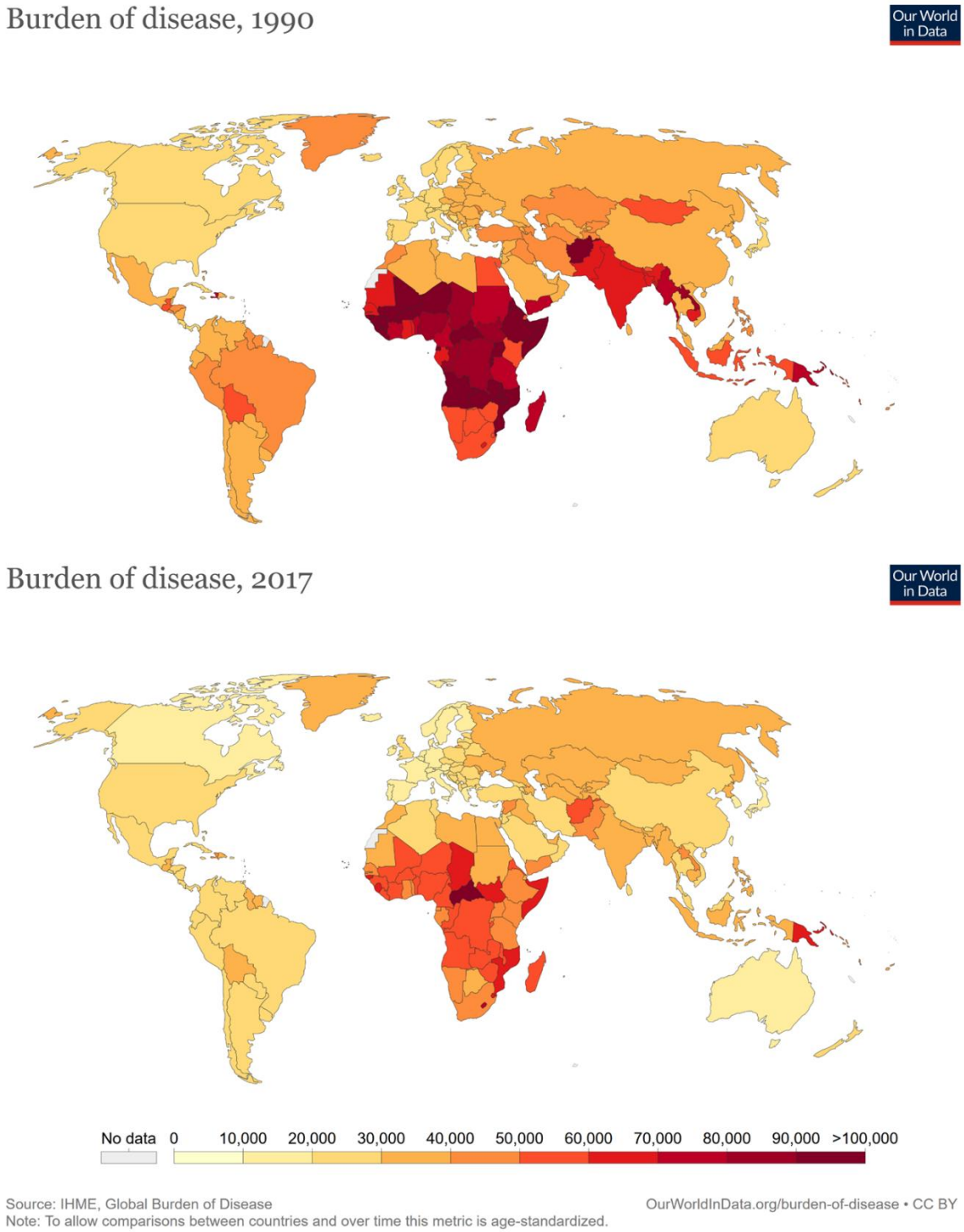
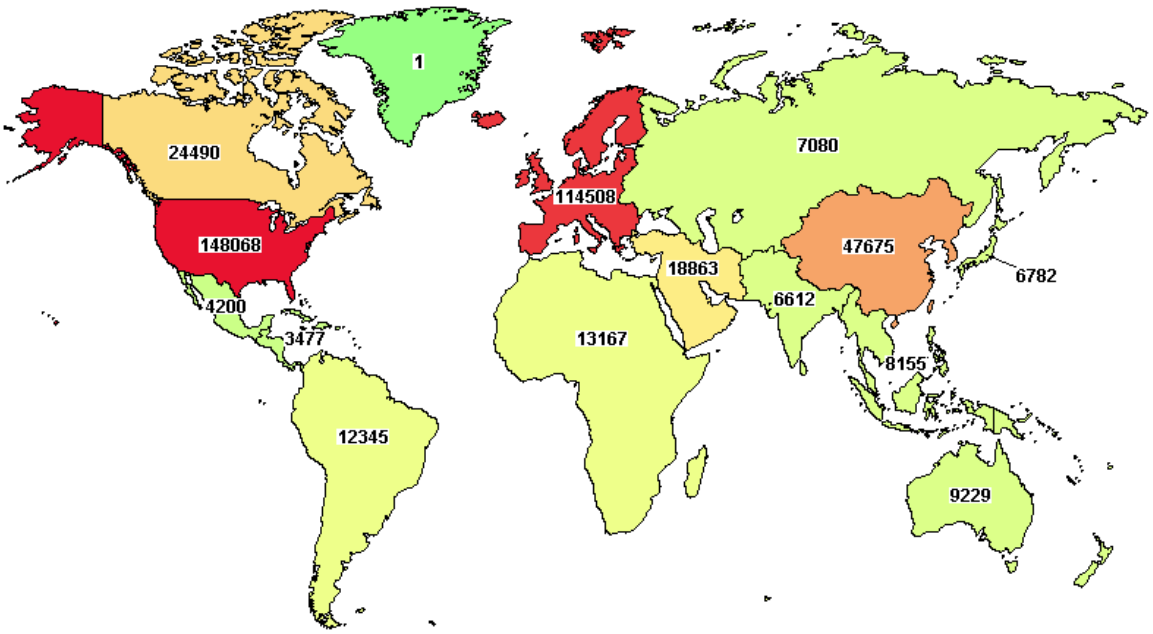


Figure 2. Number of trials per continent registered on www.ClinicalTrials.gov (accessed on December 13th, 2021) [4].



To obtain licensure of a vaccine or drug, clinical trial data need to be collected among different populations (e.g. different in age, gender, race, ethnicity) to ensure that the product is safe and efficacious in all target populations [12]. However, while efficacy trials need to be conducted in countries were exposure to the infectious disease is sufficient, clinical trial regulations are becoming increasingly complex and demanding – and consequently more expensive – limiting the possibilities for LMICs to perform high quality clinical trials [13]. Next to regulatory and financial barriers, a lack of human capacity and logistical and operational barriers are main constraints to conduct research in LMICs [14]. As a consequence, the completion of clinical trials in LMICs takes more time and vaccines and drugs take longer to be licensed, which directly impacts the possibility to reduce high morbidity and mortality rates in poor populations most affected by infectious diseases [13,15]. However, while several barriers to conduct clinical trials in LMICs have been identified, former experiences suggest that these can be overcome through international collaboration whereby partners from high income countries (HICs) can support partners in LMICs during research conduct [14].

Therefore, within the framework of the EBOVAC3 consortium [16], and funded by the European Union's Innovative Medicines Initiative (EU-IMI) and the Coalition for Epidemic Preparedness Innovations (CEPI), a randomized, open-label, monocentric, Ebola vaccine trial (ClinicalTrials.gov identifier: NCT04186000) was set up in Boende, a remote Ebola endemic area of the DRC. In an attempt to prepare this area for future outbreaks, this vaccine trial specifically targeted health care providers (HCP) and frontliners as participants, as they are not only more at risk of contracting infectious diseases but may also contribute to the spread of these diseases [17–20]. In total 700 participants were planned to be recruited and vaccinated with a two-dose heterologous vaccine regimen (Ad26.ZEBOV (Zabdeno®) as the first dose and MVA-BN-Filo (Mvabea®) as the second dose, at a 56-day interval) followed by a booster Ad26.ZEBOV (Zabdeno®) dose, administered either one or two years (randomization 1:1) after the initial dose [21]. This trial was established through an international partnership between the University of Antwerp (UAntwerp) as sponsor and the University of Kinshasa (UNIKIN) as principal investigator (PI). Further details of the trial design can be found in Larivière et al. 2021 [21].

In this article, we present the encountered challenges, mitigations, recommendations and lessons learned from setting-up an Ebola vaccine trial in a remote area of the DRC. We believe these challenges and lessons learned are useful for other researchers planning to establish a well-functioning clinical trial unit in other remote settings in the DRC or anywhere in sub-Saharan Africa.

5.3 Challenges

Table 1 presents the challenges encountered while setting up the Ebola vaccine trial, including how they were mitigated and which lessons were learned. The challenges are grouped into eight categories. The mitigations presented in this table, can be considered as recommendations when establishing a vaccine trial in a remote area with limited access to care in sub-Saharan Africa or elsewhere.

Table 1. Encountered challenges, mitigations and lessons learned while planning and setting-up an Ebola vaccine trial in Boende, Tshuapa province, DRC.

#	Challenges	Mitigations	Lessons learned
1	**Regulatory, political and ethical**		
	Lack of electoral stability	Pause vaccine trial initiation until instabilities are resolved.	Electoral instability and political hesitancy can delay or pause trial initiation. Ensure a good knowledge and permanent contact with local and national authorities to mitigate delays.
	Political hesitancy towards trial approval	Ensure advocacy and frequent diplomatic interventions of the PI and local trial staff to regain confidence in the study vaccine among the necessary authorities.	
	The regulatory capacity of the national regulatory authority (DPM) and ethics committee are highly impacted by limited available resources (e.g. communication channels, technology, human capacity, etc.)	Foresee good contacts with a focal person at the central level within the regulatory authority (DPM) and the ethics committee to ensure a swift follow-up and approval of submitted documents.	Regular contact (through phone calls and visits) and good relations with key persons of the national regulatory authority (DPM) and the ethics committee are crucial to obtain clear guidance and quick responses to submitted documents.
2	**Trial documents**		
	Protocol changes in study population and location	Ensure enough time for protocol writing and adapting.	Foresee enough time for protocol writing. Last minute changes at request from for example the pharmaceutical company to change the study population can impact the foreseen timeline.
	Lack of Standard Operating Procedures (SOPs) and plans available at the appointed site	Ensure good collaborations between stakeholders of the project team to develop all required documents in a timely manner. Ensure good management and oversight of the documents that need to be developed. Include partners in the project with expertise and available SOPs and plans that can easily be adapted according to local practice.	Foresee enough time to develop SOPs and plans when planning to initiate a vaccine trial in a new clinical trial unit. Foresee good communication and development strategies between partners. By dividing the work among stakeholders of the project team, the development will be faster. Foresee oversight of the developed documents.
	Language barriers	Ensure clear documentation for participants translated into official and local languages, as required.	Foresee essential documents that need to be completed by participants in the country's official language (e.g. French).

#	Challenges	Mitigations	Lessons learned
		Ensure staff capable of explaining essential documents in both official and local languages.	Translate essential documents into local language (e.g. Lingala) if required. Foresee staff to clearly explain documents in the official of local language, as chosen by the participant. In case of illiterate participant, ensure possibility to perform informed consent procedure via an impartial witness.
	Site readiness assessment	Ensure a site initiation checklist is available when setting up a new clinical trial site.	To make sure all necessary documents, material, etc. are in place prior to commencing a vaccine trial, a site readiness checklist can help identify any existing issues.
	Quality control plan	Foresee a quality control plan with regular quality controls through the use of a checklist to ensure high quality of data.	By considering all essential data documents prior to commencing the trial, it is possible to start the trial with the collection of high quality data.
3	**International collaborations**		
	Lack of clear role distinction between different stakeholders of the consortium	Ensure a joint decision to the relevant status for each stakeholder within the consortium.	There is a necessity of clear and correct identification of the status of partners, their planned contributions and responsibilities within the consortium prior to start of the project.
	Lack of clear role distinction between different stakeholders of the project team	Ensure clear roles and responsibilities among all stakeholders within the project.	Agreements between multiple international partners can be time consuming but are crucial for a smooth collaboration and implementation of a vaccine trial. Institutions of higher education in the North can strengthen their connection with the South, possibly through the alumni. These connections can enhance (vaccine) research projects of which implementation requires North-South partnerships.

#	Challenges	Mitigations	Lessons learned
	Time zone differences	Ensure willingness of teams to work before 9AM or after 5PM as a consequence of different time zones among involved stakeholders.	Keep in mind different time zones when assembling the different stakeholders for the project. Ensure willingness for flexible working hours.
4	**Local trial staff**		
	Limited vaccine trial experience of local trial staff	Recruit local trial staff with experience from a previous vaccine trial in the area. Reinforce the local trial staff with staff from Kinshasa (UNIKIN), more experienced in clinical research. Ensure a robust training plan. Perform dry runs of trial activities and study visits.	Foresee time and effort to thoroughly train local trial staff to ensure confidence and readiness of staff before trial initiation. Perform dry runs to assess the feasibility and acceptability of trial activities to help eliminate difficulties before actual trial initiation.
	Very limited electronic data collection experience	Thoroughly train staff on electronic data collection. Organize dry run using tablets for electronic data collection to assess feasibility and acceptability. As a back-up, prepare paper data collection and train staff on data collection using paper case report forms. Make sure data entry specialist are in place for transfer of paper case report forms into electronic case report forms.	Ensure back up approaches are in place, should an initial approach not be feasible or accepted to limit and/or avoid delays in trial initiation.
	Less available HCP in health care facilities by recruiting them as trial staff and as study population	Develop mitigation plan to ensure sufficient medical support in the province during active trial activities. Present the mitigation plan to local health authorities for approval.	When selecting a study population, ensure that this does not have serious consequences on local activities. If there is a risk of impacted local activities, it is important to develop a mitigation plan and present this to the local authorities, prior to trial initiation.

#	Challenges	Mitigations	Lessons learned
5	**Community engagement and sensitization**		
	Fear, mistrust and preconceived notions in the community	Inform and involve the local political authorities of the trial conduct. Involve medical anthropologists to discuss with representative of civil society, non-governmental organizations and health care providers of Boende on the study design and the rationale of the Ebola vaccine trial in the study area. Perform meetings and workshops with (potential) trial participants to ensure acceptance of the trial and the Ebola vaccines in the community. Develop a recruitment plan on how potential participants will be informed about the trial.	Referring to local authorities and civil society including local non-governmental organizations, medical doctors and opinion leaders of the area should be considered as a key point in enhancing community engagement for a vaccine trial. Ensure permanent communication in formal and informal settings with opinion leaders to facilitate the implementation of the trial. Involve the community and trial participants in discussions while setting up the vaccine trial to minimize or avoid trial initiation delays due to fear, mistrust of preconceived notions in the community. Training local radio journalists on community engagement messages is an important aspect of avoiding the spread of misinformation that can turn away potential volunteers from a trial.
	Limited robust participant identification and retention tools	Iris scanning and mobile messaging as a new innovative technology.	Using technology that does not work can delay or pause the progress of a trial. Perform a pilot study beforehand to assess feasibility and acceptability of new innovative technology to avoid delays or other issues.
	Lack of cell phones and cell reception 10km outside Boende	Work with community health workers to reach the participants living outside of this 10km radius around Boende.	Working in a remote area can hinder initial planned retention tactics such as the use of visit reminders via cell phone messaging. Ensure community engagement and work with community health workers to reach a high participant retention.
6	**Logistics**		
	Lack of basic infrastructure at study site	Upgrade trial site infrastructure.	Foresee enough time to make a new trial site operational. Infrastructural modifications can take time in a remote area.
	Lack of electricity	Foresee generators (including fuel) and solar panels.	

#	Challenges	Mitigations	Lessons learned
	Lack of water access	Construct a bore hole, foresee water tanks and plumbing.	If possible, foresee durable and sustainable material (e.g. solar energy). Assess the needs of the trial site to select the best options for trial infrastructure upgrades.
	Lack of internet access	Install a very small aperture terminal (VSAT). Foresee a lightening conductor to prevent damage from lightning.	
	Lack of a well-appointed laboratory for a vaccine trial	Build a laboratory to perform trial activities.	
	Lack of cold chain	Foresee functional cold chain with continuous temperature monitoring.	Material needed for trial activities can be unavailable in the country of the trial activities. Ensure good relations and clear communication between North-South partners (e.g. sponsor-PI) on who will buy which materials to limit delays in trial initiation.
	Lack of locally available study material	Make a list of all material required for study activities and reach an agreement between PI and sponsor as to who will buy which material on the list. Clear communication is required as to the availability of the material in each country.	
	Limited expertise in the area for the setup and maintenance of the trial equipment	Foresee a maintenance contract with companies in Kinshasa. Foresee the maintenance at each start of activities on the site by the key persons from Kinshasa. Foresee a back-up generator for electricity/contingencies. Foresee a back up refrigerator/freezer for vaccine and sample storage.	Local expertise on trial material may not be available in very remote research settings. Ensure an agreement with a company within the trial country to help mitigate delays in trial initiation/activities, should material break down.
	Sub-optimal healthcare infrastructure	Foresee a study pharmacy that can cover the basic health needs of participants.	A study pharmacy can indicate a temporary improvement or availability of healthcare for trial participants.
7	**Remoteness and climate conditions**		
	Lack of good-quality fuel for generators in trial site area	Buy fuel in Mbandaka and transport it by boat to the site (5 days travel).	Material needed for trial activities can be unavailable in the area of the trial. Identify these items before trial initiation and foresee back-up material on site to limit and/or avoid the halting of trial initiation or continuation.
	Lack of frequent flights to the trial site	Charter airplanes to reach the trial site at different time points.	Assess the travel options to and from the potential trial site before choosing a trial location. If no back-up

#	Challenges	Mitigations	Lessons learned
			alternatives are available, this can slow down the trial setup and initiation. Ensure back-up travelling options when the trial site cannot be easily/frequently reached.
	Lack of safe domestic flights to trial site	Charter airplanes in which staff feels more at ease. Charter airplanes and perform mock shipments, including temperature monitoring for the vaccines and serum samples. Ship the vaccines and serum samples in two separate shipments.	Assess staff fears and how to mitigate these before starting a vaccine trial to ensure good team spirit and motivation. Perform mock shipments of vaccines and samples (that need to remain below a certain temperature) to identify issues that must be mitigated before the actual shipments occur.
	Impact of the high humidity	Foresee protection for material affected by high humidity (e.g. a filing cabinet to protect the paper source documents and dehumidifiers and air conditioners for the cold chain room).	Climate conditions can impact trial activities and storage conditions. Take note of the weather conditions and ensure a mitigation plan while setting up the trial are crucial.
	Lack of internet connection due to tropical rain storms	Foresee a local server that can function without internet connection to ensure continued trial activities are possible.	
	Impact of tropical storms	Foresee potential delays due to tropical storms: impact on travel schedules, shipments of material, etc. Foresee lightning conductor to avoid damage to the dishes and antenna by lightening.	
	Lack of public transport in study area	Rent cars or motor bikes to transport staff locally.	Perform a feasibility assessment of a trial location. Make notes of all available and unavailable infrastructures and workforces in the area. Seek alternatives for missing infrastructures or workforces in a trial area that are crucial for a smooth initiation and continuation of a trial.
	Lack of banks in study area	Ensure enough cash for the continuous trial activities. Foresee alternative money transfers via trusted money wiring systems for smaller amounts.	
	Lack of available workforce for trial site renovations in Boende	Hire workforce in Mbandaka to do renovation works (e.g. borehole construction) in Boende	

#	Challenges	Mitigations	Lessons learned
8	**Financial**		
	Set adequate compensation for participants' costs during trial activities	Foresee a budget for transport, food and lodging for participants having to make long journeys (e.g. 6 hour round trip) to come to the trial site for a scheduled study visit, as well as for those that reside nearer to the site.	Participants need to be adequately refunded for their transport, lodging, food, and time, but this amount must not be coercive to participate in the trial. Determining the amounts for compensation requires careful discussions with local authorities and potential participant groups leaders.
	Large distance between PI staff based in Kinshasa and the trial site	Establish an administrative team at the trial site. Ensure close collaboration between administrators at PI level based in Kinshasa and administrators at the trial site.	Ensure good communication and agreements between different project partners prior to the start of a vaccine trial that clearly identify the status of project partners within the consortium (cf. 3. International collaborations) and consequently every institution's responsibilities related to the project funds and the reporting thereof, as well as the preconditions for availability of funding.
	Funder's administrative and financial regulations posed challenges for implementation in LMIC	Ensure close administrative cooperation between the sponsor and the PI. Ensure sufficient and skilled human capacity for financial administration.	
	Limited experience of administrative staff with funder's regulations	Foresee training of all administrative staff at PI level and at the trial site on financial regulations and reporting, put a timely reporting schedule in place and follow up closely to adhere to funder's regulations.	
	Delayed availability of project funds at PI level and incapacity to pre-finance project related costs	Set up high-level advocacy meeting at sponsor level to arrange transfer schedules. Start procurement of services, goods, and materials at sponsor level.	
	Budget changes	Ensure budget flexibility in the initial budget planning.	Setting up a vaccine trial brings unforeseen challenges along. Many of these challenges come with a change in price tag. Anticipate funding for possible shifts in infrastructure and trial set up in the original budget planning. Ensure that risk mitigation is part of the initial budget planning.

5.3.1 Regulatory, political and ethical

The political situation in the DRC at the initiation of the project was very uncertain as the outgoing president was out-of-term and the elections were delayed. Political unrest after the elections could potentially destabilize the country [22,23]. Thus, uncertainty around the ending of the parliamentary and presidential votes in 2018 interfered with the trial's preparatory phase. As a consequence of these uncertainties, agreements and key decisions between the trial's principal actors (i.e. PI and sponsor) were delayed by several months.

Furthermore, the departing government curtailed internet access throughout the country pending voting outcomes. This occurred at the moment the sponsor approved the study and the PI had to commence the submission process to the DRC ethics committee (EC) to obtain ethical clearance. The curtailment of internet access disrupted the PI's submission process to the EC and hindered participation in certain important international preparatory online consortium meetings. Further delays occurred because of an extensive approval process at the level of the EC itself and the national regulatory authority of the DRC (Direction de la Pharmacie et de Medicament, DPM). In an effort to speed this up, the PI frequently liaised with the EC-office to remind them of the standard timeline (15 days) to issue approval letters [24,25]. Delays in obtaining ethical clearance are particularly common in countries with lower clinical research experience, including the DRC [14]. The PI was aware of this potential barrier from the onset and selected the DRC Ministry of Health's National Ethics Committee, which had sufficient expertise and a relatively shorter review turnaround time than other ethics committees in the country. Additionally, it had experience in reviewing and monitoring Ebola drug and vaccine trials conducted during the 9th and 10th Ebola epidemics that occurred in the country (2018–2020) [26].

While the 10th Ebola epidemic was ongoing in the east of the DRC (August 2018-June 2020, Ituri and Nord Kivu provinces), the research team was trying to establish the Ebola vaccine trial in Boende. According to the 2017 Strategic Advisory Group of Experts on immunization (SAGE) recommendations, the registered ERVEBO® vaccine (Merck and Co, Kenilworth, United States) was considered as the priority vaccine to vaccinate individuals at high risk of

contracting Ebola (i.e. contacts and contacts of contacts, health care workers and frontline workers in affected health areas) [27]. Therefore, to interrupt the chain of transmission, the SAGE recommended the use of the ERVEBO® vaccine using a ring vaccination strategy [26]. Yet, in May 2019, these SAGE recommendations were revised and both the Ad5-EBOV vaccine (CanSino-Beijing Institute of Biotechnology, Tianjin, China) and the Zabdeno®and Mvabea® vaccine regimen (Janssen Vaccines & Prevention B.V., Leiden, The Netherlands) were also included as potential vaccines to be administered during outbreaks to individuals with a lower risk of contracting Ebola (e.g. people living in areas surrounding an outbreak) [26,27]. To avoid any confusion on the field, as per his opinion, the Minister of Health of the DRC, by issuance of a decree, banned the use of any other Ebola vaccine candidates besides the ERVEBO® vaccine [28]. This directly impacted the Ebola vaccine trial, located >2000 km away from the area affected by the 10th epidemic that prompted the Health Minister's decree. Due to the decree, neither the EC nor the national regulatory authority were in a position to authorize the Ebola vaccine trial in Boende, which planned to administer the Zabdeno® and Mvabea® regimen. In the meantime, the sponsor and the PI kept working on outstanding study documents. Following the presidential elections and the installation of a new government in September 2019, the PI advocated for the cancellation of the decree. Fortunately, the new Minister of Health indeed quickly repealed the decree, allowing the start of the vaccine trial.

As is often the case in LMICs, the DRC's national regulatory (DPM), currently has limited regulatory capacity and lacks the much needed resources to ensure effective oversight and regulation of clinical trials [29]. There is no official communication channel whereby the regulatory requirements are documented, such as a website that outlines the submission and processing timelines, the required submission documents, and/or official contact options. This led to complications in the application process, forcing the PI to make regular telephone contact with the DPM secretary and frequently visit their office during the preparatory phase of the trial. This close contact with the regulatory authority, intense in human capacity and time investment, ensured that further delays in issuing authorizations

could be prevented. Inadequate follow up could therefore potentially disrupt the deadlines for starting recruitment or importing investigational products by the research team.

5.3.2 Trial documents

Writing the protocol for the Ebola vaccine trial was a lengthy process. To abide by authorization requirements, the study population was changed from HIV-positive participants in Kinshasa to HCP in Boende. As the Boende Health District had previously experienced an Ebola outbreak in 2014 [19], this location was chosen to perform the trial in an attempt to prepare this location for future outbreaks. These changes required the protocol to be rewritten and new trial site feasibility evaluations to be performed. This delayed the setup of the trial by several months. However, Boende was at that time the study site for a Monkeypox vaccine trial [30] and thus it seemed worthwhile to capitalize on their experience in order to guarantee a fluent trial setup and initiation. Nevertheless, very few standard operating procedures (SOPs), clinical trial plans or source documents were still in place during the site feasibility assessment and almost all documents had to be redeveloped.

All documents that would be completed by a participant (e.g. test of understanding, Informed Consent Form, participant diary) were available in French (i.e. official language in the DRC). The majority of these documents were also translated and available in the local language (i.e. Lingala). Further details on informed consent and trial procedures can be found in Larivière et al. (2021) [21].

Before starting enrolment of trial participants, to ensure the site was ready, a final site readiness assessment was performed during a site initiation visit. During this assessment several key aspects were evaluated using site readiness/activation approval checklists. Using these checklists it was determined whether 1) all required regulatory approvals were obtained, 2) all protocol and study procedures were in place, 3) all necessary source documentation was developed, 4) all site facilities were adequate for the conduct of the vaccine trial, 5) back-up power to the trial site was in place, 6) the temperature monitoring

of the cold chain was stable, 7) the required regulatory documents were filed, 8) the PI and local staff were fully and recently trained on Good Clinical Practice (GCP) guidelines and study activities, 9) the Investigational Product accountability was performed, and 10) study supplies were on hand. For this trial, the issues encountered during the site initiation visit were minor and mostly related to missing documents (i.e. signed and dated CVs, practising licences and some study specific training documentation). Any observed deviations were documented in a site initiation visit report, reviewed, and approved by the relevant parties, including the sponsor and the Clinical Research Organization (CRO) and filed in the Investigator Site File and Trial Master File.

To ensure the quality of data collection, a Site Quality Control plan was developed prior to commencing the trial. In this plan, quality control activities (to be conducted during on site study activities) included day-to-day review of data generated from approved protocol procedures/activities conducted at the site. Any member of the quality control team at the site could perform quality control activities. The quality control team was appointed by the PI and delegated appropriately in the delegation log, prior to starting the trial. Quality control checklists were in place for collected data, informed consent forms, laboratory sample collection, processing, storage and transportation and the storage of the investigational product.

5.3.3 International collaborations

Several international collaborations were established during the setup and initiation of the Ebola vaccine trial. The first involved multiple consortium partners funded by the same EU-IMI grant, each performing different Ebola vaccine trials with the Zabdeno® and Mvabea® vaccine regimen in Ebola-endemic settings in West and Central Africa. The second involved the conduct of the vaccine trial itself. This vaccine trial was built on a long-lasting partnership between the PI and the sponsor, who had worked together on previous projects. This collaboration brought together broad expertise on (vaccination) trials as well as local expertise. Consequently, a socio-political network made it possible to establish good relations with the local authorities and targeted study population, which is of utmost

importance to perform a successful vaccine trial. Next to the sponsor and PI, the vaccine manufacturer (Janssen Vaccines & Prevention B.V., Leiden, The Netherlands) provided the vaccines for the trial, as well as support and advice based on their experience in previous Ebola vaccine trials in Western Africa (clinicaltrial.gov identifiers - among others: NCT02509494, NCT03820739, NCT03929757, NCT02564523). A CRO with expertise in LMIC was also involved to further support the sponsor and PI. Finally, to perform the necessary immunogenicity analyses, several laboratories were subcontracted to the sponsor. These laboratories were located in Africa, Europe and the United States of America, requiring flexible working hours from all staff involved to establish contracts, analysis timelines and data sharing agreements.

To establish a clear role distribution between all collaborators, all parties (Sponsor, PI, CRO and vaccine manufacturer) had lengthy online, as well as face-to-face meetings in Belgium, prior to the project start. Main topics discussed were project management, communication, resource management, in-country management, project meetings/teleconferences, submissions and registrations, filing, site activation, monitoring plan and site visits, pharmacovigilance activities and safety management, Investigational Product management, data management, database build and clinical sample management. For each topic it was decided who was responsible, who would provide support and who was accountable. All of the agreements were combined into a project management plan.

5.3.4 Local trial staff

The trial is being led by UNIKIN as PI, spearheaded by four former PhD students of UAntwerp and all of them are senior physicians with clinical research experience. In addition to the roles of (co-)PI and the project coordinator, setting up this trial required hiring approximately 44 local trial staff members with medical, nursing, pharmacy, laboratory technician, logistics, financial and administrative experience for a variety of responsibilities. While there was limited clinical trial experience among the initial local trial staff for the conduct of the trial, the PI identified some candidates with clinical trial experience from a previous Monkeypox vaccine trial in the study area [30] and

strengthened the team with staff from the University teaching hospital of Kinshasa with more experience in clinical trials. All the hired staff attended a two weeks training on the study protocol, study SOPs, GCP, and Human Subjects Protection organized by the sponsor, CRO and PI.

By employing HCP in the vaccine trial (approximately 4 months per year during active trial periods [21]) and by recruiting them as participants, HCP were less available at their original place of work during these active trial periods. To ensure continuation of the local health care, the PI was asked to present a mitigation plan to the local health authorities. Prior to inviting participants to the site, the site coordinator, together with the delegates from the provincial health division and the provincial health inspectorate, ensured continuity via a team on duty in all locations, while others were at the trial site. However, given the limited number of HCP working in rural and remote areas such as Boende, this was not an easy task.

5.3.5 Community engagement and sensitization

Given that the 10th outbreak of EVD (2018–2020) was ongoing when setting up the Ebola vaccine trial in Boende, some rumors claimed that vaccinating people where no Ebola epidemic was ongoing, indicated that the outbreak was used to conduct business [31,32]. This was a challenge that risked spreading mistrust for the trial in Boende. To tackle these rumors, contacts were made by the PI and the sponsor with the relevant national and local political and administrative authorities, as well as international non-governmental organizations (NGO), e.g. in-country representatives of the World Health Organization, Centers for Disease Control and Prevention, and Group Inter Bailleurs Santé (GIBS) composed of all the financial partners of the health sector in the DRC. Through these contacts the research team was able to anticipate what (not) to do, how to avoid the spread of false information that might jeopardize recruitment, how to best raise awareness and involve the right stakeholders in the process. Procedures that were applied consisted of hiring local personnel, performing refurbishment on the hospital wing that hosted the site, etc. Consequently, the local health, political and administrative authorities trusted the

research team, which made it easier for the HCP to participate in the trial and for the population to accept the Ebola trial being conducted in their community.

In order to promote the trial to potential volunteers, the PI team developed a recruitment plan in which it was foreseen to utilize the communication channels (e.g. flyers, radio messages, etc.) normally used throughout the DRC health system. All disseminated key messages were approved by the ethics committee. The main strategies targeted posting announcements in various common areas (e.g. bill boards, meeting rooms, corridors, offices, and rest areas) at the General Referral Hospital (GRH) of Boende (i.e. trial site location) as well as in all other facilities in the health district of Boende targeted by the trial. For this approach the authorization of the management staff of each facility was requested. Additionally, it was also foreseen to broadcast these messages in the form of radio spots. Finally, to attract potential trial participants (HCP and frontliners), a workshop was organized whereby presentations on health related topics were given and a video was shown explaining the Ebola vaccine trial. During this workshop, a team of researchers took the time to answer questions and concerns raised by potential participants.

To further diminish potential rumors, a community engagement strategy was implemented through a team of social scientists from UNIKIN. They trained community health workers as well as the local media to better understand and explain the study to potential volunteers and on how to address rumors in the community. The local media therefore did not play a negative role in disseminating the messages before recruitment began (nor did they afterwards).

To prevent double enrollment and confirm participant identity, it was decided to use iris scanning, an innovative biometric technology, as well as a mobile messaging system to remind participants of upcoming visits. In order to evaluate whether these elements would be accepted and feasible, a pilot study was conducted prior to the start of recruitment, in which a sample of potential trial participants (i.e. HCP) were questioned about the acceptability of the identification tool and the feasibility of the mobile messaging system [33]. Through this pilot study, the team was able to anticipate and prevent potential issues.

For example, while the iris scanning seemed to be generally accepted by the study population, it became clear that the visit reminders via mobile messaging would be impossible in the remote area of the Boende health district, due to the absence of network coverage beyond 10 km around Boende.

5.3.6 Logistics

Boende is located at the heart of the equatorial forest. Factors that impacted the trial implementation were its remoteness, poor or no road networks and the precariousness of existing infrastructure, including a lack of suitable facilities to house the study site. Alongside these issues, there was a lack of electricity, unreliable or inexistent communication (telephone and internet) networks and insufficient basic health facilities and health provision.

To obtain a suitable location for the Ebola vaccine trial, a contract was established between the PI and GRH of Boende. It was agreed that a hospital wing would be rented to house the study and that some of the hospital's medical staff was to be employed part-time for the trial. To strengthen local capacities, it was further agreed that the hospital wing, used for the vaccine trial, would be refurbished prior to the start of the trial. As there was no electricity, water supply, sanitary facilities, nor internet connection on site, these were included in the renovation activities.

To make the site fully operational, material had to be purchased for the conduct of the vaccine trial. Next to laboratory equipment (e.g. biochemistry and hematology analyzers, blood sampling equipment, etc.), a cold chain for the storage of vaccines and serum samples and a study pharmacy for (serious) adverse event management were also required. While purchasing material locally (in the DRC) was always the main goal, not all required material was easily available in the country. Therefore, some of the material purchases (e.g. cold chain equipment, benchtop centrifuges, blood sampling equipment, etc.) were done at sponsor level in Belgium. This equipment was then shipped to the DRC, allowing the trial schedule to remain as planned.

Domestic transportation of the cold chain equipment from Kinshasa, the DRC's capital city, to the trial site in Boende was particularly challenging. The dimensions of the equipment (up to 2.5 m in height) did not allow transport by air as the only domestic aircraft to Boende measured 1.5 m in height. Larger aircrafts could not land there due to a short and unmarked landing strip. As per manufacturer's recommendations however, the cold chain was to be transported in an upright position, both from Belgium to the DRC, as well as from Kinshasa to Boende. The only way to comply with the recommendations was by boat. Nonetheless, given the poor conditions of the boats, known for its precariousness and accidents, the PI took the risk of horizontally transporting the cold chain to fit the dimensions of the plane. After arriving on the site, the fridges and freezer were left unplugged to rest in an upright position for a few days. Fortunately, this approach was successful and the functioning of the cold chain equipment was unaffected.

5.3.7 Remoteness and climate conditions

Boende, capital of the Tshuapa Province, is accessible from Kinshasa, by road over 1370 km; by four major rivers (Congo, Ruki, Busira and Tshuapa), with a distance of 1194 km; or by air, about 1100 km from Kinshasa as the crow flies. There is no rail network. The equatorial forest is the dominant vegetation. It is characterized by an equatorial climate with heavy rainfall leading to risks of flooding and erosion.

As the trial was set up in such a remote area, climatic constraints such as rain, extreme humidity and heat, presence of rodents, absence of vehicles and poor road conditions were deemed to be barriers in establishing a functional cold chain, for adequate storage of study paper documents and non-disruption of internet access at the study site. In addition to that, only one small commercial flight connects Boende to the capital once a week and only a few makeshift boats carrying goods and persons with the risk of sinking, operate between Boende and Kinshasa via Mbandaka (capital city of the neighboring Equateur Province).

While renovating the site, additional challenges as a consequence of the remoteness of the trial site were the absence of banks and cash dispensers in the province and the scarcity of

qualified workforce for the reconstruction activities. All monetary transactions had to be performed by cash, imported from Kinshasa. These money shipments were only possible via the weekly domestic flight. At times, the PI had to resort to money transfers via private transfer agencies with very limited transaction ceilings. As a result, several transfers per month were required in order to meet the site's logistical needs (transportation reimbursement, accommodation, payment of staff fees, etc.). Furthermore, qualified reconstruction workforce had to be contracted from Mbandaka as this was not available in Boende.

5.3.8 Financial

To ensure that potential participants were adequately and fairly compensated for their contribution in time and for travel expenses [34], it was decided that participants would be reimbursed for transportation costs and possible food and lodging costs depending on the distance and time travelled from their residence to the trial site, according to the economic context of Boende. For this, participants were categorized into two groups; 1) participants traveling less than 6 hours (approximately less than 25 km from the site) and 2) participants traveling more than 6 hours (approximately more than 25 km from the site). The former would receive a fixed amount of 20USD for transportation (e.g. for reimbursement of fuel or motorbike rental costs), whereby food and accommodation are not covered; the latter would receive a fixed amount of 25USD for transportation to Boende and for possible food and lodging costs a sum of 40USD per participant was directly paid to accommodations foreseen for participants. All amounts were agreed upon during a feasibility assessment between the local staff, local authorities and potential participant group leaders and the PI. In addition, they were approved by the ethics committee.

Due to the large distance between UNIKIN (PI headquarters in Kinshasa) and the trial site, a separate financial administrative team needed to be established in Boende. With two administrative locations in the DRC, a regular and systematic reporting system needed to be thoroughly established. Moreover, the regulations and guidelines for financial reporting were often very extensive, complex and not developed for or anticipating the situation of

project partners in LMICs. Additionally, the PI had limited experiences with these particular financial requirements and consequent administration, which necessitated trainings of administrative staff in both administrative locations, as well as close administrative cooperation and follow up between the sponsor and the PI.

While donor's funding practices for partners in HICs regularly include reimbursement of pre-financed activities, this is not always possible, nor feasible, elsewhere in the world. Partners in LMICs often rely on the obtained funds for implementing project activities. The assumption that pre-financing is possible for all international partners can thus directly influence the trial initiation. Multiple high-level advocacy meetings at sponsor level had to be organized to discuss and rearrange transfer schedules to the PI who was highly dependent on these funds to initially kick start and henceforward continue to conduct the trial activities. While an amended transfer schedule was being discussed internally, the project team at sponsor level had to take charge of the procurement of specific services, goods (e.g. hematology and biochemistry analyzers), and materials to be used by the PI, in order not to further delay the trial preparations and set up.

When performing a clinical trial in any setting, budget changes should always be expected, both on Sponsor and on PI level. Each challenge not only requires flexibility of the study team but often also involves a budget reshuffle. Therefore, financial risk mitigation should be part of the initial budget planning, as is considered GCP for trials conducted in resource-poor settings [35]. This way, in the course of the trial, funds could be reallocated to implement additional or unforeseen activities.

5.4 Discussion and conclusion

This article outlines implications met and lessons learned by the research team in designing and setting up an Ebola vaccine trial in the remote area of Boende, the DRC.

Though many researchers have reported on their encountered challenges and lessons learned when designing, setting-up and conducting clinical trials [36–39] and others have

tried to combine these into systematic reviews [14,40,41], finding this information is currently quite an elaborate task for researchers trying to establish (a) new clinical trial(s) (units). In these challenging COVID-19 times where vaccine and drug trials were required to run smoothly and efficiently across the globe at an unprecedented speed, these papers and lessons learned were undoubtable often overlooked during trial setup and conduct. While clinicaltrial.gov is a very useful platform that is widely known and used to register privately or publicly funded clinical trials conducted around the world [42], it also allows researchers to quickly assess whether their research ideas are innovative or already ongoing. Such a similar central platform, listing the different existing trial site locations and the challenges and lessons learned from establishing these trials sites could be extremely useful to research groups looking to establish a new clinical trial (site) anywhere around the world. Research into what content such a platform should contain precisely and how it could be used needs to be further explored.

The challenges faced in LMIC (potential) trial sites, e.g. the precariousness of infrastructure and equipment, the lack of a research culture, insufficient practical research experience, a shortage of research leaders, etc. [14] have often been at the root of the underrepresentation of LMICs in clinical studies, compared to the representativeness of HIC countries [43,44]. However, LMICs represent the majority of the global population and solutions resulting from research in these countries could have the greatest impact on the burden of global morbidity [45–47]. Increasing the number of clinical trials conducted in these countries could therefore help generate local evidence that could influence local health policy.

Implementing trials according to GCP in LMIC may thus call for considerable investment in local capacity [48], as was the case in the current trial, via e.g. the training of local medical staff, the provision of an equipped pharmacy and laboratory, and refurbishment of the hospital facilities. Especially when trials are conducted in locations with poor health care facilities and limited infrastructure, the necessity and the (financial) implications of renovating local facilities should be considered by research teams. For example, the

implemented internet connection, if properly maintained, can have a considerable impact on the rapidity of the transmission of health information from Boende to the central level at Kinshasa. The health district of Boende can now connect to the network and transfer large files to the central server in Kinshasa, something that was unimaginable before. Renovating a trial site location therefore does not only benefit the study team for the duration of the trial, but it allows capacity building of the local health facilities, if complemented by relevant training for the use and maintenance of this (technical) equipment and the other infrastructural investments made [49].

The solid PI-sponsor partnership and the other organizations involved in this trial (CRO and Janssen Vaccines & Prevention B. V.) were crucial to trial implementation. Through close collaborations between all parties, leading to a transfer of knowledge and experience, the clinical research capacity in the DRC increased through the PI team. Rahman et al. (2011) and Yassi et al. (2014) described this method as one of the most effective and sustainable ways to advance a country's health and health education system in the area of clinical research [50,51]. Such partnerships should thus be made more sustainable and extended to other LMICs, as the key to scientific success lies in the empowerment of human resources [51,52]. Highly qualified personnel are needed to propose, initiate and implement trials.

Next to increasing the PI capacity, the plan to increase the level of community engagement through capacity building workshops, implies that conducting more clinical trials in the same remote area could help build the confidence and capacity of local trial staff to successfully conduct more trials in the future. Consequently, local communities of professionals can contribute as channels for disseminating recommended preventive (health) measures to respond to (new) global health threats, especially during epidemics, such as Ebola outbreaks, or even pandemics, such as COVID-19.

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5.6 Declaration of Competing Interest

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

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Chapter 6 Conducting the Ebola vaccine trial

Conducting an Ebola vaccine trial in a remote area of the Democratic Republic of the Congo: Challenges, mitigations, and lessons learned

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6.1 Abstract

Conducting a vaccine trial in a low- and middle-income country (LMIC) can present unique challenges and lessons learned. This Ebola vaccine trial, enrolling 699 healthcare providers and frontliners and jointly set up by the University of Antwerp (Sponsor) and the University of Kinshasa (Principal Investigator (PI)), was conducted in Boende, a remote city in the Democratic Republic of the Congo (DRC), between December 2019 and October 2022 (ClinicalTrials.gov: NCT04186000). While being bound by strict ICH-GCP and international funder regulations, this trial, exemplary for being a public–private partnership, required collaboration between several international stakeholders (e.g., two universities, a pharmaceutical company, and a clinical research organization), local communities and government agencies. Here we address several logistical and administrative challenges, cultural differences, language barriers and regulatory, political, and ethical considerations over the trial's 2.5-year duration, while tailoring and adapting the study to the specific local context.

Lessons learned include the importance of clear communication with participants in all phases of the study, but also within the study team and among different stakeholders. Challenges, mitigations, and lessons learned are presented in nine categories (e.g., safety management; trial documentation, tools, and materials; communication, staff training and community engagement/sensitization; financial and administrative hurdles; and more). Ultimately, to reach the successful end of the vaccine trial in this remote Ebola endemic area in the DRC, careful planning, collaboration, and great flexibility and adaptability was often required from all involved partners. Despite the encountered challenges, the vaccine trial discussed in this paper was able to obtain high participant retention rates (i.e., 92% of participants completed the study). We hope that other international teams aspiring to conduct similar trials in remote areas of LMICs can learn from the way our challenges were addressed, mitigations developed, and lessons were learned.

Keywords. Challenges, Mitigations, Vaccine trial, remote area, lessons learned, Ebola vaccine.

6.2 Background

Vaccine trials are crucial in the fight against infectious diseases. They evaluate the safety, tolerability, immunogenicity, and efficacy of candidate vaccines before they are licensed. Hence, vaccine trials should be conducted in populations of different ages, genders, ethnicities, and geographical and environmental contexts. Additionally, it is incremental to evaluate new candidate vaccines in countries where the disease is endemic [1]. Therefore, the University of Antwerp, as sponsor, and the University of Kinshasa (UNIKIN), as Principal Investigator (PI), jointly conducted an Ebola vaccine trial (hereafter referred to as the EBL2007 trial) in Boende, a city located in a remote and Ebola endemic area in the Tshuapa province of the Democratic Republic of the Congo (DRC) [2].

In 2014, the DRC's 7th Ebola outbreak took place in the Boende health district [3]. Of the 69 suspected, probable, and confirmed cases, eight cases (12%) were healthcare providers (HCP), seven of whom died (88% case fatality rate) [3,4]. HCP and frontliners represent a high risk group for contracting and spreading the disease [5]. Therefore, the EBL2007 trial enrolled 699 HCP and frontliners (i.e., medical doctors, nurses, midwives, community health care workers, first aid workers, laboratory technicians, health facility cleaners, hygienists, care givers, pharmacist aids, nutritionists and vaccination program aids) working and living in the Boende health district [2,6]. Each participant was vaccinated with the 2-dose heterologous Ad26.ZEBOV, MVA-BN-Filo vaccine regimen, followed by an Ad26.ZEBOV booster dose one or two years after the initial dose (randomization 1:1) [2] (ClinicalTrials.gov; NCT04186000). The first participant was enrolled on December 18, 2019, and the last participant visit took place on October 12, 2022. An extremely high participant retention rate of 92% was achieved by the research team over 2.5 years of follow up.

Conducting trials in remote areas of low- and middle-income coun- tries (LMICs) where infectious diseases like Ebola virus disease (EVD) occur, is challenging [7–10]. We previously described the encountered challenges, mitigations and lessons learned (at both sponsor and PI level) to set up the EBL2007 trial in Boende [11]. As a follow up, we describe

here the challenges, mitigations and lessons learned encountered while conducting the trial. To ensure consistency, we maintained the 8 categories where possible or adapted where required. Some categories, specific to the trial conduct, were added (safety and pharmacy management; influence of other infectious diseases; participant's recruitment and follow-up visits). Our main aim is to expand on what was previously published with the experiences and lessons learned from the actual trial implementation and further progress towards its successful completion (Table 1).

6.3 Challenges, mitigations & lessons learned (Table 1)

Table 1. Encountered challenges, mitigations and lessons learned during the conduct of an Ebola vaccine trial in Boende, Tshuapa province, DRC.

#	Challenges	Mitigations	Lessons learned
1	**Regulatory, political, and ethical**		
	Financially support unforeseen regulatory and ethical institutions' visit requests to inspect the study site.	The study budget was reshuffled to allow the site visit of the regulatory and ethical institutions, at their request. The principal investigator (PI) ensured his presence at the site when the visit occurred.	Keep in mind unforeseen (organizational and budgetary) requests from regulatory and ethical institutions. Include a buffer for risk mitigation or contingencies in the trial budget.
	Unvaccinated study staff against Ebola virus disease, working in an Ebola endemic area.	Not applicable.	Depending on the disease, the availability of vaccines and the trial design, vaccination of study staff should be considered either at onset of the trial or as a post-trial measure.
	Trial participants suffered from (serious) adverse events throughout the trial, but the local healthcare system was dysfunctional and operates largely on out-of-pocket contributions.	Provision on ancillary care via the development of a *(non-)related (serious) adverse event ((N)R-(S)AE) algorithm and policy* for participants for the duration of the trial.	Algorithms and policies can help guide the PI and local staff on financial and medical ancillary care decision making and management.
2	**Trial documents, tools, and material**		
	Archiving source documents by the principal investigator (e.g. case report forms (CRFs),	A storage method/system was developed using the study visit and subject identification number, so that information remained coded.	Develop an archiving system for study documents before the start-up of the study.

#	Challenges	Mitigations	Lessons learned
	informed consent forms (ICFs), etc.) between Boende (site location) and Kinshasa (headquarters PI).	A travel plan was developed together with the sponsor in which it was determined which documents were needed at the site before each active period started.	Develop a list of the documents needed per visit. Develop a travel plan and ensure timely shipment of the required documents to and from the site.
	The design of the CRFs information such as the dates of form completion or clinical visits.	Source document notes were utilized to gather the missing dates of study visits.	Always ensure that all information is recorded, with specific attention to dates. Clinical notes, next to the CRF can be essential to document all information/performed actions, as they may be needed for reference in the future. If data is not collected electronically (with a time stamp of completion) but on paper, ensure that each source document and CRF page has the date of the performed action.
	Identifying treatment and trial disposition dates for participants that did not complete the study (e.g. lost to follow-up, moved, etc.).	*Treatment and trial disposition algorithms* were developed by the sponsor to help the PI and monitors remain consistent when identifying treatment and trial end dates.	Algorithms can be useful tools to create clarity in complex situations.
	High numbers of (severe) arterial hypertension in enrolled study participants.	The sponsor and PI developed *hypertension algorithms* that helped guide study doctors on what to do/how to treat participants with (severe) hypertension during a study visit where participants were supposed to be vaccinated.	Algorithms can be useful tools to help guide local staff during vaccination visits. Identify a referral hospital/treatment centre where participants with severe arterial hypertension can go after being diagnosed.
	GCP compliance - Storage of thousands of study documents for 25 years.	Digitization of the source documents: • Source documents containing personal information (e.g. ICF) was stored by the PI. • All other documents were digitized by the sponsor using a specialized company. Digitized documents were stored on two password protected hard drives. One for the sponsor and one for the PI.	Ensure digitization of the source documents to prevent humidity and long-term storage challenges. If digitization is not possible, ensure a large enough storage area with dehumidifiers and humidistat (to regulate the humidity) in tropical climates.

#	Challenges	Mitigations	Lessons learned
		This set-up was reported in a note to file to the investigator site file and the trial master file of the study.	
	Axillary temperature measurement led to impossible temperature measurements results among some participants.	The recorded temperature measurements in participant adverse event diaries that seemed medically impossible (e.g., hypothermic measurements) were discussed by the study medical doctor with the participant during a reactogenicity assessment. A medical decision by the medical doctor was then made to determine whether a participant was truly hypothermic based on clinical assessment and interrogation.	Always ensure clear explanations to the participant on how to conduct study related activities. Foresee oral temperature measurements (instead of axillary temperature measurements) to minimize measurement bias when possible. After ensuring clear explanations of the required study activity, verify whether oral temperature measurements are culturally accepted. Perform a pilot study if necessary. Re-test calibrated material (e.g., thermometer) together with the participant before sending the participant home with the thermometer.
	Terminology that is usually used, was not applicable in the study population.	Erythema (redness) that had more of a brown discoloration after vaccination at the injection site than a red discoloration was not always considered as erythema by some of the participants. Reactogenicity assessment of the medical doctor was required to identify those participants that did have erythema but did not report it as such.	Ensure that the medical jargon used is applicable for your study population.
3	**Safety and pharmacy management**		
	Difficulty to report some SAEs to the sponsor within the required 24 hours after becoming aware of the SAE.	If delays in SAE reporting were expected (later than 24 hours after becoming aware), the PI informed the sponsor of this via WhatsApp. This allowed the sponsor team to be aware that an SAE report would be shared by the PI as soon as possible.	Think about the use of social media (e.g., Whatsapp) to improve the speed of the necessary initial communication between the PI and sponsor, pharmaceutical company, etc.

#	Challenges	Mitigations	Lessons learned
	Impossible to fully rely on the hospital pharmacy (or other external pharmacies)	An adapted version of the World Health Organization (WHO) *Interagency Emergency Health Kit* was used as basis for a study pharmacy construction but had to be adapted throughout the trial to consider the most common pathologies in the area.	Provision of a study pharmacy was essential. The WHO *Model List of Essential Medicines* can be a good starting point. Good contact with the local health authorities and pharmacies can assist in adjusting the list of medications needed, before the start of the trial. Adapting the pharmacy to the local research context, trial population and usages throughout the trial can be achieved with the help of and connections with local health authorities.
4	**Communication, staff training and community engagement/sensitization**		
	Long passive study periods within a >2.5-year study duration.	Study staff was retrained prior to each active study phase on applicable study procedures, protocol amendments, ICF amendments, etc. Participants were invited for workshops on the eve prior to each study visit. Workshops included sessions on the trial activities and basic and more advanced medicine. A test of understanding (TOU) was performed yearly, before each active study stage, to assess the knowledge of study participants on the conduct of the trial.	Re-inform trial participants and staff about the trial study procedures before each active study stage (i.e., what will happen during the next few visits). If a long study duration applies, use this opportunity to train local health care providers through workshops.
	Attempt of study participation fraud.	Iris scanning was used to identify members of the community that pretended to be a participant.	Use biometric identifications tools to help identify attempts of fraud that would otherwise be missed.
	A yellow fever vaccination campaign in Boende led to a vaccine related death.	Prior to starting the trial, community health care providers (*relais communautaire*) were trained by social science professors from UNIKIN to help distribute correct information to communities during the conduct of the trial. Additionally, participants were invited for a workshops 24 hours before each study visit. During this workshop, the PI took the	Continued and clear communicating with the community throughout the conduct of a trial can be challenging. By training local community HCP before the start-up of the trial, rumors and uncertainties in the community can be timely addressed while conducting the trial.

#	Challenges	Mitigations	Lessons learned
		opportunity to respond to any questions, rumors, and uncertainties regarding the study vaccines.	Be alert to what is ongoing in the trial surroundings and anticipate and mitigate dropouts before they happen.
	Participants indicated on several occasions to want to know the outcome of this study and their contribution to it.	Sponsor and PI are organizing a dissemination conference in Boende for study participants, local health authorities, national EC-members, and international stakeholders once all trial results are available.	Foresee a communication channel to distribute study results to the participants and other relevant parties.
5	**Participant's recruitment and follow-up visits**		
	Complaints from some participants about the length of time they had to stand by while being screened, consented, bled and vaccinated.	Staff debriefing by the site coordinator on a daily basis. Readjustment of the participants flow initially designed to accommodate and improve the participants' mobility within the study site during screening and follow up visits.	For better preparation and scheduling of each participant visit, provide notice of the estimated duration of the screening and participant inclusion process and other follow-up visits to the participants.
	Participants residing in area without network coverage.	Obtain information on how to reach participants and remind them of upcoming study visits before a visit window was about to be exceeded. Prompt (or real-time) notification to the PI or study site of the occurrence of a problem with safety.	The cooperation of the local health committee is a key factor in optimizing enrolment and follow-up within a trial in a remote area.
	One year after the start of the trial, recruited first aid worker coordination members wanted to be compensated in terms of equipment, operating funds, etc.	First aid worker members were invited to contribute to the community engagement and capacity building strategy of the study.	Be alert for any rumors and anticipate and mitigate conflicts before they happen.
6	**Remoteness and climate conditions**		
	Changed flight schedules; Multiple plane crashes in the East; Weather condition hindering flights.	A plane was chartered with a trustworthy airline if vaccines needed to be transported to Boende site or if enough staff had to fly as it was safer than flying with the local airline.	Always assess the safety of the staff that is flying to remote study sites and develop a risk benefit assessment of each airline. Ensure flexibility of study staff in remote locations with uncertain weather conditions.

#	Challenges	Mitigations	Lessons learned
			Foresee enough time between domestic flights and international flights, when applicable.
	Internet connectivity issues	The PI often switched providers based on cost-efficacy. To avoid data collection delays, a local server was set up that transmitted the data to a central server as soon as internet connectivity was available.	Know available providers in the study area and make a cost-effectiveness evaluation prior to starting the study. Set up a local server that transmits data when internet is available, if possible.
	Damaged generators and unavailability of high-quality fuel in Boende for generators	Despite having several generators (back-ups of each other), this method of foreseeing electricity was not fully reliable. An expert in repairing generators was sought in Kinshasa and had to fly to Boende to repair damaged generators. High quality fuel was shipped from Kinshasa to Boende to ensure the generators would run smoothly.	Mitigations to avoid low-quality fuel in such a setting were difficult to establish. Local capacity building on all levels may be required to ensure a smooth continuation of the study trial. Foresee budgetary implications for repair and capacity building in remote study locations. Alternative energy sources to generators (e.g., solar energy) should be explored when setting up a study in a remote location.
7	**Influence of other infectious diseases**		
	Ebola outbreak in Mbandaka	The protocol contained a section on next steps in case of an Ebola epidemic in the study area.	Always be alert for a new outbreak when conducting research in an endemic area. Foresee a contingency plan in the event an epidemic occurs in the study area.
	COVID-19 Pandemic and Site implications : • Travel ban in DRC. • Power supply fail mid-covid. • Rumors on mix-up between Ebola booster dose and COVID-19 vaccine. • Sample shipment analysis delayed. • Worldwide stock ruptures in	Travel ban: The network of the PI was used to obtain a plane to Kinshasa at the end of the first active study period (during the national lockdown period). Power supply: expanded program on immunization generators were used as back-up. Rumors: When COVID-19 occurred, rumors were addressed during workshops, for which participant were invited 24 hours prior to their study visit.	Try to establish a good relationship with political authorities. Foresee a resilient contingency plan and travel plan (for staff, samples, and source documents). Taylor community engagement to include unexpected events that could have an impact on participant perception of the trial. Flexibility from all parties is required and a solution driven approach should be practiced when

#	Challenges	Mitigations	Lessons learned
	laboratory material and medical consumables. • Sponsor staff unable to travel to DRC for support (international travel ban). • Monitors unable to travel to the site. • Longer sponsor travel visits required after travel ban removal because of testing and quarantine. • Additional cost of testing to travel to site (UNIKIN)/DRC and site (sponsor).	Impossibility to order certain required laboratory material: The University of Antwerp network was used to obtain the necessary material. Delay in sample shipment: Readiness of samples and courier were ensured as soon as borders opened up, and air transport was possible. Cancelled sponsor visit: Continuous online contact between site, PI and sponsor was ensured and the sponsor tried to help remotely where possible. Cancelled monitor visit: Monitoring visits was delayed until it was possible to perform the monitoring at PI headquarters in Kinshasa. Additional costs: Pay the additional costs for testing and plan longer study visits to include quarantine days.	coming across unexpected situations. Foresee a buffer in study budgets for unforeseen additional expenses (e.g., Covid testing, longer research stays due to quarantine).
8	**(Inter)national collaborations**		
	Large staff turnover in some teams.	Turnover documents were developed to ensure adequate information was passed on to a successor. The sponsor team ensured that each staff member had a back-up within the team. This way, no issues were left unaddressed when someone went on holiday for example.	Ensure clear communication, plans and SOPs for a smooth continuation of the study during high staff turnover. Develop turnover documents to ensure the most important details are passed on to successors. Foresee trained back-up personnel in each team.
	Data coding responsibilities and discussions.	Many meetings were needed to discover the reason for inconsistencies in expectations concerning coding of the data.	Ensure clear communication, including clear guidelines on which software versions to use and expectations of each involved institution.
	Medical writer selection.	The required budget was higher than initially foreseen. Three companies had to be contacted according to Belgian law.	When subcontracting, check the requirements of the funder before approaching companies. Involve your institute's processing department before approaching

#	Challenges	Mitigations	Lessons learned
			qualified companies if budget implications are unknown.
	Language barrier.	On site, translators were hired when required. Some of the study team members spoke the necessary languages and could function as translators during meetings.	If possible, hire local staff that speak the necessary languages. If this is not possible for the established international collaborations, ensure that some of the staff in the sponsor and PI team can function as translators.
	Delay in sample analysis: • Covid-19. • Moving locations of laboratory: FDA approval required after moving; no sample results could be shared until approval was obtained.	The sponsor ensured frequent communication and meetings with the pharmaceutical company and the analyzing lab to discuss progress and potential solutions to delays.	When funding lasts for a certain amount of time, ensure enough wrap-up time or potential delays before funding is scheduled to end. If not, keep in mind that a no cost extension request with the funder may be required.
9	**Financial and administrative hurdles**		
	Funders' reporting requirements can burden the capacity of partners' administrations in LMIC.	Sponsor's administrators provided close follow up and capacity training for the partners and collaborated with the financial teams (Boende and Kinshasa) in the field to develop a project-specific accounting system.	The partners' administrative coordinators should to be involved from the initial set up of the project in order to develop an adapted project reporting system that enables a smooth operational roll out, while simultaneously adhering to the funders' binding guidelines.
	Differences between the administrative set up of the funder and financial auditors and the local reality and practices in LMIC can lead to financial uncertainties and delays in funding.	Consortium coordinator and partners cooperated closely by unifying the experience and know-how of audits in order to find solutions to the funder's and auditor's requests.	Consortium partners are advised to exchange their experiences and know-how of audits conducted in projects in LMICs. The most experienced partners in the consortium should provide support to others for the benefit of the project as a whole.
	Funder-designed processes can be bureaucratic when correcting flaws or amending research activities in consortium set up.	Lengthy, recurrent exchanges and discussions between funder, consortium coordinator and involved partners, with frequent references to initial proposals and contract clauses.	Enable a sound financial and administrative set up of the research consortium at the project's proposal stage by involving the administrative project coordinators.

#	Challenges	Mitigations	Lessons learned
	Running a project using three different currencies in a cash-reliant country.	Very close follow up of the cash movements by means of the cash ledger and monitoring of the exchange rate risks.	Encourage a disciplined use of the cash ledger and a close cooperation with the financial administrators is paramount in controlling the substantial cash movements within the project.

6.3.1 Regulatory, political, and ethical

In agreement with national guidelines on medical research involving humans [12], the National Health Ethics Committee of the DRC (EC-DRC) conducted a 3-day inspection of the EBL2007 trial site in Boende to ensure that ethical standards were respected and all study procedures were conducted according to the approved protocols. Next to this visit, inspectors from the Laboratory Directorate of the DRC Ministry of Health visited both the trial site and the UNIKIN's cold chain facilities in Kinshasa to verify that the collection, processing, and storage conditions of clinical trial samples followed good clinical & laboratory practices (GCLP), before authorizing the shipment of the samples to laboratories outside the DRC. These visits were expected to be financially supported by the PI, an unexpected responsibility which was thus unforeseen in the budget planning. As pointed out by Kass et al. (2007), funding of EC activities in Africa is generally experiencing significant bottlenecks [13]. Adequate, transparent, and sustainable funding is essential for the effective functioning of an EC, to ensure its independence, and to avoid potential conflicts of interest with investigators.

Despite its challenges, conducting the EBL2007 trial in an Ebola endemic area was relevant and important. Firstly, it was pertinent that the investigational product (IP) was evaluated in a high-risk area. Secondly, participating HCP were likely to be better protected and show clinical efficacy, should an outbreak occur. However, to avoid evaluation bias, hired study staff were not allowed to participate in the study, limiting their own protection against a possible Ebola infection. Consequently, while the risks initially seemed low, several mitigations and measures had to be in place to adequately support and protect study staff (e.g., training national and international staff on sanitation and safety precautions, liaising

with local and national public health authorities) and when the trial was ongoing, a suspected (but eventually not confirmed) Ebola case was reported near the study area. Therefore, depending on the disease, the availability of vaccines and the trial design, vaccination of study staff should be considered either at onset of the trial or as a post-trial measure.

In remote and resource-constrained areas in LMICs, access to quality healthcare may be challenging. However, when quality healthcare is inadequate, legislation or binding regulations require sponsors to provide care to conditions unrelated to the IP, also referred to as Ancillary Care (AC) [14,15]. Hence, our research team developed a policy, combined with a decision algorithm, to systematically and non-arbitrarily approach and support participants' concomitant medical events [16]. The development and modalities of this specific AC approach, as well as its implementation challenges, are described elsewhere [16,17].

6.3.2 Trial documents, tools, and material

Since the PI was based in Kinshasa, the capital city of the DRC and approximately a 3h30min flight from the trial site in Boende, the archiving of paper source documents (e.g., informed consent forms (ICFs), case report forms (CRFs), logs) came with its unique challenges. General lessons included; (1) the necessity to have a predefined travel plan to keep track of source document mobility; (2) due to rodents and weather conditions, high level documents such as ICFs are best stored in a safe or lockable cupboards; (3) documents are best filed by participant ID so that records can be easily identified when needed (this study stored source documents per visit and document type); (4) the study visit date should be reported on each source document and CRF page, as this may be crucial to reconstruct a participant's study timeline when assessing treatment and trial disposition timelines during analysis; (5) algorithms can provide guidance (e.g., AC algorithm and policy; how to identify reasons and dates for treatment/trial disposition; etc.). Though algorithms/guidelines offer a framework, they should not replace rational thinking and decision-making for each individual case.

As the vaccine trial was conducted under the Innovative Medicines Initiative (IMI), which is a European Union (EU) public–private partnership, it had to abide by EU pharmaceutical legislation [18]. While this legislation indicates that medical records of participants must be archived in compliance with national law [19], the storage duration mandated by the DRC law is unclear. For this reason, the research team (consisting of sponsor and PI) decided that all source documentation would be stored for the same duration as the trial master file, which follows EU legislation, and amounts to 25 years [19]. To achieve long-term storage without the constraints of weather or storage limitations, all paper source documents without patient identifiers were digitized. The digital source documents replaced the paper versions, with the approval of the EC-DRC. The Good Clinical Practice (GCP) guidelines (ICH E6, 4.9) further highlight the importance for the archiving system to enable document identification, version history, search, and retrieval [20]. To allow anyone to find a specific term within a PDF, the documents contained optical character recognition. After digitization and quality checks, the source documents were destroyed by the digitization company. The sponsor and PI are both in possession of a password protected hard drive, on which the digitized source documents are stored. Only delegated staff within both institutions have access to the password. The PI stored documents with patient identifiers (e.g., ICFs) elsewhere.

Culturally accepted practices need to be taken into consideration when developing documents and determining procedures to be carried out during a trial. In this study, axillary temperature measurements were taken. The PI determined that the use of axillary temperature measurement would be culturally acceptable as it is a globally recognized non-invasive standard, although it may be less accurate and precise than oral measurements [21]. However, discrepancies were noted among some study participants who recorded hypothermic readings below 35.0°C, which were later invalidated by the study physician’s reactogenicity assessment, attributing them to improper axillary thermometer usage. While several mitigations were taken to prevent inaccurate axillary temperature measurements (i.e., provision of a personal thermometer per participant and providing clear instructions on its proper use), such inaccuracies still occurred.

Consequently, we posit that oral temperature assessment might be less prone to user error and thus more reliable than axillary methods.

Furthermore, when creating trial documents, it is crucial to consider the overall appearance of potential study participants. In this trial, solicited adverse event terminology was included in the participant journal as it had been used in previous studies assessing the safety of the vaccines. One of the symptoms documented was *erythema* (described as redness of the skin in journal guidelines). However, after booster vaccination some participants did not report any redness at the injection site in their participant journal. Yet, when questioned during a follow-up visit, they reported a more brown discoloration at the injection site instead. Hence, they did not measure this discoloration because it was not really red as described in the guidelines and as elucidated to study participants. Therefore, we recommend ensuring that the assessed (medical) symptom terminology and guidelines apply to the study population being assessed and to adapt terminology and guidelines accordingly, if required.

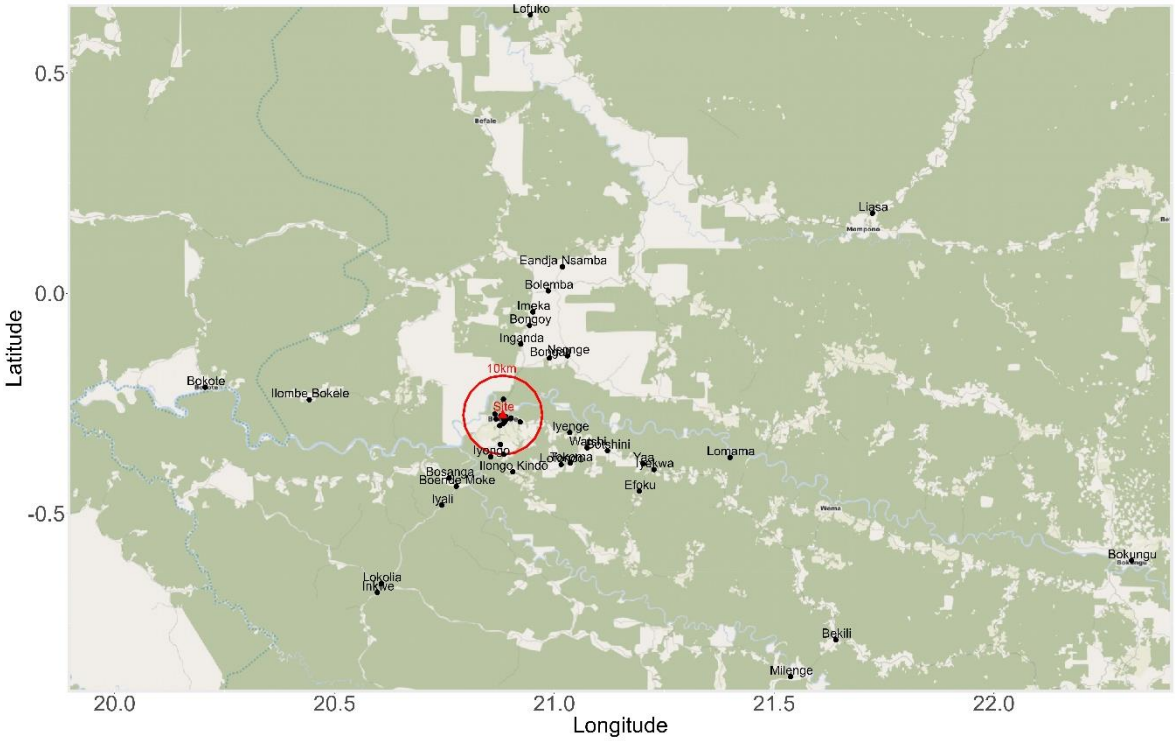
6.3.3 Safety management

Since the trial was conducted in a remote area with frequent disruptions in mobile network communication and in internet connection used at the study site, there was significant risk of a delay (more than 24h) in reporting serious adverse events (SAEs). To anticipate this, the SOP for SAE reporting allowed the sponsor to be informed via WhatsApp before a more detailed report followed. In addition, to prevent any missing SAEs, participants were informed at the beginning of the study that a toll-free number was available to contact the site. Health facility managers (Nurse Attendants) were also asked to notify the study site coordinator upon receiving a study participant with a health problem at their health facility. This was particularly important for participants residing outside of the mobile phone network range (10 km radius from the trial site; Fig. 1).

To temporarily improve the availability of basic healthcare for trial participants, a study pharmacy was foreseen to provide AC. This pharmacy was set up using the *Interagency*

Emergency Health Kit of the World Health Organization as a starting point [22]. However, not all medication and supplies were relevant nor included to provide basic healthcare to the study participants. Therefore, adaptations in medications and amounts were made before the trial started and were refilled based on consumption as the trial progressed. In hindsight, while the emergency health kit served as a worthwhile starting point, the *Model List of Essential Medicines* may have been more applicable as this list includes the minimum medication requirements to deliver primary healthcare [23].

Fig. 1. Mobile phone network range around Boende.

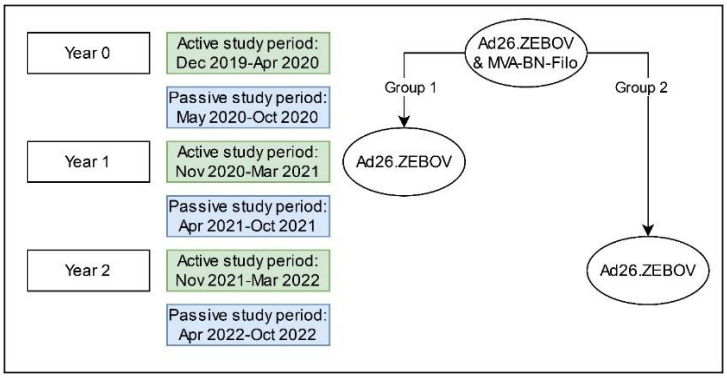


The site location in Boende is shown in a red triangle, the 10 km mobile phone network radius is indicated with a red circle. Participants living in villages outside of this red circle, could not be contacted via mobile phone. Villages of participants' residence outside the 10 km radius are indicated with a black dot and the village name. The map was made in R version 4.3.1 and is presented as type osm-public-transport. The circle was added using the packages rgeos and sp.

6.3.4 Communication, staff training and community engagement/sensitization

The EBL2007 trial had long intermittent study periods where no active study visits took place. In total, the trial was split into three stages (Fig. 2). While long passive periods were essentially not a problem, retraining study staff on the protocol, GCP, SOPs, etc. was essential before the start of each active study stage. Each year the training courses were updated to include the necessary procedures according to the upcoming study visit and were taught by clinical research associations, the sponsor-team and the PI-team.

Fig. 2. Simplistic overview of the EBL2007 Ebola vaccine trial.



The EBL2007 trial can by split up into active (green) and passive (blue) stages. During active stages, vaccination, blood sample collection and safety assessments took place during scheduled visits. In passive stages, serious adverse events were collected during unscheduled visits or scheduled phone call visits.

In line with retraining staff after long passive periods, we found that it was important to (re)explain the upcoming study activities and ICF content to trial participants prior to their next study visit. This was learned through a test of understanding collected prior to enrollment and before each active study period. To maximize understanding of the trial, capacity-building workshops were held on the eve of the screening and recruitment day and all other follow-up visits scheduled in the trial. These capacity-building workshops with participating HCP, covered educational topics on non-medical preventive measures against EVD or other diseases or health issues, coupled with explanatory sessions and necessary information related to the conduct of the clinical trial. This was followed by a question-and-answer session to address any questions or concerns of participants.

Because the study vaccine regimen was to be administered in two doses, followed by a booster (Fig. 2), it was imperative to ensure that the correct individual was vaccinated. For this reason, an iris scan tool was used throughout the trial to ensure correct identification of participants [24]. The iris scans were captured on tablets and transferred to a portable server via local Wi-Fi. Iris scans were recorded in a binary code and the code was encrypted in rest and transit from the tablet to the portable server. These encrypted data were backed up on an external hard drive daily. Both the portable server and hard drive were stored securely at the study site. Access to the main server and back-up hard drive was restricted to designated trial staff, ensuring participant identity protection. Incidents of fraud were detected by this scanning tool when family members tried to present themselves as a substitute for participants who were unable to attend a scheduled visit at the clinical trial site. Therefore, biometric identification should be considered for longitudinal studies.

Other challenges were encountered during a yellow fever preventive vaccination campaign when a yellow fever-vaccine related death (classified by the pharmacovigilance center) took place in the Boende health district. This occurred between the heterologous two-dose vaccine regimen and the booster dose (Ad26.ZEBOV) administrations at Year 1 (Fig. 2). Interestingly, this incident did not have an impact on the EBL2007 vaccine retention rates. We formulated three hypotheses for this observation. First, we enrolled HCP, a (relatively) well-educated population who was able to discern that the study vaccines used were different from the yellow fever vaccine. Second, capacity-building workshops and sensitization sessions between the communication task force and participants on the eve of each scheduled visit built participant confidence and anticipated the spread of false messages or rumors. Third, with the 2014 Ebola outbreak in mind, participants considered the risks of Ebola vaccines acceptable.

Finally, in the spirit of open communication and community engagement, the sponsor and PI team found it important to communicate to the participants, local health authorities and the EC-DRC what the outcomes of the trial are. For this reason, a face-to-face dissemination conference is planned in Boende when all study results are available and analyzed. The

conference planning is ongoing at the time of this writing. This step, though ethically relevant and often important to participants, is often omitted in scientific research.

6.3.5 Participant's recruitment and follow-up visits

During the initial enrollment visits, participants spent an average of 2 h at the trial site. This time was eventually reduced to less than 45 min per participant through morning briefing sessions, and staff experience. To avoid complaints, we recommend warning participants about the duration of the screening and enrolment processes so they can prepare and schedule their work activities on that day. Additionally, morning briefing sessions between study staff and the site coordinator are important to discuss difficulties encountered on previous days, so solutions can be sought.

Several trial participants lived in villages beyond the mobile network coverage in the Boende health district (Fig. 1). Their only means of accessing the site was on foot, by bicycle, with dugout canoes or by motorcycle. This presented a challenge in terms of localizing and/or reminding participants of upcoming study visits. To minimize the loss of follow-up of these participants and to maximize their comfort and well-being throughout the trial, the PI reimbursed travel expenses for all participants and additional accommodation and meal expenses for any participants travelling more than 6 h (approximately >25 km) to the trial site. Additional reminders were made through the health district's community health workers (identified at the beginning of the trial) to locate participants who did not attend scheduled study visits.

At the very beginning, first aid workers of the Boende health district were contacted to participate in the EBL2007 trial, given their status as stakeholders in the process of safe burial during Ebola epidemics. A meeting was held with the first aid worker coordination team, to explain the main objectives and procedure of the study and to compile a list of potential study participants. When starting recruitment, several members agreed to participate in the study and very good adherence to the various appointments was noted. However, at the start of the Year 1 visits (Fig. 2), the coordination team of first aid workers

contacted the PI and asked to be compensated in terms of equipment, operating funds, etc. As it would be unethical to compensate institutions for their members to participate, the PI could not respond to these requests. Consequently, the coordination team countered by suggesting all first aid worker participants leave the study. After lengthy discussions, a solution was found; some coordination team members would be hired to give capacity building workshops planned in the study. This experience demonstrates that unexpected circumstances can arise, and that flexible and at times creative solutions need to be sought to maximally avoid dropout rates from escalating.

6.3.6 Remoteness and climate conditions

Boende can be accessed from Kinshasa either by river, which can take up to two weeks using makeshift boats transporting goods along the Congo River, or by air, which takes approximately 1h45min to 3h30min depending on the type of plane and airline company. However, considering the high risks associated with the river routes, domestic flights to Boende - operated by two commercial airlines (limited to one flight per week) - are in high demand. Unfortunately, flight cancellations can occur due to weather conditions (e.g., heavy rain, strong winds), technical issues (e.g., maintenance failures, lack of kerosene, failure to confirm the flight 24 h in advance) or unavailability of the aircraft (e.g., leased to officials for travel within the DRC). A well-designed travel plan, and collaboration with charter companies for personnel transportation, vaccine delivery, and sample shipment, helped mitigate the negative impact of these constraints.

In terms of high-speed internet access, the DRC as a whole lags behind [25]. Access to a submarine cable system is limited to a few areas (primarily concentrated in major cities), but is non-existent in Boende. Furthermore, mobile internet access in Boende is extremely limited and more complex compared to Kinshasa. A thorough understanding of the internet provider landscape, enabling better planning and minimizing potential disruptions in the continuity of the study was important. However, while some suppliers offered good services at the beginning of their contract, this often declined over time and new solutions/providers had to be sought. For data collection, the limited internet connection

was resolved through the setup of a DFdiscover local server, on which data entry took place. Data were copied over from the local server to the central server on a daily basis as connectivity permitted using a satellite uplink. Both servers were fully 21 CFR Part 11 compliant.

Three generators operated daily, with a shift change every 12 h to foresee the study site of electricity. Despite these arrangements, several breakdowns occurred during trial activities (e.g., because of lightning strikes or bad quality of local fuel). Therefore, high quality fuel had to be imported from Mbandaka (Equateur Province) to Boende. Furthermore, the lack of technical expertise in Boende for generator maintenance and upkeep posed a challenge. The PI had to subcontract a company from Kinshasa for regular maintenance missions to Boende. In hindsight, it might have been more advantageous to have a solar power source as a backup to the generators. Having a solar power source would have provided a reliable and sustainable alternative energy option, ensuring an uninterrupted power supply and reducing dependence on external resources in critical situations.

6.3.7 Influence of other infectious diseases

During the EBL2007 trial in Boende, a total of six outbreaks of EVD occurred in the DRC. These outbreaks alternated between two provinces (North Kivu and Equateur). While no Ebola outbreak was officially declared in the Boende area, Mbandaka has a robust commercial connection with Boende via the river. Additionally, the index case of the DRC's 14th outbreak in Mbandaka had returned from a medical internship at the GRH in Boende, where the trial site was located. These outbreaks and the strong connection between Boende and Mbandaka, likely led to a heightened perception of the risk of EVD occurrence in Boende, motivating the study population to accept the investigational vaccine.

Seen the overabundance of (mis)information and related vaccine-hesitancy during the global COVID-19 pandemic, there was a very negative perception of COVID-19 vaccines and their deployment in the DRC, which faced numerous challenges [26]. Some HCP participants in the EBL2007 trial were convinced that the deployment of COVID-19 vaccines

was unnecessary in Boende. Their perception was influenced by several factors, including a perceived low-risk of the pandemic due to the absence of reported cases in the region until a year after the pandemic began, and the erroneous belief/misconception that having received the study's Ebola vaccine would provide sufficient protection against COVID-19.

The first COVID-19 case in the DRC was reported in March 2020 in Kinshasa, four months after the start of the EBL2007 trial. Unfortunately, this period coincided with active participant visits at the study site in Boende (Fig. 2) and the containment measures of the public health emergency decree, issued in the DRC, banned national and international flights and national transport by boat with passengers. This emergency status complicated logistical support to the clinical trial staff in Boende; cash transfers could not come from Kinshasa (no bank exists in Boende), serum samples could not be shipped to the destined laboratories and the supporting trial staff from UNIKIN, Kinshasa, was grounded in Boende. However, thanks to the support of the Provincial Health Division on the one hand, and the connections of the PI with relevant national political and administrative authorities on the other, the local team was able to ensure the continuity of trial activities. Fortunately, Boende being very remote, the site and study activities were only slightly affected by the pandemic. Only one participant missed his/her study visit because of the national travel ban whereby the participant could not return from travels for a scheduled visit. Once trial activities terminated during national lockdowns, the UNIKIN staff working in Boende and the collected samples were exceptionally able to return to Kinshasa by means of a chartered flight that had received special authorization from the political-administrative authorities of DRC.

Unfortunately, once the samples reached Kinshasa, these could not be sent on to the international laboratory for testing until the international flight ban was lifted and the backlog of cargo flights was resolved. While sample collection for the first active period ended on the April 25, 2020, the samples could not be shipped to the United States (San Juan Capistrano, CA) until October 31, 2020. Additionally, the capacity to analyze samples was further delayed due to lock downs and diminished staffing availability in the laboratory

as well as the prioritization of COVID-19 testing. Therefore, final sample results were not obtained until January 28, 2022.

Another consequence of the lockdowns and flight restriction was the impossibility for others to reach the site location. Support and trainings from the sponsor that was foreseen on site, had to be cancelled and given online. Additionally, monitors could not reach the site and remote monitoring methods had to be set up.

Once lockdowns had lifted and travelling was possible again, new challenges arose. Negative COVID-19 PCR tests were required prior to both domestic and international travels, leading to unforeseen costs and travel time, as a quarantine period in Kinshasa before leaving for and after returning from Boende was obligatory.

When preparing for the active study period in Year 1 in August-October 2021 (Fig. 2), COVID-19 was still in full swing. Factories making laboratory and medical equipment/material had to go into lockdown or were brought down to limited staffing, leading to limited stock availability. The world's available stock had been redirected to fight the pandemic and to COVID-19 related research, impacting other ongoing research. For example, between August-October 2021, cryotubes were impossible to find on the market. In the end, this could only be resolved by obtaining excess stock from other studies of other research teams within the University of Antwerp. Luckily, this allowed the EBL2007 trial to continue as planned.

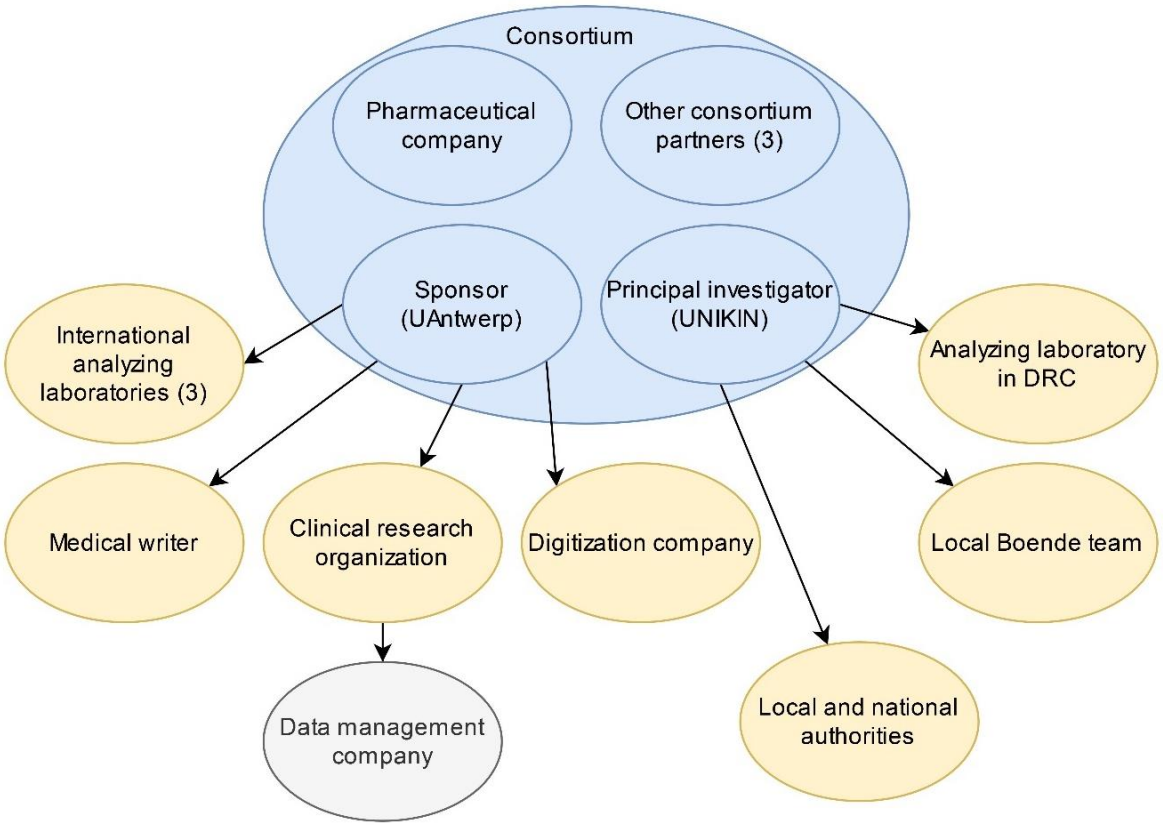
Once trial activities resumed for the second active phase (Year 1, Fig. 2), preventive public health measures were incorporated into the trial activities. These included reducing the number of participants at the site, mandatory wearing of masks by all staff and participants, and the wearing of protective face visors and lab coats by laboratory personnel. This was based on an update of the biosafety SOP in relation to COVID-19. Furthermore, a negative COVID-19 test was required for anyone travelling from outside of the Tshuapa province. Some additional precautions were taken within the trial team, including the requirement that study staff with COVID-19 symptoms refrain from coming to the site, and consult the

health services in Boende for diagnosis and appropriate management. Finally, once possible, COVID-19 self-tests were made available for participants or staff presenting with symptoms. In total, five participants tested positive during the trial. However, no participants experienced severe symptoms or hospitalization as consequence of a COVID-19 infection.

6.3.8 (Inter)national collaborations

With many international teams involved directly or indirectly in the EBL2007 trial activities (Fig. 3), several challenges and difficulties were encountered. First, some teams had large staff turnovers throughout the trial, at times making it difficult to ensure continuity for other partners. Second, though roles and responsibilities were clearly defined in a project management plan at the beginning of the trial, the study and the teams evolved. In doing so, the clearly allocated roles and responsibilities sometimes became blurry. In a project that lasts several years, we therefore recommend to reassess, redefine and reassign these roles and responsibilities at predefined time points or more frequently when needed. Thirdly, while the main language used in the consortium and among partners was English, the local languages in Boende were French and Lingala. Language differences and barriers needed to be considered when developing study material that reached the study staff and participants or when hiring staff that worked in these different language environments. Finally, the most important aspect of working with such many partners was clear and frequent communication, to avoid misunderstandings. This was ensured through daily, weekly, or monthly meetings (depending on the need) between the relevant partners and stakeholders. For example, within the EBL2007 trial, weekly meetings within the sponsor team but also between the sponsor and the PI teams; the sponsor and pharmaceutical company; the sponsor, PI, and data management company; and the sponsor and the clinical research organization were held.

Fig. 3. International collaboration diagram of the EBL2007 trial based on contractual links



This diagram shows the contractual links between different stakeholders. However, the communication between the different stakeholders was even more intertwined. For example, the sponsor team also had contact with the national analyzing laboratory (meetings), the local Boende team (training), and the data management company (meetings), while the Principal Investigator team also had contact with the clinical research organization (meetings and monitoring visits) and data management company (meetings).

6.3.9 Financial and administrative hurdles

Conducting research projects in resource-poor LMICs, with two financial chairs (e.g., one in Boende and one in Kinshasa) was challenging. Adhering at the same time to elaborate and binding funder's financial and administrative guidelines added additional challenges for the PI's administrations.

Funders and financial auditors, based in Europe or other 'Western' high income countries, tend to draft agreements and guidelines based on their own - often complex - administrative and financial practices. However, these agreements and practices do not always consider the local realities, legal situation, or usual accounting practices of the

reporting entities in LMICs. For the PI to abide by these guidelines and agreements, it was paramount for sponsor administrators to provide close follow-up and capacity training, and to collaborate closely with the teams in the field in Boende and Kinshasa to develop an almost tailor-made, project-specific accounting system. Therefore, we recommend that all partners' administrative coordinators are involved from the start of the project, ideally already in the proposal phase, to develop an adapted project reporting that enables a smooth operational roll out in all involved countries.

The forementioned differences between the specific administrative set up, of the funder and its financial auditors and the local reality and practices of partners in LMICs, can increase the potential for misunderstandings and inaccurate conclusions. Practically, this risked stalling the project due to delays in funding and the entailing financial uncertainties. Therefore, anticipating auditors' requests, while documenting everything meticulously, is a way to avoid delays or even a possible (temporary) blocking of the funding in a project. Additionally, we recommend that consortium coordinators should assist less experienced partners in finding solutions to auditor's requests by combining the experience and know-how of their financial and administrative staff. The most experienced partners in the consortium should provide support to others for the benefit of the project as a whole.

When drafting project proposals and grant and consortium agreements, there is a tendency to focus on the research and operational field work, inadvertently paying less attention to the organizational and administrative aspects. Involving and consulting the administrative project coordinators already at the early stages is therefore strongly recommended.

Finally, operational, and logistical tasks in a cash-reliant environment (as is often true for LMICs) were made more difficult because three currencies were involved for the EBL2007 trial; the funders' Euro, the local currency (Congolese Franc) and the US dollar which often replaces the local currency. This set-up required a very close follow up of the cash movements for the different currencies by means of a well-structured cash ledger and close monitoring of the exchange rates. A continuous close cooperation with the financial

administrators, and their empowerment, was paramount in controlling the substantial cash movements while at the same time complying with the funder's guidelines.

6.4 Conclusion

Overall, the EBL2007 trial was a great success. After more than 2.5 years of visits and follow-up, 92% of participants completed the study. We believe open, honest, and frequent communication among partners, with local authorities, trial staff and participants contributed greatly to this success. By assigning roles and responsibilities in the very beginning of the trial, all partners were aware of what was expected of each other. Frequent meetings (weekly or monthly) between partners ensured agreements were followed and adapted when necessary. In doing so, logistically the trial was well-organized and able to stay on track, even during unexpected events such as the COVID-19 pandemic. Additionally, we recommend other researchers to ensure participants and relevant authorities are informed of trial results through dissemination activities. This way, good relations can be maintained and future research opportunities in the area will have more likeliness of success. This paper was written in the same spirit of open communication and by sharing the challenges we encountered, how we mitigated them and the lessons that were learned, we hope to help other researchers aspiring to perform successful trials in similar settings of LMICs.

6.5 Funding

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6.6 Declaration of Competing interest

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

6.7 Data Availability

No data was used for the research described in the article.

6.8 Acknowledgements

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All authors attest they meet the ICMJE criteria for authorship.

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Chapter 7 Evaluation of a study-specific ancillary care policy

An ancillary care policy in a vaccine trial conducted in a resource-constrained setting: evaluation and policy recommendations

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7.1 Abstract

Introduction. Clear guidelines to implement ancillary care (AC) in clinical trials conducted in resource-constrained settings are lacking. Here, we evaluate an AC policy developed for a vaccine trial in the Democratic Republic of the Congo, and formulate policy recommendations.

Methods. To evaluate the AC policy, we performed a longitudinal cohort study, nested in an open-label, single-centre, randomized Ebola vaccine trial conducted among healthcare personnel. Participants' demographic information, residence distance to the study site, and details on the financial and/or medical support provided for any (serious) adverse events ((S)AE) were combined and analysed. To assess the feasibility of the AC policy, an expenditure analysis of the costs related to AC support outcomes was performed.

Results. Enrolment in this evaluation study started on 29 November 2021. The study lasted 11 months and included 655 participants from the Ebola vaccine trial. In total, 393 participants used the AC policy, mostly for AE management (703 AE and 94 SAE) via medication provided by the study pharmacy (75.3%). Men had a 35.2% (95% CI: 4.0-56.6%) lower likelihood of reporting AE compared to women. Likewise, this was 32.3% lower (95% CI: 5.8-51.4%) for facility-based compared to community-based healthcare providers. The daily AE reporting was 78.8% lower during the passive versus the active trial stage, and 97.4% lower during unscheduled versus scheduled visits ($p<0.001$). Participants living further than 10km from the trial site more frequently reported the travel distance as a reason for not using the policy ($p<0.04$). In practice, only 1.1% of the operational trial budget was used for AC policy support.

Conclusion. The trial design, study population, and local health system impacted the use of the AC policy. Nonetheless, the AC policy implementation in this remote and resource-constrained setting was feasible, had negligible budgetary implications, and contributed to participants' health care options and well-being.

7.2 Key questions

What is already known on this topic. Implementing ancillary care (AC) in clinical trials is encouraged through international guidelines, but remains non-compulsory. Whereas several research groups have provided models and theoretical approaches, there is a dearth of strategies for practical implementation, thus AC is mostly defined on a case-by-case or ad hoc approach. We previously developed and published an AC algorithm and policy that was systematically applied in an Ebola vaccine trial in a remote and resource-constrained area of the Democratic Republic of the Congo.

What this study adds. This is, to the best of our knowledge, the first study that evaluates the outcomes of a study-specific AC policy. It demonstrates the feasibility of a well-structured AC approach to provide medical and/or financial support to trial participants who experience medical events, with limited budgetary implications for the sponsor, investigators and funders. Based on the study results, i.e., the participant utilization of the AC policy and the related support outcomes, we provide recommendations to other research groups and sponsors willing to implement a similar approach.

How this study might affect research, practice or policy. This AC approach can be adapted to other contexts and clinical trials, and inspire policy makers, ethics committees, and funders to call for adequate AC provisions in clinical trial settings with access to care constraints.

7.3 Introduction

The development of vaccines and medicines for diseases affecting low- and middle-income countries remains crucial to reduce global health inequalities. Ebola vaccines are primarily needed in remote areas of West and Central Africa, where outbreaks are more prevalent. Therefore, the University of Antwerp (UAntwerp; Belgium), as sponsor, and the University of Kinshasa (UNIKIN; Democratic Republic of Congo (DRC)), as principal investigator (PI), conducted a large Ebola vaccine trial in Boende, DRC, between December 2019 and October 2022. In 2014, the Boende health zone experienced an Ebola outbreak, with healthcare personnel disproportionately affected [1]. To enhance the outbreak preparedness of this Ebola-endemic region, the trial assessed the safety and immunogenicity of a heterologous Ebola vaccine regimen (Ad26.ZEBOV, MVA-BN-Filo) in this at risk population, with a booster dose (Ad26.ZEBOV), administered one (Arm 1) or two (Arm 2) years after the first dose (randomisation 1:1) [2].

In the early stages of the trial, the sponsor and PI were confronted with the substandard quality healthcare routinely available and accessible to the population in Boende, including the trial participants [3]. This context-related vulnerability may impact individuals' motivation to participate in research [4]. However, it is important not to overemphasize the effect of structural coercion on the decision of prospective participants to enrol in clinical trials, as it underestimates the role of the individual agency. Enrolment may offer the best possible outcome for socially vulnerable participants and be an active choice, in expectation of better care [5]. As such, researchers should take adequate measures to mitigate the effects of socio-economic vulnerability, by promoting quality healthcare and address participants' therapeutic expectations based on their specific (health) needs [6, 7]. According to the Council for International Organizations of Medical Sciences, offering medical care that goes beyond the scope of a scientific study – also referred to as ancillary care (AC) – should not be considered an undue influence to participate in research. On the contrary, it may contribute to optimizing the balance between burdens and benefits of research participation [8]. Hence, the Uantwerp and UNIKIN team developed an AC policy,

to provide medical and/or financial support for all medical events experienced by trial participants, irrespective of their relatedness to the investigational product (IP) or to trial procedures, which has been published elsewhere [3].

In this paper, we share the findings from the assessment of this trial-specific AC policy, which aimed to treat and financially cover the treatment expenses of participants' unrelated (S)AE. The primary objective was to evaluate the participants' use of the AC policy; including its policy outcomes in terms of medical and/or financial support, geographic determinants, budgetary implications, and formulate recommendations for AC strategies in similar settings.

7.4 Methods

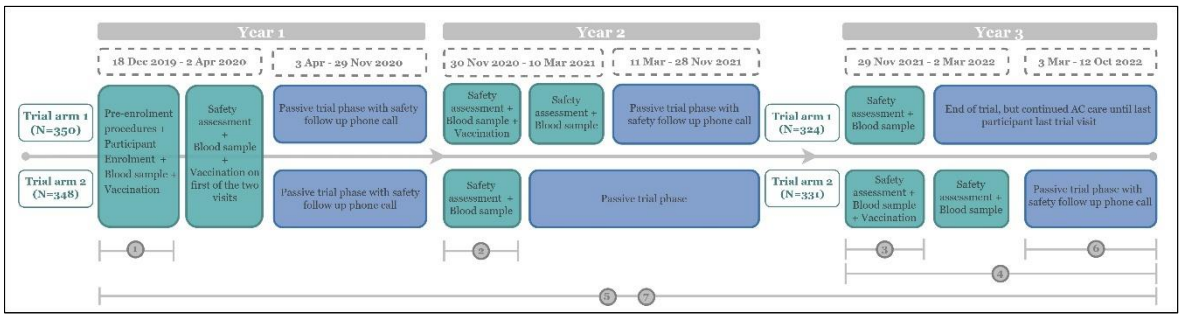
7.4.1 Study design and participants

This was a longitudinal cohort study, nested in an open-label, single-centre, randomized Ebola vaccine trial (clinicaltrials.gov: NCT04186000; Figure 1) conducted among healthcare providers (HCP). In the context of this study, HCP were categorised into two groups. The first group comprised facility-based healthcare personnel who may be exposed to infectious diseases through their professional activities. It included doctors, nurses, midwives, lab technicians, health facility cleaners, and others. The second consisted of community-based healthcare personnel; professionals who may be exposed in community settings, and included community healthcare workers, first aid workers, stretcher bearers, and similar roles. The main trial was conducted in the General Reference Hospital (GRH) of Boende (hereafter referred to as the trial site), in the Tshuapa Province of the DRC [2].

7.4.2 Ethical approval and consenting procedure

Both the trial's AC policy and the policy evaluation study were approved by the National Ethics Committee (EC) in the DRC (n°231/CNES/BN/PMMF/2021 and n°313/CNES/BN/PMMF/2020). The AC policy was introduced in the Ebola vaccine trial in November 2021, before the start of Year 3 (Figure 1). All trial participants still enrolled at that timepoint were informed of the policy and enrolled for the policy evaluation study (Figure 1, Crossbar 3). Consenting to the AC policy was not mandatory to remain in the trial. Nevertheless, all participants consented and potentially benefited from the policy until the trial's conclusion. No formal sample size calculation was performed for the policy evaluation study, which included all consenting participants.

Figure 1. Overview of the study design of the Ebola vaccine trial and ancillary care policy evaluation study with data collection periods in Boende, Democratic Republic of the Congo.



AC = ancillary care; Trial Arm 1 and Trial Arm 2 indicate the different trajectories of the two arms in the Ebola vaccine trial. Green boxes indicate scheduled visits during the active trial stage, blue boxes indicate a passive stage where no trial activities were planned. The grey crossbars refer to the data collection periods of the below specified studies:

1 Ebola vaccine trial: collection of demographics and baseline characteristics;

2 Geographic study: mapping of participants' village of residence;

3 AC policy in Ebola vaccine trial: informed consent procedure & policy evaluation study enrolment;

4 AC policy evaluation study: Adverse Event collection;

5 AC policy evaluation study: Serious Adverse Event collection;

6 AC policy evaluation study: collection of survey questions during follow up and safety phone calls in the Ebola vaccine trial;

7 AC policy evaluation study: collection of direct expenditures of policy implementation costs.

7.4.3 The AC policy in practice

The AC policy outcomes were : 1) provision of medication and/or certain diagnostic tests (e.g., malaria rapid diagnostic test) from the study pharmacy; 2) direct payment of medical invoices (e.g., for consultations or surgical interventions at GRH Boende or elsewhere) or of medication obtained from another pharmacy (e.g., if the required medication was unavailable in the study pharmacy); 3) reimbursement of medical invoices (e.g., when the participant prefinanced medication, consultations, surgical interventions or hospitalization costs); and 4) no support possible (e.g., no proof of payment). Only healthcare services provided by formal health structures were covered by the AC policy, as the researchers had insufficient legitimacy and knowledge regarding local traditional medicine practices [9].

Before AC policy implementation, (serious) adverse events ((S)AE) were managed on a case-by-case basis. Upon policy implementation, all newly reported (S)AE were systematically approached as per the AC policy and related algorithm [3]. As such, SAE that had occurred before policy implementation could be supported as well, upon availability of proof of payment. Once an (S)AE was reported during a scheduled (transport reimbursed) or unscheduled (transport not reimbursed) trial visit, the AC algorithm would determine if the medical event qualified for support.

7.4.4 Patient and public involvement

This evaluation study was set up to quantitatively assess the implementation, use and feasibility of the AC policy in the Ebola vaccine trial in Boende, DRC. The protocol of the evaluation study also included a qualitative component, i.e., participant and staff acceptability, through focus group discussions, surveys and interviews. However, the latter aspects will be published later. The study results will be presented to the national EC in the DRC in April 2024.

7.4.5 Data collection

Data from different sources were combined. First, participants' demographic information was obtained from the Ebola vaccine trial database (Figure 1, Crossbar 1). Second, coordinates of participants' residence village were collected as part of an ecologic sub-study and obtained from its database (Figure 1, Crossbar 2). Third, information on medical and financial support provided through the AC policy was collected. For AE, this was done prospectively (after AC policy implementation). For SAE, this was done both retrospectively (before AC policy implementation) and prospectively (after implementation) (Figure 1, Crossbar 4 and 5). The different AC approach for SAE and AE was based on their distinctive characteristics. Irrespective of a causality link to the IP, participants were *advised* to report all AE related or unrelated to the IP, as per the AC policy, and *required* to report all SAE related or unrelated to the IP as soon as possible, as per the main trial procedures. (S)AE definitions used within the trial and AC policy study (Supplementary Material 2) were according to the ICH E2A clinical safety data management scientific guideline [10]. Each individual (S)AE could have been treated at multiple healthcare facilities, using different treatment methods, or at different timepoints, often leading to multiple support outcomes. Fourth, six months after booster vaccination, trial Arm 2 participants were invited to partake in a short telephone survey, with multiple choice and open-ended questions that enquired (a) whether AC support was requested and received for experienced medical events, (b) if not, the reason(s) why, and (c) any change in residence (to update the geographic database) (Figure 1, Crossbar 2 and 6). Participants of Arm 1 were not contacted as this telephone visit was not in their trial schedule. Finally, all direct expenses related to the AC policy were aggregated throughout the trial as a part of the financial project administration (Figure 1, Crossbar 7).

7.4.6 Data Analysis

Demographic and baseline characteristics and (S)AE data (i.e., number of (un)related (S)AE, place of treatment, support provided via the AC policy, reasons for not using the policy) were tabulated and summarized with descriptive statistics (n (%), mean (standard deviation), or median (range)). A binary logistic regression was performed to assess whether age, sex, profession, medical history or the randomisation arm influenced participants' reporting of AE for AC policy support (yes or no). Professions were categorized as facility-based or community-based HCP.

Incidence rates were calculated for all reported (S)AE. Negative binomial regression models, assessing the mean number of AE reported per day during the trial stage (active or passive) and visit type (scheduled or unscheduled), were used to assess when AE were more frequently reported for AC support. Active trial stages denote a timeframe during which trial visits were scheduled (full trial staff capacity on site), while passive stages represent periods with no planned trial activities (less staff present).

To identify the distance between the participants' residence village and the trial site, the residence villages were geographically mapped with the trial site at the centre of circles that had a 1km, 5km, 10km, 20km, 30km and 40km radius. Unidentified villages were considered missing data. Within Boende, the capital city of the Tshuapa province, a more detailed distinction was made to include the different communes (e.g., Motema Mosantu, Marie Louise).

The telephone survey assessed whether participants felt they lived too far from the trial site to report AE for AC support. This was then compared with the actual residence distance, using Fisher Exact tests. To compare the proportion of participants using the AC policy during scheduled and unscheduled visits per residence location (≤1km, >1-5km, >10-20km, >20-30km, >30-40km, and >40km), a two-sample z-test for proportions was used. It was impossible to assess the difference against a >5-10km radius as no participants living within this area reported unsupported AE.

All data analyses were performed using R software version 4.3.1. For the geographic mapping the package *OpenStreetMap* version 0.3.4 was used, with *osm-public-transport*, and circles were added using the packages *rgeos* version 0.6-4 and *sp* version 2.0-0.

Finally, a post-trial cost analysis focusing on the management of (S)AE was conducted. Data were obtained from the trial's accounting records, and included all direct expenditures related to (S)AE management, i.e., medication and diagnostic tests from the study pharmacy, direct payment of medical invoices for medical interventions at the trial site or for medication from external pharmacies, and reimbursement of medical invoices from other health facilities pre-financed by participants.

7.5 Results

7.5.1 (S)AE reporting

In total, 655 participants were enrolled in the policy evaluation study. Overall, 393 participants (60.0%) reported at least one (S)AE. Multiple (S)AE could be reported at one timepoint. For AE, 370 participants (56.5%) reported a total of 703 cases (2.4% related, either to the IP (n=16) or to trial participation (n=1)). Overall, 196 participants reported (an) AE once (53.0%), 97 twice (26.2%), and 37 thrice (10.0%), whereas 40 participants used the policy 4 to 10 times (10.8%). For SAE, 61 participants (9.3%) collectively experienced 94 SAE from enrolment in the trial (18 December 2019) until conclusion of the trial (12 October 2022). One SAE (post-booster vaccination fever case, identified as ≥40.0°C) was considered related to the IP and two were considered related to trial participation (i.e., motorcycle accidents while traveling to or from the site for a scheduled trial visit) [9, 11].

The trial stage (active or passive follow-up) influenced the mean daily number of AE reporting ($p<0.001$; Supplementary Material 1, Table 1a). Overall, 467 AE (66.4%) were reported during the active trial follow-up stage (n=321 participants) and 236 (33.6%) during the passive trial stage (n=142 participants). The daily reporting rate of AE was reduced by 78.8% (95% CI: 71.3-84.3%) during the passive versus the active trial stage.

Likewise, the type of trial visit (scheduled or unscheduled) influenced the mean daily number of AE reporting (p<0.001; Supplementary Material 1, Table 1b). For a total of 370 participants reporting AE for AC support, 164 reported them during scheduled visits and 274 during unscheduled visits, with 68 of them reporting AE during both scheduled and unscheduled visits. The daily reporting rate of AE was reduced by 97.4% (95% CI: 90.7%-99.7%) during unscheduled versus scheduled visits.

7.5.2 Demographic and baseline characteristics (Table 1, Supplementary Table 2 and 3)

Of the 655 enrolled participants, 395 participants were community-based HCP (60.3%) and 260 were facility-based HCP (39.7%). The odds of reporting AE was not influenced by age (OR=1.01; 95% CI: 1.00-1.02), nor by medical history at enrolment (OR=1.01; 95% CI: 0.66-1.53). Compared with women and community-based HCP, men and facility-based HCP had 35.2% (95% CI: 4.0%-56.6%) and 32.3% (95% CI: 5.8%-51.4%) less likelihood of reporting AE for AC support, respectively. The odds of reporting AE for AC support was 2.12 (95% CI: 1.54-2.92) times higher for Arm 2 participants compared to Arm 1 participants. However, Arm 2 participants had twice the amount of scheduled visits compared to Arm 1 participants (two scheduled visits versus one scheduled visit). Consequently, 148 AE were reported in Arm 2 during scheduled visits compared to 41 AE in Arm 1. For unscheduled visits, Arm 1 and Arm 2 participants reported a similar number of AE (48.8% (n=251) vs 51.2% (n=263), respectively).

Table 1. Demographics and baseline characteristics of participants in the Ancillary Care policy evaluation study, Boende, Democratic Republic of the Congo.

	Participants enrolled in AC policy evaluation study (N=655)	Participants with reported AE (N=370)	Participants without reported AE (N=285)	Participants with SAE (N=61)	Survey participants with reported unsupported AE (N=111)
Race, n (%)					
Black	655 (100.0)	370 (100.0)	285 (100.0)	61 (100.0)	111 (100.0)
Sex, n (%)					
Male	508 (77.6)	280 (75.7)	228 (80.0)	44 (72.1)	90 (81.1)
Female	147 (22.4)	90 (24.3)	57 (20.0)	17 (27.9)	21 (18.9)
Age					
Median (range)	46.0 (19.0-75.0)	47.0 (19.0-74.0)	45.0 (20.0-75.0)	45.0 (21.0-68.0)	47.0 (20.0-65.0)
Mean (sd)	45.2 (11.9)	45.5 (12.1)	44.6 (11.6)	44.7 (11.5)	46.1 (10.9)
Profession all categories, n (%)#					
Community health worker[#]	225 (34.4)	122 (33.0)	103 (36.1)	13 (21.3)	37 (33.3)
Nurse*	170 (26.0)	83 (22.4)	87 (30.5)	19 (31.2)	36 (32.4)
First aid worker[#]	161 (24.6)	109 (29.5)	52 (18.3)	18 (29.5)	22 (19.8)
Hygienist*	36 (5.5)	23 (6.2)	13 (4.6)	4 (6.6)	3 (2.7)
Midwife*	28 (4.3)	18 (4.9)	10 (3.5)	2 (3.3)	4 (3.6)
Doctor*	11 (1.7)	5 (1.4)	6 (2.1)	1 (1.6)	1 (0.9)
Health facility cleaner*	10 (1.5)	4 (1.1)	6 (2.1)	2 (3.3)	3 (2.7)
Care Giver[#]	7 (1.1)	2 (0.5)	5 (1.8)	1 (1.6)	3 (2.7)
Lab Technician*	2 (0.3)	0 (0.0)	2 (0.7)	1 (1.6)	0 (0.0)
Pharmacist aid[#]	2 (0.3)	2 (0.5)	0 (0.0)	0 (0.0)	1 (0.9)
Other*	3 (0.5)	2 (0.5)	1 (0.4)	0 (0.0)	1 (0.9)
Medical history, n (%)					
Yes	125 (19.1)	73 (19.7)	52 (18.3)	12 (19.7)	96 (86.5)
No	530 (80.9)	297 (80.3)	233 (81.8)	49 (82.3)	15 (13.5)
Unknown	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Trial arm, n (%)					
Arm 1	332 (50.7)	156 (41.6)	169 (59.3)	27 (44.3)	0 (0.0)
Arm 2	323 (49.3)	216 (58.4)	116 (40.7)	34 (55.7)	111 (100.0)

*AE = adverse event; SAE = serious adverse event; N = the total number of participants in a given category; n (%) = the number (percentage) of the participants corresponding to the demographic or baseline characteristic category; sd = standard deviation; [#]Professions categorized as community-based healthcare providers during logistic regression analysis; *Professions categorized as facility-based healthcare providers during logistic regression analysis.*

7.5.3 AC policy support outcomes (Table 2)

For AE, 621 cases (75.3%) were supported with medication from the study pharmacy. For 161 AE (19.5%), medication was either not available in the study pharmacy but obtained from external pharmacies, and/or another form of medical care was provided (e.g., consultation at the GRH) and directly paid by the study funds. For 24 AE (2.9%), participants presented their medical invoices and were reimbursed. Two AE (0.2%) were supported with methods initially not foreseen in the policy. First, one participant received a consult when a neuropsychiatrist from UNIKIN was present in Boende. Second, a COVID-19 test was performed by study staff for a participant experiencing symptoms. Finally, for 17 AE (2.1%) treated elsewhere and reported afterwards, reimbursement was not possible because of the unavailability of invoices and/or other proof of payments. However, only one AE did not receive any support. Sixteen others still partially benefited from the policy through another form of support (e.g. study pharmacy medication; Supplementary Material 1, Table 4). During the active trial stage, a lower proportion of AE (69.2%, n=400) was treated with medication from the study pharmacy compared with the passive stage (89.5%, n=221) ($p<0.001$). Additionally, medication for AE treatment was more frequently sought in external pharmacies, or another form of medical care was provided and paid for directly with study funds (24.9%, n=144) compared with the passive trial stage (6.9%, n=17) ($p<0.001$). Lastly, no differences were observed for 'reimbursements of medical invoices' ($p=0.89$), 'other support' ($p=0.88$) or for 'no support possible' ($p=0.06$) for AE between both trial stages.

Overall, 23 SAE cases (29.1%) were supported via the reimbursement of medical expenses bore elsewhere, 17 (21.5%) via study pharmacy medication and 13 (16.5%) via the direct payment of medical expenses (i.e., medications or interventions). Two SAE (2.5%) related to trial participation received partial financial support for treatment via traditional medicine practices, as these were preferred by the participants over conventional medicine [9]. This was provided ad hoc and not foreseen in the AC policy. Unfortunately, 24 SAE (30.4%) could not be (fully) supported because of the unavailability of invoices and/or proof

of payments (Supplementary Material 1, Table 4). Most of these cases (n=19) occurred before the AC policy implementation. When comparing the AC support for SAE, the proportion of participants treated with study pharmacy medication before (18.2%, n=8) and after (25.7%, n=17) policy implementation was not significantly different (p=0.59). However, there was an increase in the proportion of medical invoices for SAE that were directly paid by the study funds after policy implementation (34.3%, n=12), compared to before policy implementation (2.3%, n=1; p<0.001), making this the most applied AC support outcome for SAE after policy implementation. Additionally, a significant decrease (43.2% to 14.3%, n=19 and n=5, respectively) in the proportion of SAE that could not be (fully) supported was seen after policy implementation (p=0.01). No differences were observed for the 'reimbursements of medical invoices' (p=0.40) or 'other support' (p=1.00) outcomes before versus after policy implementation.

7.5.4 Treatment location/method (Table 2)

Despite the long distances that some participants had to travel to reach the study site (Supplementary Material 1, Figure 1) with limited transportation means (e.g., dugout canoe, bicycle, motorbike, on foot), a higher proportion of AE was treated at the trial site during the passive trial stage (96.6%, n=227) than during the active stage (92.1%, n=453; p=0.03; Table 2). Additionally, more participants reported self-medication to treat AE during the active trial stage (n=26, 5.3%), compared to the passive stage (n=2, 0.9%). No other differences in healthcare seeking behaviour were observed between the active and passive trial stages for AE.

For SAE, participants' healthcare seeking behaviour in terms of treatment location and methods was similar before and after AC policy implementation.

Table 2. Ancillary care (AC) support provided for serious adverse events before/after AC policy implementation (per trial stage) and treatment location/method, Boende, Democratic Republic of the Congo.

	Before AC implementation	After AC policy implementation						Overall (before and after)	AE Active vs Passive trial period	SAE Before vs after AC implementation
		Active trial stage		Passive trial stage		Overall after AC policy implement-tation				
AC support provided, n (%)	**SAE N = 44**	**AE N = 578**	**SAE N = 24**	**AE N = 247**	**SAE N = 11**	**AE N = 825**	**SAE N = 35**	**SAE N = 79**	**p-value#**	**p-value#**
Study pharmacy medication	8 (18.2)	400 (69.2)	6 (25.0)	221 (89.5)	3 (27.3)	621 (75.3)	9 (25.7)	17 (21.5)	**<0.001**	0.59
Direct payment of medical invoices	1 (2.3)	144 (24.9)	6 (25.0)	17 (6.9)	6 (54.6)	161 (19.5)	12 (34.3)	13 (16.5)	**<0.001**	**<0.001**
Reimbursement of medical invoices	15 (34.1)	16 (2.8)	6 (25.0)	8 (3.2)	2 (18.2)	24 (2.9)	8 (22.9)	23 (29.1)	0.89	0.40
No support possible	19 (43.2)	16 (2.8)	5 (20.8)	1 (0.4)	0 (0.0)	17 (2.1)	5 (14.3)	24 (30.4)	0.06	**0.01**
Other	1 (2.3)	2 (0.4)	1 (4.2)	0 (0.0)	0 (0.0)	2 (0.2)	1 (2.9)	2 (2.5)	0.88	1.00
Treatment location/method, n (%)	**SAE N = 40**	**AE N = 492**	**SAE N = 20**	**AE N = 235**	**SAE N = 12**	**AE N = 727**	**SAE N = 32**	**SAE N = 72**	**p-value#**	**p-value#**
Medical doctor of GRH Boende or trial site	17 (42.5)	453 (92.1)	11 (55.0)	227 (96.6)	9 (75.0)	680 (93.5)	20 (62.5)	37 (51.4)	**0.03**	0.15
Medical doctor or health care personnel outside of GRH Boende	18 (45.0)	9 (1.8)	8 (40.0)	6 (2.6)	2 (16.7)	15 (2.1)	10 (31.3)	28 (38.9)	0.72	0.34
Self-medication	1 (2.5)	26 (5.3)	0 (0.0)	2 (0.9)	0 (0.0)	28 (3.9)	0 (0)	1 (1.4)	**0.007**	1.00
Traditional healer/ medicine*	3 (7.5)	2 (0.4)	1 (5.0)	0 (0.0)	1 (8.3)	2 (0.3)	2 (6.3)	5 (6.9)	0.83	1.00
Other/ Unknown	1 (2.5)	2 (0.4)	0 0.0)	0 (0.0)	0 (0.0)	2 (0.3)	0 (0.0)	1 (1.4)	0.83	1.00

*AC= ancillary care; AE = adverse event; SAE = serious adverse event; GRH = General Reference Hospital; N = the total number in a given category; n (%) = the number (percentage) corresponding to a specific sub-category; For each (S)AE there were multiple treatment locations/methods and AC support outcomes possible; * Traditional healer/medicine indicates that a traditional healer was consulted, or that the participant reported to have taken traditional medicine through self-medication; # Two-sample z-test for proportions or Fisher exact test were used where applicable.*

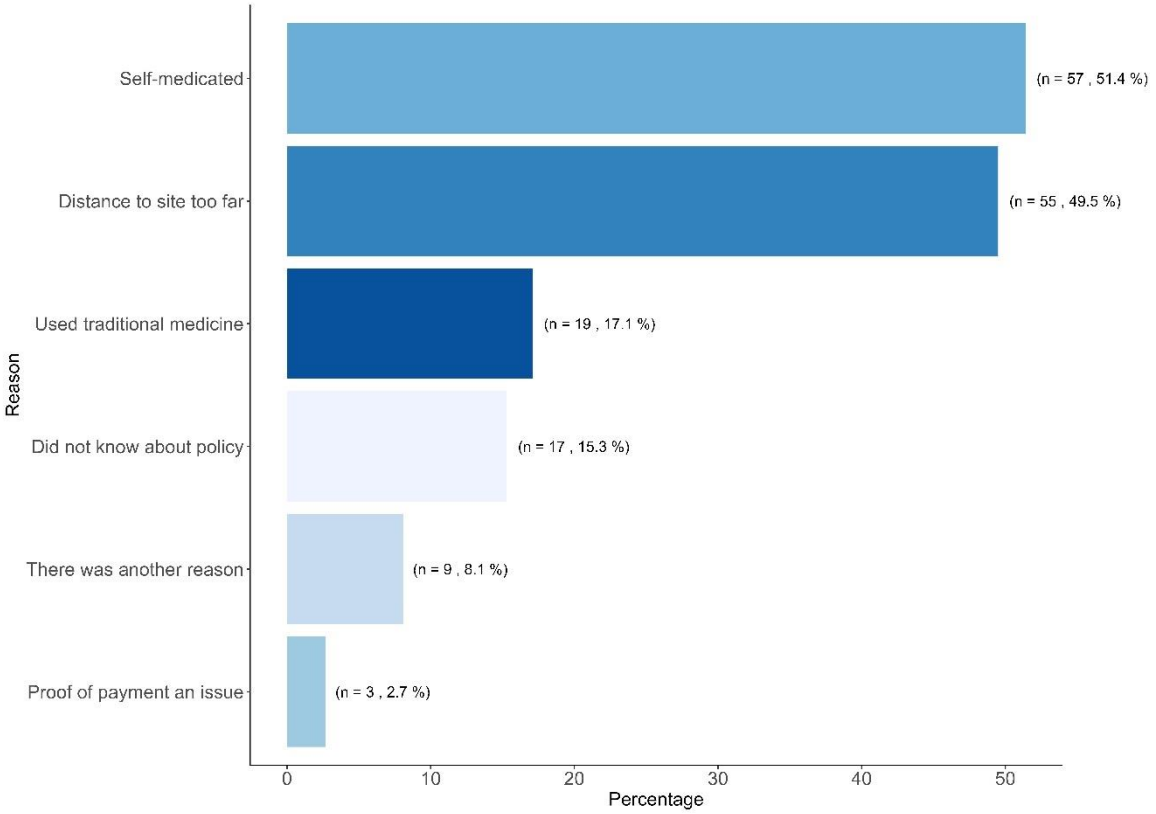
7.5.5 Telephone survey outcomes and geographic mapping

A total of 314 participants from Arm 2 were contacted by telephone 6 months after booster vaccination. Of them, 311 participated in the AC policy evaluation survey. Of these 311, 111 (35.7%) reported an AE for which support was not sought, or was not possible according to the AC policy. Figure 2 shows participants' reasons for not using/receiving AC policy support when an AE occurred. In total, 57 participants indicated to have self-medicated (51.4%), followed by 55 who perceived the distance to the trial site to be too far (49.6%), and 19 used traditional medicine instead of conventional healthcare covered by the AC policy (17.1%). Although all participants were informed of the AC policy and consented to use it, 17 participants (15.3%) indicated not to know that their AE could have been supported. However, only three participants expressed this as the sole reason; 14 did so in combination with other reasons (i.e., 'I live too far', 'I had no proof of payment', 'I self-medicated', 'I used traditional medicine'; Supplementary Material 1, Table 5). Nine participants (8.1%) specified other reasons for not using the AC support, including alternative support provided by the community or other sources [12], stock-outs at the study pharmacy, time constraints to travel to the site, and long waiting times for consultations at the trial site. Finally, three participants (2.7%) could not be supported because they could not present a proof of payment.

When assessing whether the actual residence distance from the trial site influenced the use of the AC policy, analysis shows that participants living less than 1km or >1-5km from the site seldomly (n=7 and n=5, respectively) indicated the distance as an issue (Figure 3, Supplementary Material 1, Table 6). When comparing the type of visit, participants more frequently used the AC policy during unscheduled (30.7%) versus scheduled visits (16.5%) when living within a 1km radius from the trial site (p=0.001; Supplementary Material 1, Table 7). However, a higher proportion of AE were reported during scheduled visits compared to unscheduled visits for participants living between >10-20km (24.4% vs 14.6%, p=0.02 and >20-30km (14.6% vs 7.3%, p=0.02) from the site. This was not significant for participants living at a distance of >30-40km and >40km, but this could be due to the

smaller sample size in these groups. Participants living at a >1-5km distance made use of the policy during scheduled and unscheduled visits similarly (p=0.36). When living more than 10km from the trial site, between 71.4% and 100.0% of the surveyed participants perceived the distance to the site as an issue (Supplementary Material 1, Table 6). However, 90 participants (32.9%) did travel more than 10km for AC support during unscheduled visits (Supplementary Material 1, Table 7).

Figure 2. Participants' reasons for not using/receiving ancillary care policy support for an experienced adverse event (N=111); ancillary care policy evaluation study, Boende, Democratic Republic of the Congo.

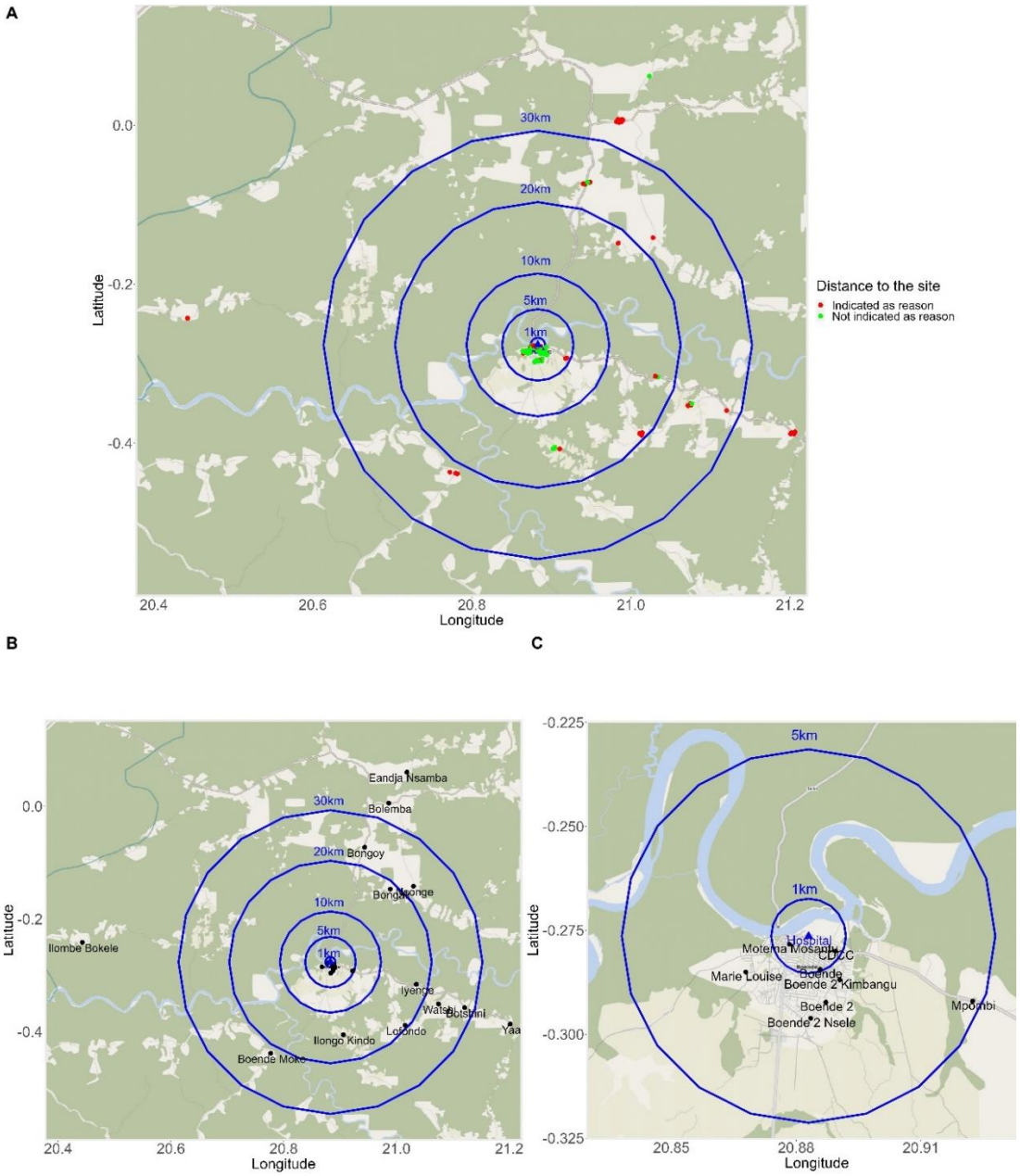


7.6 Cost assessment

Upon policy implementation, an initial budget of €10,000 from the PI funds was allocated for AC support. The total cost ultimately amounted to €33,196 representing 1.1% of the operational budget available to the PI. When also taking into account the sponsor budget, this percentage dropped to 0.4% of the total study budget, highlighting the limited financial impact of the AC policy for this trial.

The total expenditure included 1) the set-up and replenishment of the study pharmacy, created at the start of the trial, independently of the AC policy, and 2) the direct payment or reimbursement of AE treatment costs during the AC implementation period, and of SAE during the entire trial duration. In practice, this came down to health care sought in the surrounding areas of the participants' residence location, which is the "best care locally-available". The study pharmacy medication took up the largest part of the AC budget (28,448 euro or 85,7%), whereas the reimbursement and/or direct payments of medical invoices for both AE and SAE (e.g., prefinanced consultations, medication, surgical interventions or hospitalization) amounted to 4,748 euro (14.3%). However, 41 (S)AE could not be (fully) reimbursed when proof of payment was not available (Supplementary Material 1, Table 4).

Figure 3. Villages of participants reporting an adverse event for which ancillary care policy support was not sought or provided (N=111; Boende region, Democratic Republic of the Congo).



▲ *Trial site location; Panel A shows the residence location of participants. Red dots represent locations of those who indicated that their residence was "too far" from the study site and therefore did not use the ancillary care (AC) policy support for an experienced AE. Green dots represent the participants who did not report distance as a reason for non-policy use. The bottom left panel (B), shows the residence village names of participants shown in panel A. In panel A and B, the distance from the site is indicated up to a 30km radius. The bottom righ pannel (C) zooms in at a 5km radius from the site location to show the village names/Boende communes that are not readable on panel B. Five participants were not included in these plots as they had relocated to the health zones of Bokungu and Mbandaka, located more than 100km distance in radius from the trial site. All but one of these participants indicated distance to the site as the reason for not using the AC policy.*

7.7 Discussion

We previously described the development process of this study-specific AC policy, as well as some particular implementation challenges which triggered ethical dilemmas regarding insurance coverage gaps for mobility-related injuries, the use of traditional medicine practices and the strong family involvement in participants' SAE management [3, 9]. Previous studies have assessed stakeholder representatives' perceptions and experiences of AC, or discussed models or decision-making processes involving AC practices [13-16]. Researchers have called for a quantitative assessment of AC needs and for the evaluation of AC delivery services [17, 18]. Other scholars presented practical case studies, or reported on their experiences with the provision of AC when participants developed a life-threatening illness during research, suggesting that AC provision is feasible, if supported by the commitment of investigators and funders [18, 19]. To the best of our knowledge, this is the first formal quantitative evaluation of a study-specific AC approach.

The policy evaluation lasted 11 months. During this period, over half (n=370, 60.0%) of the enrolled trial participants made use of the AC policy. All three initially-planned AC support options (i.e., study pharmacy medication, reimbursement of medical invoices, and direct payment of medical invoices) were used in both AE and SAE management. For AE, the most frequently used support was the provision of medication via the study pharmacy (n=621, 75.3%). For SAE, many costs were directly paid for (n=12, 34.3%) once the policy was implemented.

Providing AC could offer a significant benefit, not only from an ethical perspective, but also from a scientific prospective, as it is likely to result in a more comprehensive reporting of AE. Still, we observed a lower likelihood of AE reporting for facility-based HCP compared to community-based HCP, and for men compared to women. This observation may be due to a difference in educational background and/or the place of work (i.e., HCP work in health facilities with access to healthcare services) [20, 21]. Additionally, a comprehensive study conducted across 59 countries revealed that women generally use healthcare services at

rates equal to or surpassing those of men [22]. The authors argue that the gender gap in self-reported health appears to be shaped by a combination of societal (e.g., disparities in employment and education, coupled with gender inequality) and biological factors [22].

In this study, Arm 2 participants were twice as likely to report AE compared to Arm 1 participants. However, as their AE reporting was similar during unscheduled visits, this can largely be explained by the higher number of scheduled visits in Year 3 for Arm 2 participants compared to Arm 1 participants (two versus one scheduled visit, respectively). Additionally, one of the two scheduled visits was seven days after booster vaccination, which could lead to a higher use of the AC policy if an AE after vaccination was still persisting. Conversely, we estimate that the administration of the vaccine did not greatly influence the AC policy use, as only 2.3% (n=16) of AE and 1.1% (n=1) of SAE were considered related to vaccination. Thus, the population and design of the Ebola vaccine trial impacted the use of the AC policy by the participants. Study findings indicated a 78.8% (95% CI: 71.3-84.3%) reduction in the daily AE reporting rate during the passive trial stage compared to the active stage, and a 97.4% (95% CI: 90.7%-99.7%) reduction during unscheduled versus scheduled visits. Consequently, a trial with more scheduled visits or lengthier active stages is likely to see a higher frequency of participants utilizing the AC policy. However, it is noteworthy that many AE were also reported during unscheduled trial visits (n=514) and the passive follow-up stage of the trial (n=236).

The trial design influenced the application of the AC policy by study personnel. Unexpectedly, study doctors made less use of the study pharmacy medication for AE treatment in the active compared to the passive study stage. Several reasons could apply. First, as reported by participants during the telephone surveys, the study pharmacy experienced periodic stock-ruptures which impacted medication availability. Second, more study staff (e.g., (co-)PI, financial administrator) was present during the active trial stage, facilitating the obtention and direct payment of medication from external pharmacies (e.g., if the preferred medication was unavailable at the study pharmacy) and/or other methods of medical care (e.g. consultation at the GRH of Boende) with study funds. Following this,

we hypothesize that stock-outs in the study pharmacy during the active study stage were mediated by obtaining medication from external pharmacies or directly paying for other medical care, i.e., decreasing the study pharmacy use, while alternative medication available in the study pharmacy was provided during stock-outs in the passive study stage, i.e., increasing its use.

When it comes to participants' self-reported reasons for the non-use of AC policy support for AE, just over half of the surveyed participants (n=57, 51.4%) reported to have resolved their AE through self-medication. Considering that the study population were HCP and frontliners, this outcome was not unexpected [20, 23]. It is uncertain, whether other population groups would have self-medicated similarly for AE treatment.

Nearly half of the surveyed participants (n=55, 49.6%) indicated the distance to the study site as a reason for not using the AC policy. Upon further geographic analysis, we found that distance was more commonly reported as an issue when participants lived >10km from the study site. Though this may seem relatively near, the local setting needs to be considered: most participants travelled on foot, by bicycle, motorbike or dugout canoe, facing natural obstacles in the environment to reach the study site [24]. Unfortunately, details on the type of AE (e.g., severity or urgency) could not be taken into account in the analysis as they were not collected as part of the survey. A further limitation was that the specific location of the health centres in the area, and their type and quality of healthcare were unknown to the researchers. These aspects could therefore not be taken into account during analysis. However, while health centres are typically available at a 5km travelling distance for DRC's residents [24], we observed that several participants who lived more than 10km from the site still reported AE for AC support during unscheduled visits (n=90).

Even though the AC policy was thoroughly explained during an informed consent procedure, and all participants consented to it, seventeen surveyed participants (15.3%) expressed a lack of awareness regarding the potential support for their AE. Although recall bias could be at play, we suggest, based on the replies to the telephone survey, that *interpretation bias* may have occurred. To the specific multiple choice question, "Why did

you not come to the trial for treatment or a reimbursement of expenses?", 14 out of 17 participants responded 'I didn't know it was possible', in combination with other reasons (i.e., 'I live too far', 'I had no proof of payment', 'I self-medicated', 'I used traditional medicine'). As the latter reasons could indeed not be supported, it is unclear whether participants thought support was not possible *because* of the other reason, or that they, in general, did not know/recall the AC policy at all. Moreover, language barriers or illiteracy may have contributed as well. This would suggest that consent documents and procedures were either too lengthy and complex, or too hurried, leading to poor understanding and recall [25-27].

Three elements were initially not integrated in the AC policy, but emerged as needs and were addressed during the trial. First, as the COVID-19 pandemic could not be anticipated, COVID-testing performed by a study physician was not foreseen, but later implemented free of charge when participants presented with symptoms. If tested positive, they were referred to the GRH of Boende for a free consultation, as per local guidelines at the time. Second, conventional psychological and mental healthcare services are not available in Boende. However, a consultation was provided when a neuropsychiatry professor from UNIKIN was in Boende for a trial-related workshop [28]. Third, some participants turned to traditional medicine for the treatment of their SAE, which were discussed elsewhere [9]. Altogether, these three experiences point to the necessity of flexible AC procedures, policies and guidelines, that are adaptable to a complex environment or unforeseen elements, such as the COVID-19 pandemic.

Seen the relative small expenditures for AC support (€33,196; 1.1% of the operational budget, and 0.4% of the overall study budget), this evaluation study shows that the health benefits for participants greatly outweighed AC policy costs. Therefore, depending on the trial budget, the attributes of the local health system, participant characteristics (e.g., chronically ill participants compared to our generally healthy (based on vital signs and physical examination)), and participant needs, our AC policy could be adapted accordingly for other clinical research in resource-constrained settings.

This study had some limitations. First, the demographics and baseline characteristics of the participants were collected at enrolment in the main trial, two years before the start of the AC policy evaluation study. As such, parameters such as medical history might have changed. Second, there was a *reporting bias*, i.e., participants who for various reasons did not come to the site for AE reporting and AC policy support, were missed. Only the reported events were included in the policy evaluation. However, this limitation was partially addressed when enquiring about any non-reported AE during the telephone survey, including the reasons for non-reporting. Third, the geographic mapping used to represent distance from the trial site made use of the Euclidean distance only, and was not triangulated with social determinants of health (e.g., education or socio-economical background) and other travel barriers that affect access to care (e.g., seasonal variation, land use, road network, geographic factors) [29, 30]. Fourth, the AC policy was only implemented in the last year of the trial, which may have impacted the assessment of costs related to the reimbursement and/or direct payment of medication. Fifth, the AC policy restricted the provision of health care services to those available locally, and omitted specialized care available outside of the research setting. This limitation also had implications for the policy's cost assessment. Lastly, no indirect costs (e.g., cost of additional manpower to ensure (S)AE management (of unrelated cases)) were included in the costs analysis.

7.8 Conclusions and recommendations

This study indicates that an AC policy can be introduced in a clinical trial without excessively burdening the research team and local health system. We believe, in light of the high uptake, applicability, and financial feasibility of the AC policy, that it is feasible and ethically commendable to implement a study-specific AC policy during clinical trials in resource-constrained settings. This evaluation study demonstrates that the characteristics of the trial design, study population, site accessibility, local context, and local health system altogether influence the use and applicability of an AC policy. All possible support options of our trial-specific policy (i.e., provision of medication from a study pharmacy, and direct payment or

reimbursement of medical invoices of locally available healthcare services) were crucial in providing adequate, equal and systematic medical care for (S)AE to trial participants. The policy was most applied for AE with the provision of medication from the study pharmacy. Our findings can inform the development of study-specific AC policies for other clinical trials in resource-constrained settings, in order to reconcile the achievements of research objectives with the protection of the health and wellbeing of participants. We hope that the results of this study can inspire and motivate policy makers, national EC, and funders to require feasible but adequate AC measures in global health research.

7.9 Declarations

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Conflict of interest. Authors have no conflicts to declare.

Authors' contributions. GL and YL wrote the manuscript; GL developed the data collection tools for the policy evaluation study; YTB, ED, SM, and PK were involved in the data collection; YL and GL performed data management of the data included in this article; YL performed all analyses included in this article; BIO performed data management of the trial and checked the data analysis included in the manuscript; RR assisted in effectively translating the analysis into the manuscript and improving the clarity of writing; HMM and PM were (co)-principal investigators, VM was project coordinator, TZM was site coordinator; PVD and JPVG were the sponsor of the Ebola vaccine trial. All authors reviewed and contributed to the final manuscript.

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7.11 Supplementary material

7.11.1 Tables

Table 1a. Negative binomial regression model assessing the average number of visits per day adjusted for trial stage; ancillary care policy evaluation study, Boende, Democratic Republic of the Congo.

	Estimate	Std. Error	P-value
(Intercept)	1.60	0.12	**<0.001**
Passive stage	-1.55	0.15	**<0.001**

Table 1b. Negative binomial regression model assessing the average number of visits per day adjusted for type of visit; ancillary care policy evaluation study, Boende, Democratic Republic of the Congo.

	Estimate	Std. Error	P-value
(Intercept)	4.14	0.64	**<0.001**
Unscheduled visit	-3.66	0.65	**<0.001**

Table 2. Odds ratios with 95% confidence intervals per predictor as per the logistic regression model (outcome: reporting of adverse events Yes or No); ancillary care policy evaluation study, Boende, Democratic Republic of the Congo.

	OR	2.5%	97.5%
(Intercept)	0.98	0.48	2.00
Profession facility-based HCP	0.68	**0.49**	**0.94**
Gender Male	0.65	**0.43**	**0.96**
Age	1.01	1.00	1.02
Medical history Yes	1.01	0.66	1.53
Study Arm 2	2.12	**1.54**	**2.92**

HCP= Healthcare provider; OR = Odds ratio

Table 3. Adverse event reporting rate according to the visit type per trial arm; ancillary care policy evaluation study, Boende, Democratic Republic of the Congo.

Trial arm, n (%) – number of days where this type of visit applied	Scheduled visits (N = 189)	Unscheduled visits (N = 514)	All visits (N = 703)
Arm 1	41 (21.7) – 1 day	251 (48.8) – 318 days	292 (41.5)
Arm 2	148 (78.3) – 2 days	263 (51.2) – 318 days	411 (58.5)

Table 4. (Serious) adverse events with 'no support possible' as outcome; ancillary care policy evaluation study, Boende, Democratic Republic of the Congo.

AC (non-)support outcome(s), n (%)	AE (N = 17)	SAE with 'no support possible' outcome, according to timepoint of AC policy implementation		
		Before policy implementation (N=19)	After policy implementation (N=5)	Overall (N=24)
No invoice or proof of payment	1 (5.9)	12 (63.2)	3 (60.0)	15 (62.5)
Combination 1: • No invoice or proof of payment • BUT direct payment of medical expenses	3 (17.6)	0 (0.0)	0 (0.0)	0 (0.0)
Combination 2: • No invoice or proof of payment • BUT medication from study pharmacy	9 (52.9)	6 (31.6)	2 (40.0)	8 (33.3)
Combination 3: • No invoice or proof of payment • BUT direct payment of medical expenses • BUT medication from study pharmacy	4 (23.5)	0 (0.0)	0 (0.0)	0 (0.0)
Combination 4: • No invoice or proof of payment • BUT medication from study pharmacy • BUT reimbursement of medication expenses	0 (0.0)	1 (5.3)	0 (0.0)	1 (4.2)

AC = ancillary care, AE = adverse event; SAE = serious adverse event; N = the total number of events with 'no support possible'; n (%) = the number (percentage) of events corresponding to a specific sub-category of 'no support possible'

Table 5. Amount of survey participants indicating 'I did not to know support was possible', in combination with other reasons for non-support; ancillary care policy evaluation study, Boende, Democratic Republic of the Congo.

Surveyed participants' reasons for non-support of an AE (N=17)	
Support outcome	**n (%)**
I did not know support was possible	3 (17.6)
Combination 1: • I did not know support was possible • AND I live too far	6 (35.3)
Combination 2: • I did not know support was possible • AND I did not have no proof of payment	1 (5.9)
Combination 3: • I did not know support was possible • AND I self-medicated	3 (17.6)
Combination 4: • I did not know support was possible • AND I used traditional medicine	1 (5.9)
Combination 5: • I did not know support was possible • AND I live too far • AND I self-medicated	3 (17.6)

AE = adverse event; N = the total number of participants that indicated having an AE for which no support was possible/sought from the AC policy; n (%) = the number (percentage) of participants corresponding to a specific sub-category.

Table 6. Comparison of perception of distance as "too far" to the trial site versus the distance based on residence coordinates; ancillary care policy evaluation study, Boende, Democratic Republic of the Congo.

Distance	**≤1km**	**>1-5km**	**>10-20km**	**>20-30km**	**>30-40km**	**>40km**	**N**
≤1km	7 (25.0)	0.52	**0.004**	**<0.001**	**<0.001**	**0.03**	28
>1-5km	FE	5 (16.1)	**<0.001**	**<0.001**	**<0.001**	**0.008**	31
>10-20km	FE	FE	11 (73.3)	0.69	0.09	1.00	15
>20-30km	FE	FE	FE	13 (81.3)	0.23	0.62	16
>30-40km	FE	FE	FE	FE	14 (100.0)	0.10	14
>40km	FE	FE	FE	FE	FE	5 (71.4)	7

N = total number of participants that experiences an adverse event for which treatment was not sought or possible per actual residence distance to the site; Diagonally, the n (%) – number (percentage) – of participants indicating the distance as too far to use ancillary care support; below the diagonal the used statistical test is shown; FE = Fisher Exact test; above the diagonal the p-value of test FE test shown

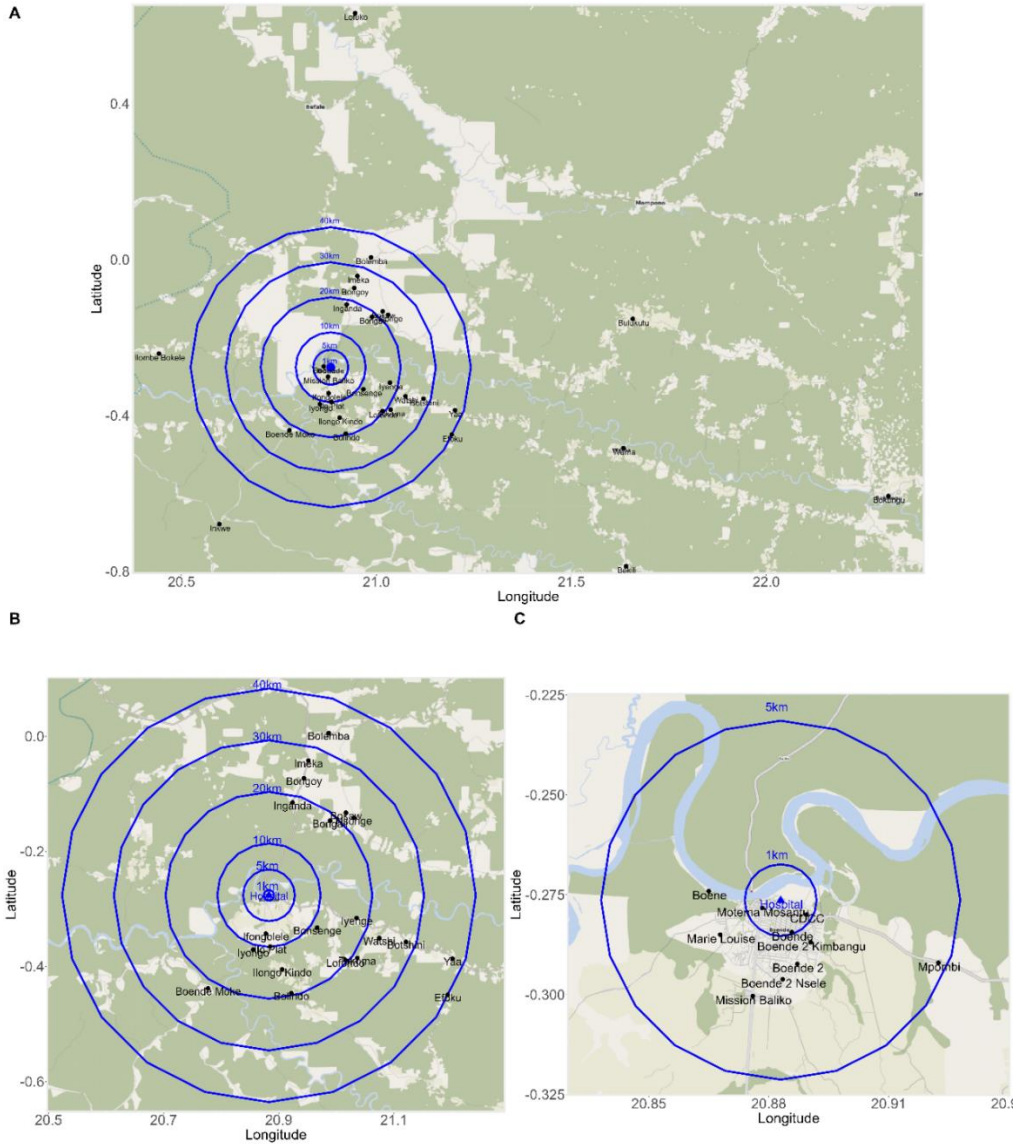
Table 7. Distance of residence from the study site for participants with adverse events; ancillary care policy evaluation study, Boende, Democratic Republic of the Congo.

	Participants with AE reported to the site (N = 370)	**Arm 1 and Arm 2 participants coming for unscheduled visits (N=274)**	**Arm 1 and Arm 2 participants coming for scheduled visits (N=164)**	**Unscheduled vs scheduled***	**Arm 2 participants not using/supported by AC policy for an AE (N=111)**
Residence distance	**n (%)**	**n (%)**	**n (%)**	**p-value**	**n (%)**
≤1km	100 (27.1)	84 (30.7)	27 (16.5)	**0.001**	28 (25.2)
>1-5km	109 (29.5)	88 (32.1)	45 (27.4)	0.36	31 (27.9)
>5-10km	5 (1.4)	5 (1.8)	0 (0.0)	-	0 (0.0)
>10-20km	63 (17.0)	40 (14.6)	40 (24.4)	**0.02**	15 (13.5)
>20-30km	35 (9.5)	20 (7.3)	24 (14.6)	**0.02**	16 (14.4)
>30-40km	28 (7.6)	15 (5.5)	16 (9.8)	0.13	14 (12.6)
>40km	20 (5.4)	15 (5.5)	9 (5.5)	1.00	7 (6.3)
Unknown	10 (2.7)	7 (2.6)	3 (1.8)	-	0 (0.0)

*AC= ancillary care; AE = adverse event; N = the total number of participants with an AE reported for which support was sought; n (%) = the number (percentage) of participants corresponding to a specific sub-category; *Two-sample z-test for proportions*

7.11.2 Figure

Supplementary Figure 1. Villages of participants indicating to have had an Adverse Event for which Ancillary Care policy support was sought (N=370; Boende region, Democratic Republic of the Congo)



▲ *Trial site location; The upper panel (A) shows all villages in the Boende health zone, or its surrounding health zones (Befale, Wema, and Bokungu), from where participants travelled to obtain medical and/or financial support for (an) AE(s). Participants living further than the surrounding health zones (N=6), or for which the village of residence was unknown (N=3), were not included in these analyses. For six additional villages (five in the Boende health zone, and one in the Wema health zone) coordinates could not be obtained. The lower left panel (B) zooms in at a 40km radius from the site location to show the village names that were not readable on panel A. The lower right panel (C) zooms in at a 5km radius from the site location to show the villages and Boende communes that were not readable on panel B.*

Part IV – General discussion

Chapter 8 General discussion

8.1 Trial in context

8.1.1 The vaccine trial results

The Ad26.ZEBOV, MVA-BN-Filo vaccine regimen followed by an Ad26.ZEBOV booster dose showed promising results in terms of safety and humoral immune (memory) response in HCP and frontliners working and living in Boende, a remote, rural, and Ebola endemic area of the DRC. This general discussion consolidates our findings from two separate publications, highlighting the safety, immunogenicity, and antibody persistence of the vaccine regimen, and subsequently, the impact of booster doses administered one or two years after the first dose, and puts it into context within existing literature.

The administration of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen administered 56 days apart, was observed to be safe, with no SAEs considered related to the IP up to six months after the second dose. Additionally, a high immune responder rate of 96% among per-protocol vaccinated HCP and frontliners was found at 21 days after the second dose. These findings are consistent with what has previously been observed and published in adults receiving the regimen [1-8]. Additionally, the Ad26.ZEBOV booster dose showed to be generally well tolerated and, as expected for an anamnestic response, elicited a fast (i.e., within seven days) and robust (i.e., 39-times higher post-booster than pre-booster, and approximately 2.5-times higher than 21 days after the second dose) EBOV GP-specific binding antibody response both one and two years after the first dose. While serious adverse events were not collected after the primary vaccine regimen for this study and post-booster AEs could thus not be compared against the reactogenicity of the regimen, we found that injection site pain and headache (followed by fatigue and myalgia) were most commonly reported as local and systemic AEs, respectively. This is in line with findings from previous studies in adults that have assessed the reactogenicity of the Ad26.ZEBOV,

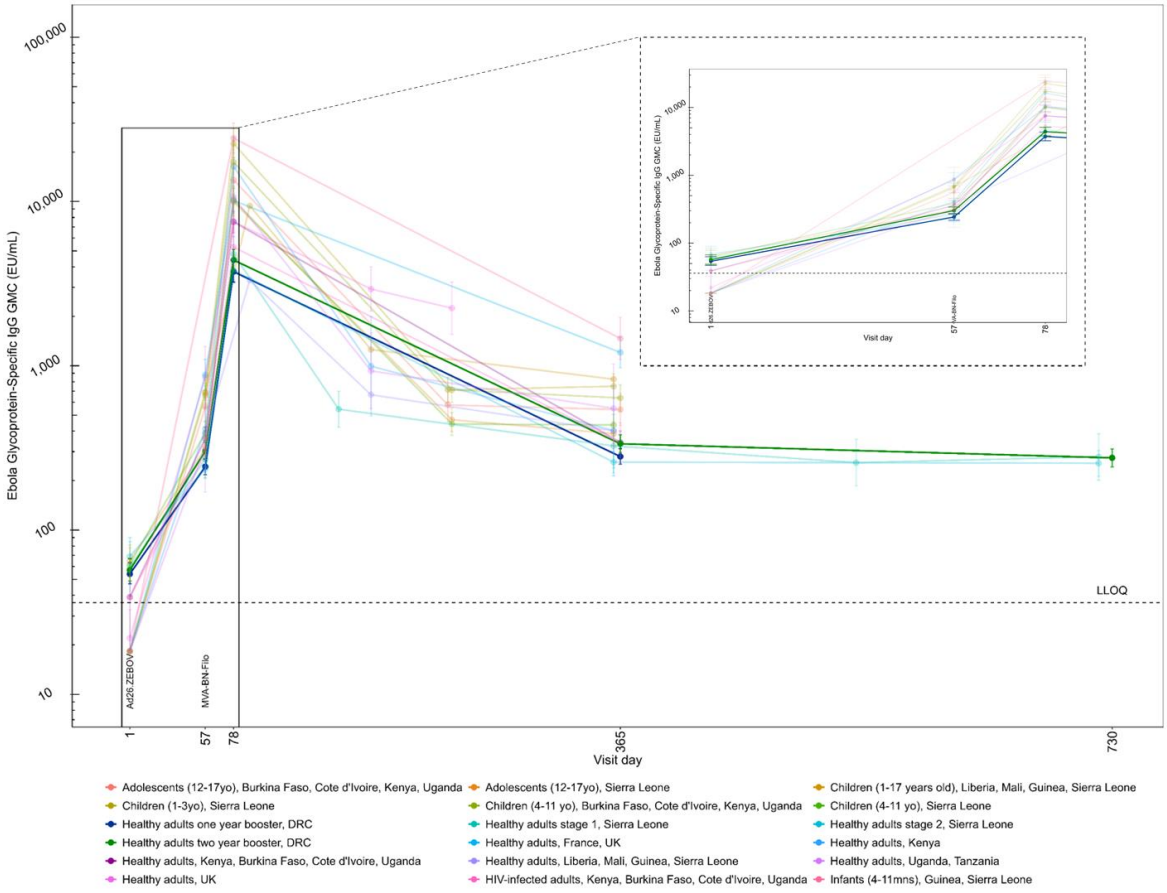
MVA-BN-Filo vaccine regimen [1-6, 9]. Additionally, two studies have previously compared the reactogenicity of the first Ad26.ZEBOV dose with the Ad26.ZEBOV booster dose and found no notable differences [1, 6].

One SAE of fever above 40°C after booster vaccination (Ad26.ZEBOV booster dose administered two years after the first dose) was considered related to the investigational product and reported as a "medically important event". No hospitalisation was required to treat this SAE and it resolved without sequelae the day after onset. This was the first time that fever was reported as a SAE after Ad26.ZEBOV vaccination. In general, the proportion of adults experiencing fever after vaccination with the Ad26.ZEBOV was low in both our trial (3%) and previous vaccine trials (0-12%) [1, 2, 4-6, 9]. When assessed at a broader scale during the UMURINZI Ebola vaccination campaign in Rwanda, whereby 216,113 children, adolescent, and adults were vaccinated, fever was one of the most reported unsolicited AEs (61% of reported unsolicited AEs) after vaccination [8]. However, while this was most reported, in total this accounted for <1% of vaccinated individuals. Noteworthy, during this campaign 17 SAEs were considered related to vaccination. Each of these were in children 2-8 years old and consisted of postvaccination febrile convulsions/fever and/or diarrhoea/vomiting. However, the incidence of febrile seizures among young vaccinees showed a decreasing trend once an acetaminophen suppository was routinely administered at the time of vaccination and again approximately six hours later. Additionally, cases that were still reported after this routine implementation of acetaminophen had not been administered the second acetaminophen suppository.

When looking at the EBOV GP-specific GMC values after vaccination with the two-dose vaccine regimen of our trial compared to previously published trials, they tend to be in the lower range of what has previously been observed (Figure 1). Authors of one publication, combining results of three trials conducted in eight different African countries (i.e., Burkina Faso, Kenya, Uganda, Sierra Leone, Côte d'Ivoire, Guinea, Liberia, and Mali), found that GMCs seemed lower in countries where vaccination occurred in rural areas (e.g., Kambia district in Sierra Leone) [10]. As Boende is also located in a rural and remote area of the

DRC, our findings are in line with this observation. Reasons for the lower response in rural areas could be numerous. For example, high concomitant parasitic disease (e.g., malaria, helminth infection), chronic malnutrition leading to micronutrient deficiencies (e.g., Vitamin A), and a poor overall health status may impact the immune response [11].

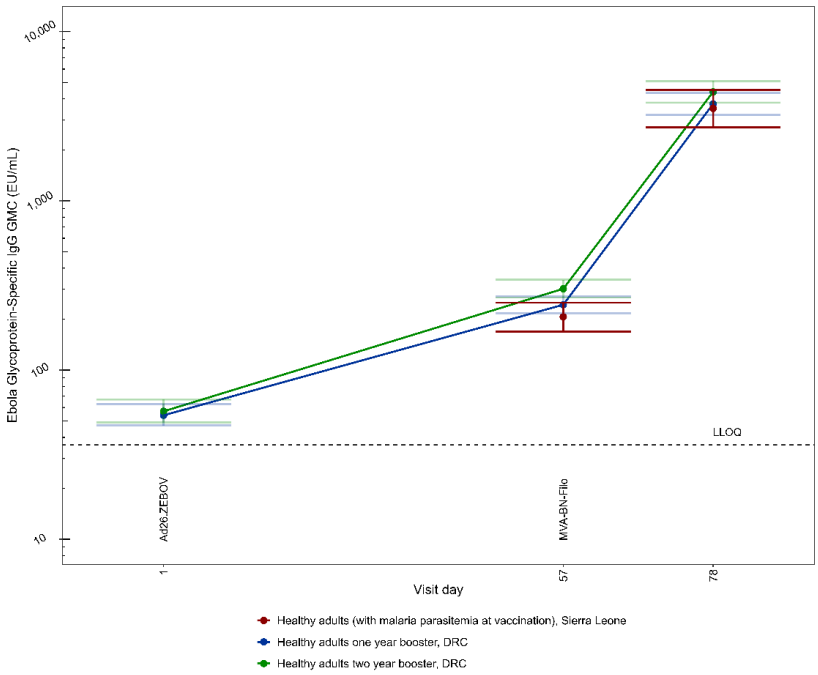
Figure 1. EBOV GP-specific IgG GMCs in EU/mL for all published studies that have evaluated the Ad26.ZEBOV, MVA-BN-Filo regimen, administered at a 56-day interval and EBOV GP-specific IgG GMCs in EU/mL of our Ebola vaccine study per randomisation arm.



Colours represent the different studies that have been conducted in different populations. Some studies were conducted in multiple populations (e.g., adults and children); results of these are presented separately. Only results of the Ad26.ZEBOV, MVA-BN-Filo regimen administered at a 56-day interval have been included. The results of our vaccine trial are presented in darker colours (blue = Group 1; green = Group 2) to highlight their responses compared to other published studies. This figure was created in R v4.3.1. Data was obtained from several publications [1-7, 12-14].

In general, impairment of the immune response to vaccination has been described in individuals with malaria, including those with asymptomatic malaria [11, 15]. The effect of (asymptomatic) malaria on the EBOV GP-specific binding antibody response after Ad26.ZEBOV, MVA-BN-Filo vaccination has previously been explored in adults and children, and did not seem to have a significant influence [16, 17]. However, though the antibody response was not found to be significantly different between those with and without malaria parasitaemia at the vaccination timepoint, one study did describe a trend towards a lower EBOV-GP specific binding antibody response in participants with malaria parasitaemia at the time of vaccination (GMR: 0.82; 95% CI, 0.67-1.02) [16]. As malaria in the DRC is endemic and Boende experiences perennial transmission [18], the lower GMC observed in our study findings compared to previous studies (many in locations where malaria is not endemic) could perhaps be explained by this phenomenon (Figure 2). However, this is a hypothesis and cannot be confirmed as malaria parasitaemia was not assessed among our study participants at any timepoint throughout the trial. Nevertheless, it is important to emphasize that the potentially modest impact of malaria parasitaemia at the time of vaccination on the antibody response should not compromise the vaccine efficacy [16], especially in regions highly endemic for malaria, where Ebola vaccination is likely to be most critical in the future.

Figure 2. EBOV GP-specific IgG GMCs in EU/mL with 95% confidence interval for our Ebola vaccine trial and of the adult EBL3001 trial participants with malaria parasitaemia at the time of Ad26.ZEBOV or MVA-BN-Filo vaccination.



This figure was created in R v4.3.1. Data for the EBL3001 malaria infection sub-study was obtained from [16].

As malnutrition is a state of immunodeficiency, this could also influence the immune response to vaccination [11, 19]. While our trial included participants that were apparently healthy at enrolment – based on vital signs and physical examination – it may be possible that some participants were actually not completely healthy. This can be observed based on haematology and biochemistry results collected at baseline[6]. When looking at these results, 306 out of 698 participants (44%) that received a first dose, seemed to have some haematology values outside of the normal range (e.g., low haemoglobin). For biochemistry, abnormal values (e.g., low urea) were found in 64 out 698 participants (9%). These findings

[6] Haematology and biochemistry results were not evaluated prior to the decision to enrol participants. The protocol states that participants would be enrolled when they were apparently healthy based on vital signs and physical examination. However, these samples were collected. The reason being that if participants reacted severely to vaccination, the baseline blood samples would be available to provide a full and more in-depth picture of the participant's health before vaccination. Haematology and biochemistry results have not been published.

show that the potential presence of malnutrition or poor overall health in our study population is possible and that as a consequence the immune response of these participants may have been lower, explaining the lower overall GMC compared to other studies. However, this is a hypothesis and further in depth evaluation of study participants' health status (including nutritional status) would need to be performed to confirm this.

The EBOV GP-specific binding antibody GMCs observed in our trial were similar to those observed in the Kambia district, a remote, malaria-endemic area in Sierra Leone (Table 1) [1]. Immunobridging has indicated a generally lower mean predicted survival probability of 30.9% (95% CI: 13.6-47.0) in individuals from the trial conducted in Sierra Leone compared to those vaccinated in Europe, the US, and several other African countries (i.e., Burkina Faso, Côte d'Ivoire, Kenya, and Uganda), in which the mean predicted survival probability ranged from 51.1% to 64.9% (95%CI range: 18.0-79.2) [20]. However, the authors highlight the important difference between the mean predicted survival probability and the actual vaccine efficacy, which is most likely higher than the mean predicted survival probability due to the stringency of the NHP model (i.e., animal model is fully lethal, NHPs have a shorter time to symptom onset, and faster disease progression with shorter time to death) on which the immunobridging is based [20]. Nevertheless, as shown by epidemiologic modelling, even in the worst case scenario whereby there is a one-to-one translation of the mean predicted survival probability to vaccine efficacy, a considerable amount of deaths could still be avoided through preventive vaccination of HCP and frontliners with Ad26.ZEBOV, MVA-BN-Filo due to the highly lethal nature of EVD [21]. However, in populations where the antibody response is generally lower after vaccination with the heterologous two-dose regimen, an Ad26.ZEBOV booster dose may be indicated to achieve an increased level of binding antibodies and likely a higher mean predicted survival probability. Unfortunately, this cannot be confirmed by the current immunobridging model as this model does not account for antibody persistence nor for vaccine-induced immunological memory [20, 22].

Table 1. GMCs in EU/mL of EBOV GP-specific binding antibodies of an Ad26.ZEBOV, MVA-BN-Filo vaccine trial conducted in the Kambia district, Sierra Leone, and our Ebola vaccine trial conducted in Boende, DRC.

Location	Trial stage or Arm	Sample size	Day	GMC	Lower 95% CI	Upper 95% CI
Sierra Leone	Stage 1	43	1	60	40	90
Sierra Leone	Stage 2	189	1	69	56	85
DRC	Arm 1	342	1	54	47	63
DRC	Arm 2	342	1	57	49	67
Sierra Leone	Stage 1	43	57	269	208	347
Sierra Leone	Stage 2	191	57	236	206	270
DRC	Arm 1	343	57	243	217	272
DRC	Arm 2	342	57	302	268	341
Sierra Leone	Stage 1	42	78	4784	3736	6125
Sierra Leone	Stage 2	187	78	3810	3312	4383
DRC	Arm 1	343	78	3740	3227	4335
DRC	Arm 2	341	78	4402	3798	5102
Sierra Leone	Stage 1	31	360	325	238	445
Sierra Leone	Stage 2	171	360	259	223	301
DRC	Arm 1	314	365	280	251	313
DRC	Arm 2	305	365	335	296	379
Sierra Leone	Stage 1	31	720	279	201	386
Sierra Leone	Stage 2	159	720	255	212	306
DRC	Arm 2	310	730	275	242	312

GMC = geometric mean concentration; DRC = Democratic republic of the Congo; Ad26.ZEBOV vaccinations were administered on day 1, followed by MVA-BN-Filo vaccination on day 57. GMCs for Sierra Leone in healthy adults were obtained from [1].

Post-hoc statistical modelling on our data provided insights into variations in vaccine response among individuals based on factors such as sex and age. Influences of age and sex on vaccine immune responses have been described for many different vaccines [11]. However, while sex and age differences were observed in our study population, the high overall immune response rate at 21 days after the second dose (96%) suggests that these variations may be clinically irrelevant.

This was the first trial that identified a considerable proportion (70.5%) of seropositive participants for pre-existing neutralising antibodies against the MVA-vector. We hypothesize that this could be due to factors such as prior smallpox and mpox vaccination, and local exposure to mpox virus among our study participants. First, 50 out of 95 (52.6%) participants in which MVA-neutralising antibodies were assessed, had previously received

an MVA-based mpox vaccine in the context of a different clinical trial conducted one year prior to the start of our trial in the same study population in Boende [23]. Second, as the age of our study population ranged between 19 and 75 years old, the older generation of participants could have been vaccinated against smallpox (i.e., an MVA-vector vaccine) in the past. Though the DRC was declared smallpox-free in June 1977, surveillance teams remained active and smallpox vaccination was administered sporadically in the DRC until 1984 [24]. Finally, natural exposure to mpox could also be a possible explanation. A recent study found that annual incidence of human mpox in the Tshuapa province is estimated to be 3.5–5/10,000 individuals, and that there was approximately one HCP infection for every 100 confirmed mpox cases [23]. While ours was the first study to find a high proportion of participants with neutralising antibodies against the MVA-vector, we found that this did not considerably influence the vaccine-induced EBOV GP-specific binding antibody response [25].

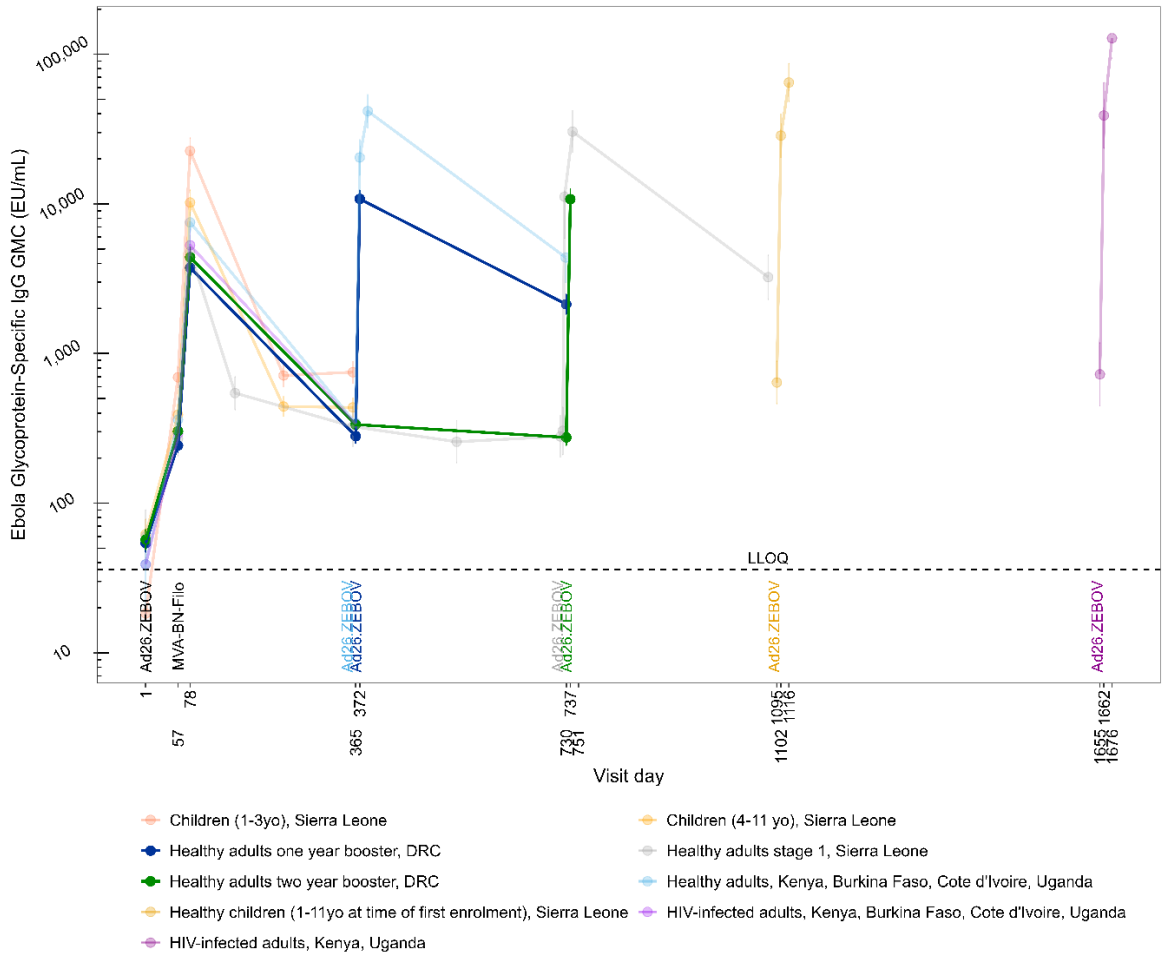
Binding antibodies persisted up to two years after vaccination with the vaccine regimen (Figure 1). Despite a decline in antibody levels between Day 78 and Day 365, stabilisation occurred thereafter, suggesting a persistent immune response up to two years after the initial vaccination. This has also been observed in another study (conducted in Sierra Leone) where Ad26.ZEBOV, MVA-BN-Filo was administered at a 56-day interval and antibody persistence was assessed up to two years after the initial regimen dose [1]. Whether the level of persisting antibodies will also lead to sufficient protection during a next EVD outbreak is uncertain and a booster dose may be indicated to sufficiently protect previously vaccinated individuals at imminent risk of infection [22].

We found that an Ad26.ZEBOV booster dose administered one or two years after the initial dose was generally well-tolerated, with a strong (39-fold increase in GMC before versus after booster vaccination) anamnestic response observed within seven days after booster vaccination. This was similar to what has previously been observed in studies administering booster doses in adults with considerable smaller sample sizes (N≤39) at one or two years after the first dose [1, 6]. Interestingly, as for the regimen, the immune response after

booster vaccination in children seems generally higher than in adults and, though not confirmed in our trial, a trend in an increasing anamnestic response with increasing time between regimen and booster vaccination may be present (Figure 3) [6, 16, 26]. While a definite reason for this age-related difference has not yet been determined, it is possible that the lower immune responses in adults could be attributed to a higher likelihood of recurrent or multiple chronic infections, such as malaria and helminth infections [11]. These conditions are prevalent in the study area and are recognized for their impact on the humoral immune response [11, 18, 27]. Additionally, a very high immune response was found in children and HIV-infected individuals that were boosted with Ad26.ZEBOV more than 3 and 4 years after the first dose, respectively. Though an increasing immune response over time was not confirmed with our booster study results, an increased immune response after longer time intervals between vaccination may be possible based on results from boosted children (1-11 years old when first vaccinated with the regimen) and HIV-positive individuals. An increasing immune response with an increased time interval between doses has also been described after Hepatitis B and measles, mumps, rubella, and varicella virus vaccination [11]. However, the booster studies in children and HIV-positive adults consisted of a small number of vaccinated individuals (N=50 and N=26, respectively) and this hypothesis warrants further investigation as this was not confirmed by our larger sample of adults boosted either one or two years after the first dose.

Although a correlate of protection determining the required antibody threshold that infers likely protection against EBOV has not been identified, and an immunobridging model to assess the probability of protection after booster Ad26.ZEBOV vaccination has not been conducted, the EBOV-GP binding antibody responses induced seven days after booster vaccination were approximately 2.5 times higher than observed 21 days after the heterologous two-dose regimen. As immunobridging has shown a likely protective effect in humans at 21 days after vaccination with the two-dose vaccine regimen [20], it is likely that a booster dose could contribute to an enhanced likelihood of protection against EVD.

Figure 3. EBOV GP-specific IgG GMCs in EU/mL of all published studies that have evaluated an Ad26.ZEBOV booster dose and of our Ebola vaccine study per randomisation arm.



The timing of the booster dose for each study is shown in colour at the bottom of the figure in the same colour as the portrayed immune memory response of that study. In children (orange) and HIV-infected individuals (magenta) only a subset of participants was boosted. The original immunogenicity results of the Ad26.ZEBOV, MVA-BN-Filo regimen from which this selection of participants was boosted has been depicted in a slightly different but similar colour. In children 1-11 years old, no pooled GMC was available for 1-3 and 4-11 year olds and thus GMC for both groups up to 21 days after Ad26.ZEBOV, MVA-BN-Filo vaccination are reported. However, the booster results for these groups were pooled. The results of our vaccine trial are presented in darker colours (blue = Arm 1; green = Arm 2) to highlight their responses compared to other published studies. This figure was developed in R v4.3.1 and EBOV-specific GP binding antibodies were obtained from [1, 6, 12, 26, 28] .

Overall, the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen, administered 56 days apart, appears safe and induces a robust immune response in HCP and frontliners, as also observed among several other study populations (i.e., healthy adults, adolescents, children, infants, and HIV-infected individuals) [1-8, 12-14, 25]. This aligns with results from

various vaccine trials evaluating heterologous two-dose schedules, employing an Ad-vectored vaccine followed by an MVA-vectored vaccine for other infectious diseases like malaria [29-31], HIV [32], and Hepatitis C [33]. These trials demonstrated the combination to be well-tolerated and effective in eliciting both humoral and cellular immune responses. Additionally, the persistent immune response of the Ad26.ZEBOV, MVA-BN-Filo regimen up to two years after the first vaccination supports its use for prophylactic vaccination of at risk populations in Ebola endemic areas. We also found that a booster dose administered one or two years after the regimen was well-tolerated and elicited a rapid (i.e. within seven days) and robust (39-fold increase pre-booster versus post-booster) anamnestic response, suggesting flexibility in booster administration timing. Therefore, given the unpredictable nature of Ebola outbreaks, prophylactic vaccination strategies targeting HCP and frontliners in Ebola endemic areas, including booster doses, could significantly reduce Ebola incidence and mortality.

Our trial demonstrated high retention rates (92%), contributing valuable data from an Ebola endemic area to already existing data. While the Ad26.ZEBOV, MVA-BN-Filo regimen is a two-dose regimen and questions may arise on its feasibility and uptake, our Ebola vaccine trial shows that many HCP and frontliners living and working in an Ebola endemic area were eager to get vaccinated. Overall, 92% completed not only the two-dose regimen but remained in the trial for a more than 2.5 year follow-up period, and if not medically contraindicated as per PI judgement, these participants also returned for a booster dose one or two years after the first dose. Therefore, these results show that even in remote resource-constrained locations, vaccination with the heterologous regimen is feasible. This was also confirmed by the UMURINZI vaccination campaign whereby 203,267 out of 216,113 (94%) individuals returned for their second dose with MVA-BN-Filo at a median 57 days and a mean of 70 days later [8]. Additionally, three studies have found that a delayed second dose with MVA-BN-Filo did not negatively impact the immune response and that the immune response was at least as high in those with delayed second dose vaccination as in those vaccinated at the recommended 56-day interval [1, 3, 6]. These findings,

combined with our findings, suggest flexibility in the timing of the administration of the second dose as well as the booster, an important finding for real-life settings.

Limitations, such as sex imbalance and possible incomplete HIV-status records or unknown malaria parasitaemia were/may have been present in our trial. Additionally, the absence of neutralising antibody measurements and the impossibility to compare the long-term persistence of binding antibodies after booster vaccination at one or at two years after the first dose were study limitations. Nevertheless, several studies have demonstrated a strong correlation between the elicited binding and neutralising antibody responses through the Ad26.ZEBOV, MVA-BN-Filo regimen [1, 3, 6, 12, 13]. Additionally, the study team is looking into further funding possibilities to follow-up this vaccinated cohort and to assess potential differences in post-booster GMCs over time.

8.1.2 The trial's challenges and lessons learned

Conducting clinical trials in LMICs presents both challenges and opportunities. This thesis described the challenges, mitigations taken and lessons learned from an Ebola vaccine trial, conducted in a remote area in the DRC, and an AC policy evaluation study [34-36]. In doing so, the importance of addressing local challenges, building sustainable partnerships, and tailoring approaches to the specific context were addressed.

One of the major factors that contributed to a successful trial implementation consisted of continued community engagement. For our trial, this included participants being invited to attend workshops prior to each scheduled trial visit. During these visits, participants were re-explained the procedures that would be performed the next day during their scheduled study visit. Additionally, during these workshops participants were able to voice any questions or concerns they might have, which could then be addressed by study staff. Participants' interest to attend the workshops was high as they also provided learning possibilities. Namely, to achieve local capacity building and participant engagement, UNIKIN arranged professors with expertise in different health-related topics (e.g., paediatrics, psychiatry, etc.) to provide a lecture during the workshops. Several other

studies, implementing the same Ad26.ZEBOV, MVA-BN-Filo vaccine regimen have described their own version of community engagement and stress it as a crucial part for successful vaccine trial implementation [37, 38]. In Sierra Leone, social scientists contributed to community engagement by identifying certain potential challenges and mitigating them through open dialogue before the start of the vaccine trial [37]. For a phase 3 trial implemented in Goma, DRC, community engagement was achieved by establishing a Community Advisory Committee (including local political and administrative leaders, traditional and religious leaders, non-governmental organisations, and social groups and organisations) that had biweekly meetings to identify and address certain risk, rumours, perceptions among the community [38]. While community engagement is applied in different ways throughout different trials, its importance is generally well accepted.

LMICs represent the majority of the global population, and solutions derived from research in these regions can have a profound impact on global morbidity and mortality [39]. Increasing the number of clinical trials in LMICs is imperative to generate local evidence that influences health policies and addresses the specific health challenges faced by these populations [39]. However, at this moment there is a gap in scientific leadership, capacity and infrastructure to conduct early vaccine trials in Africa and a global challenge to continue early testing of vaccines during non-epidemic periods [40, 41]. This often leads to delayed clinical development, causing missed opportunities for assessing vaccine efficacy when an outbreak does occur. Consequently, vaccine candidates are prepared for late-phase trials when epidemics are subsiding (cfr. Part I, Chapter 1, Figure 4), necessitating an alternative approach to address vaccine efficacy in the tail end of outbreaks (e.g., immunobridging). Delays, partially attributed to initiating phase 1 trials when epidemics are already causing significant harm, were evident during the West Africa Ebola epidemic, impacting the evaluation of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen [42]. This trend persists with more recent outbreaks, including Ebola in Sudan and the emergence of SARS-COV-2 variants. To address these shortcoming, we ensured capacity building by providing a better healthcare infrastructure (e.g., providing running water, a safe and larger medical waste incinerator than previously available, implementation of a local functional cold

chain, rebuilding the hospital laboratory, and providing laboratory equipment such as centrifuges, machines for biochemistry and haematology analyses) for the GRH in Boende. Additionally, by recruiting local doctors, nurses, and logistic and financial administrators to conduct the Ebola vaccine trial and enhancing their clinical research training and knowledge, this trial site is now recognised by other (inter)national research groups as a location where both infrastructure and trained HCP are available to conduct research [43]. The facilities are currently used for several ongoing projects [43]. The funds invested in the context of this Ebola vaccine trial therefore not only generated essential clinical trial data but also contributed to the development of the local health infrastructure and services, and enhanced the local clinical research capacity by offering training opportunities and employment for staff in the region.

Next to local capacity building, this trial also allowed for a strong North-South collaboration whereby two doctoral students (one working for the trial's sponsor at the University of Antwerp – myself – and one working for the trial's PI at the University of Kinshasa) were able to complement each others work either through methodological input or through more in depth understanding of the local infrastructure. This was mainly possible because the sponsor of the trial (University of Antwerp) was an academic sponsor, rather than the more frequent pharmaceutical sponsor, which allowed for this form of capacity building as well.

While conducting research in LMICs is crucial, the highest benefit of vaccination (such as against EBOV) can most likely by achieved in areas with a resource-poor healthcare structure (e.g., Boende). As a result, researchers often feel the need to provide healthcare that goes beyond the scope of the research being conducted. For example, the phase 3 Ebola vaccine trial implemented in Goma, DRC, provided a short medical consultation to potential participants presenting at the triage centre with fever and/or illness [38]. These individuals were offered a malaria test and were treated when tested positive. If negative, they were offered free treatment for other minor illnesses as per the national medical protocols. However, while many researchers feel the need to implement some type of AC

in clinical trials conducted in LMICs, this is most often provided ad hoc. The current Ebola vaccine trial addressed this issue by developing a study-specific AC algorithm and policy in order to provide structured, systematic, and equal care to study participants in need [36]. In Part III, Chapter 7, of this thesis we evaluate this algorithm and policy and identify certain aspects that could influence the use of the algorithm (i.e., trial design, study population, residence distance from the site, etc.). To the best of our knowledge, this was the first study whereby an AC policy was specifically developed to provide medical and/or financial support to trial participants presenting with (S)AEs in a systematic way. We hope that by publishing and evaluating the AC algorithm and policy, other researchers can be more confident to apply a similar approach, adapted to their own specific research and location.

Combining everything reported in Part III of the doctoral thesis, some key recommendations deserve repetition. First, when considering conducting research in LMICs, researchers should foresee a budget to invest in local infrastructure. While it is highly important to conduct research in remote areas of LMICs to generate local evidence, local infrastructure is rarely at par to conduct the required research. Therefore, researchers should not only focus on the immediate needs of the trial but also on the long-term benefits of capacity building for local health facilities. Second, not only local infrastructure should be considered but also local capacity building should be provided. This can be achieved through community engagement workshops, allowing the local community to expand their knowledge and confidence. For this trial, some general healthcare courses were provided during workshops to HCP and frontliner participants, creating a pool of skilled professionals that can contribute to an enhanced local healthcare and thus global health. Third, an open and honest communication with local, national, and international stakeholders is key. Clearly defined roles and responsibilities, coupled with regular meetings, contributed to the logistical organization and success of our trial. Transparent communication does not only contribute to a successful trial but also promotes sustainable partnerships. Finally, tailored (S)AE policies can greatly increase research participants' healthcare options and general well-being and should be considered by researchers conducting trials in remote and resource-poor setting of LMICs. These recommendations aim to guide future

researchers in navigating the complexities of clinical trials in LMICs, ultimately contributing to global health advancements in an inclusive and equitable manner.

8.2 Next steps

8.2.1 For the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen

Continued research, including long-term follow-up studies, will contribute to a more comprehensive understanding of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen and booster dose, and their role in Ebola preparedness. Proactive vaccination strategies, guided by ongoing research, should be considered for at-risk populations, including HCP and frontliners, to mitigate the impact of future Ebola outbreaks. Therefore, our research team is currently investigating the possibility to extend the follow-up of the vaccinated cohort and evaluate longer term antibody persistence after booster vaccination.

Persistent endeavours are required to assess the effectiveness of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen. However, direct demonstration of clinical efficacy and effectiveness remains a challenge in the absence of a rapidly expanding, large Ebola epidemic. While this was for example attempted in a phase 3 trial, set-up in Goma, during the Ebola epidemic in the DRC between 2018 and 2020, the outbreak intensity had already decreased considerably by the time the trial was sufficiently up and running, and vaccine effectiveness could no longer be determined [38]. Of the 500,000 doses that were planned to be administered, only 20,427 doses were actually administered [38]. Therefore, smaller and more widely dispersed outbreaks may require a different strategy to obtain convincing effectiveness data. As vaccination is meant to prevent or minimalize EVD, it may be possible that outbreaks will no longer occur or be identified in areas where at risk populations have previously been vaccinated, and are thus protected against EBOV, hindering the disease to spread. Several studies have highlighted the probability of asymptomatic or minimally symptomatic EVD [44, 45]. This however does not mean that these individuals are not exposed to Orthoebolaviruses. Therefore, some outbreaks may go undetected. To obtain vaccine effectiveness data in these contexts, a long-term follow-up of previously vaccinated

individuals, and more specifically HCP and frontliner as at risk professions, may be indicated using a cluster randomized case-control design. For this design, an EVD case definition may need to be redefined to an individual with a “spike” in EBOV GP-specific binding antibodies (to be defined by immunology experts in the field), compared to what was previously observed during follow-up. Hypothesising that when “spikes” in antibodies are observed the individual may not become sick or report symptoms but may still be infected (as a kind of breakthrough infection versus breakthrough disease). As asymptomatic cases have thus far not been known to transmit EBOV, this may lead to undetected EVD outbreaks and a missed opportunity to evaluate vaccine effectiveness. In this context, when a “spike” in antibodies is observed in a previously vaccinated individual, an outbreak investigation, including a test-negative case-control study, can be conducted in this cluster to assess whether EVD cases may have been missed (e.g., misclassified as a different disease or not reached the health system) and to assess protective effectiveness.

8.2.2 For Ebola vaccination and research in general

During the 2013-2016 West Africa epidemic, the single-dose rVSV△G-ZEBOV-GP vaccine proved to be 100% effective during ring vaccination (i.e., identification and vaccination of contacts and contacts of contacts) in Guinea [46]. As a consequence of this field knowledge, the Strategic Advisory Group of Experts (SAGE) on Immunisation of the WHO recommended the use of a ring vaccination strategy during the 2018-2020 outbreak in the DRC [47]. This strategy was primarily focused on contact tracing and vaccination of high-risk individuals (i.e., primarily HCP and frontliners) using rVSV△G-ZEBOV-GP [47]. However, some argue that while ring vaccination may help reduce the transmission of EVD, it relies on the immediate identification of EVD, followed by the immediate vaccination of all exposed contacts and potential contacts of contacts, and an incubation period of more than 10 days (rVSV△G-ZEBOV-GP has been found to be effective from 10 days after vaccination [46]) [48]. Yet, as discussed in the introduction of this doctoral thesis, the incubation period can be as short as 2-4 days, which means that human-to-human transmission is still possible until this 10-day limit is reached, all the while continuing the spread of an outbreak.

Additionally, during the 2018-2020 outbreak in the DRC, serious security, social, and epidemiological challenges occurred and ring vaccination could not always be adequately implemented [49, 50]. Therefore, under exceptional circumstances, SAGE recommended geographic targeted vaccination as an alternative strategy [47]. Consequently, with this strategy, a considerable number of vaccines were required, which prompted further consideration for additional vaccine programmes with different EBOV vaccines under development that might contribute to fighting the spread of the epidemic. Hence, modifications were made to the SAGE recommendations in May 2019 [51]. These changes involved extending the vaccination coverage to HCP and frontliners with the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen in nearby areas susceptible to the outbreak's spread. Furthermore, concurrent efforts included the initiation of population-based vaccination campaigns using Ad26.ZEBOV, MVA-BN-Filo in the vicinities surrounding the outbreak [38]. However, any efforts made to deploy these vaccines needed to be in the context of scientifically and epidemiologically justified studies, that had obtained the necessary regulatory and ethical approvals, with predefined endpoints in safety, immunogenicity, and efficacy that could contribute to obtaining vaccine licensure for these vaccines [51]. These requirements considerably delayed some of the vaccination efforts, hindering the possibility to assess vaccine efficacy when campaigns started near the tail-end of the outbreak [38].

Since then, the rVSV△G-ZEBOV-GP was able to obtain licensure based on efficacy data obtained in Guinea during the 2013-2016 West Africa epidemic and in the DRC during the 2018-2020 epidemic. The vaccine was approved for use in adults older than 18 years of age by the EMA and pre-qualified by the WHO in November 2019, followed by approval of the FDA in December 2019 and afterwards by several African regulatory authorities in their respective countries [52-55]. Additionally, based on immunobridging analysis, the Ad26.ZEBOV, MVA-BN-Filo regimen was approved by EMA for use in epidemic emergencies against EBOV in July 2020 for adults and children older than one year old and by several African regulatory authorities (i.e., Ghana, Côte d'Ivoire, Rwanda, Uganda, Sierra Leone, Nigeria, and Gabon) in 2022 and 2023 [9, 56, 57].

The most recent SAGE recommendations on Ebola vaccination date back to June 2021 and currently primarily recommend the use of rVSV△G-ZEBOV-GP ring vaccination during EVD outbreaks due to its proven 97.5-100% effectiveness in Guinea and the DRC [46, 58, 59]. However, recent modelling research has shown that preventative vaccination of HCP and frontliners in Ebola endemic areas prior to an outbreak, in addition to nonpharmaceutical interventions and ring vaccination during an outbreak, has the potential to decrease the occurrences of Ebola, diminish hospital admissions, and lower mortality rates, while allowing more time for reactive ring vaccination and nonpharmaceutical interventions to be implemented [60]. While, the 2021 SAGE recommendation incorporated a strategy suggesting the prophylactic vaccination of national response teams using the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen, in addition to individuals falling into the following categories: international responders actively involved in supporting EVD outbreak response efforts, laboratory personnel with potential exposure to EBOV, individuals working in specialized research units handling EBOV, and those engaged in Ebola Treatment Units who may provide care to EVD patients [59], this does not protect local HCP and frontliners living and working in Ebola endemic areas. The recommendation indicates vaccine supply constraints as the reason for not routinely including preventive vaccination of HCP and frontliners living and working in Ebola endemic areas [59].

In August 2022, a resurgence of the 2018-2020 outbreak took place in North Kivu, almost two years after the outbreak was declared [61]. At the time of this resurgence, some HCP would have received their rVSV△G-ZEBOV-GP vaccine, administered during the epidemic, more than two years earlier. Therefore, with increasing sizes of Ebola outbreaks and consequently a relatively high number of survivors, HCP and frontliners continue to be at risk of exposure in these areas. At this moment, the duration of protection of the rVSV△G-ZEBOV-GP vaccine, and the Ad26.ZEBOV, MVA-BN-Filo regimen is still unclear. While McLean et al. hypothesize based on animal models that booster vaccination may not be required for some time after vaccination with the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen [22], this has not yet been confirmed. Our booster study indicates that booster doses can be administered up to two years after the initial dose and present a similar and

robust anamnestic response at both timepoints. Therefore, it seems necessary to start exploring new vaccination strategies. Rather than reactive ring vaccination and vaccination of HCP and frontliners at the time an outbreak is detected, it may be more cost-effective to consider routine prophylactic vaccination of HCP and frontliners in areas where EVD is considered endemic and relapse or recurrence of EVD cannot be ruled out [62]. Next to HCP and frontliners, vaccinating sexual partners of male survivors may also be indicated to avoid unexpected resurging outbreaks. Finally, different vaccination strategies should take into account the different characteristics of the available Ebola vaccines and consider them as complementary; keeping in mind the onset and duration of protection, the number of required doses, the possibility to boost, logistic and cold chain requirements, adverse events, and safety in pregnant women, children and immunocompromised patients [48].

Unfortunately, how aspects of different vaccination strategies with different vaccine platforms would interact is currently unclear as real-world evidence is limited or unavailable [63]. By acknowledging the risk of unpredictable future EVD outbreaks, it is however imperative to continue prioritizing additional efforts in vaccine development and deployment of different vaccination strategies against EBOV. Nevertheless, organizing a prophylactic vaccination campaign for HCP and frontliners working in Ebola endemic areas would be a complex endeavour and whether this would be feasible is a relevant policy question. In this sense, the role of implementation research may be crucial to assess how prophylactic vaccination of HCP and frontliners could be implemented and continued follow-up would be needed to assess the need, and if so, required frequency and timing of booster vaccinations. In this implementation research, unresolved questions would include determining the financial responsibility for vaccine distribution in these settings, assessing the challenges of implementation, identifying the appropriate infrastructure in respective countries to spearhead the effort, specifying the required materials for successful execution, and evaluating the implications on local, national, and international infrastructures. With such implementation research, where the focus would shift from randomized trials towards real-world settings, a crucial step towards outbreak prevention could be taken.

8.3 References

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List of publications and presentations

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Poster presentations

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