University of Antwerp

Faculty of Medicine and Health Sciences

Dealing with challenges in an Ebola vaccine trial in a remote and endemic Ebola setting of the Democratic Republic of the Congo

Omgaan met uitdagingen in een Ebola-vaccinonderzoek in een afgelegen en endemisch Ebola-gebied van de Democratische Republiek Congo

PhD thesis submitted for the degree of **Doctor in Medical Sciences** at the University of Antwerp to be defended by Trésor Zola Matuvanga

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Dedication

To the Eternal, Almighty God, the source of all knowledge, for Your unwavering support and blessings throughout my many journeys,

In loving memory of my dear mother, Marceline Nzumba Zola. To my tender and charming wife, Joséphine Mboma Padial, and my beloved sons and daughter, Yosif Zola Matuvanga, Moses Mboma Zola, and Messias Nzumba Matuvanga,

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Abbreviations

4PL: Four-Parameter Logistic Curve

Ad26ZEBOV: Adenovirus type 26 ZEBOV

ADI: Alpha Diagnostic International

AE: Adverse Effect

BDBV: Bundibugyo ebolavirus

Bio-Plex 200: Bio-Rad's Bio-Plex 200 hardware

BOMV: Bombali ebolavirus

BSL: Biosafety Laboratory

BSL-4: Biosafety Level 4

BVD: Bundibugyo virus disease

CEPI: Coalition for Epidemic Preparedness Innovations

CFR: Code of Federal Regulations

CFR: Code of Federal Regulations

CHW: Community Health Care Workers

CI: Confidence Interval

CIOMS: Council for International Organizations of Medical Sciences

COVID-19: Coronavirus Disease 2019

CRFs: Case Report Forms

CRO: Clinical Research Organization

DALY: Disability-Adjusted Life Year

DPM : Direction de la Pharmacie et de Medicament

DRC: Democratic Republic of the Congo

EBL2007: Ebola Vaccine Trial Name

EBOV: Ebolavirus

EBOVAC 3: Ebola Vaccine Consortium 3

EC: Ethics Committee

EC-DRC: National Health Ethics Committee of the DRC

ELISA : Enzyme-Linked Immunosorbent Assay

EMA: European Medicines Agency

EU: European Union

EU/ml: ELISA Units per milliliter

EU-IMI: European Union's Innovative Medicines Initiative

EVD : Ebola virus Disease

FANG: Filovirus Animal Nonclinical Group

FAW: First-Aid Workers

FDA: Food and Drug Administration

FGD: Focus Group Discussion

GCLP: Good Clinical & Laboratory Practices

GP: Glycoprotein

GP-EBOV-k: Glycoprotein Ebola Virus - Kikwit strain

GP-EBOV-m: Glycoprotein Ebola Virus - Mayinga strain

GRH: General Referral Hospital

HCP: Healthcare provider

HCP-P : Healthcare provider participant

HGR : Hôpital Général de Boende

HGR: General Referral Hospital

HIC: High-Income Country

HIV/AIDS: human immunodeficiency virus/acquired immunodeficiency syndrome

IC95: 95% Confidence Interval

ICD-11: International Classification of Diseases 11th revision

ICFs: Informed Consent Forms

ICH: International Conference on Harmonization

ICH E6: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, Guideline E6

ID: Identification

IDIs: In-Depth Interviews

IEC: International Electrotechnical Commission

IFAT: Indirect Fluorescent Antibody Test

IgG: Immunoglobulin G

IHME: Institute for Health Metrics and Evaluation

IL: Interleukins

IMI-EU: Innovative Medicines Initiative - European Union

LED: Light-Emitting Diode

LMICs: Low and middle income countries

MFI: Median Fluorescence Intensity

MSF: Médecins Sans Frontières (Doctors Without Borders)

MVA-BN-Filo: Modified Vaccinia Ankara - Bavarian Nordic Filovirus

N(S)AE: (Non) Related (Serious) Adverse Event

NGO: Non-Governmental Organization

NICE: National Institute for Health and Care Excellence

NIH: National Institutes of Health

NP: Nucleoprotein

NV: Naamloze Vennootschap(Anonymous Society; a type of corporation in Belgium)

NVIVO: Qualitative Data Analysis Software

OD: Optical Density

PCR: Polymerase Chain Reaction

PI: Principal Investigator

PPI: Patient and Public Involvement

R&D: Research and Development

REBOV: Reston ebolavirus

Redcap: Research Electronic Data Capture

RT-PCR: Reverse transcription polymerase chain reaction

rVSVΔGZEBOV-GP: recombinant, vesicular stomatitis virus-based vaccine expressing the glycoprotein of a Zaire Ebolavirus

SAE: Serious Adverse Event

SAGE: Strategic Advisory Group of Experts (on immunization)

SD : Standard Deviation

SOP: Standard Operating Procedure

SPSS: Statistical Package for the Social Sciences

SUDV: Sudan ebolavirus

SVD: Sudan virus disease

TAFV: Tai Forest ebolavirus

TOU: Test of Understanding

Uantwerp: University of Antwerp

UK: United Kingdom

UNIKIN: University of Kinshasa

VA: Virginia (a state in the United States)

VP40: Viral Matrix Protein 40

VSAT: Very Small Aperture Terminal

Summary

Introduction

Since the discovery of the Ebola virus in 1976, the Democratic Republic of Congo has experienced 15 outbreaks of Ebola virus disease. This thesis delved into the challenges of conducting Ebola Virus Disease (EVD) vaccine trials in endemic and remote areas, focusing on the Boende Health District in the Democratic Republic of Congo (DRC). The EBL2007 vaccine trial, part of the European Union Innovative Medicines Initiative, aimed to evaluate a new Ebola vaccine regimen in a high-risk group of Healthcare providers (HCPs) and frontline workers. This research is pivotal for enhancing preparedness against potential EVD outbreaks, particularly in regions with fragile healthcare systems and limited resources.

Objectives

The thesis was guided by the following objectives: to evaluate the acceptability, feasibility and accuracy of an iris scanning system for identifying HCPs in the EBL2007 Ebola vaccine trial, to investigate the long-term perceptions and experiences of HCP-participants and staff regarding iris scanning technology, to identify and document the primary challenges and lessons learned from setting up an Ebola vaccine trial in a remote setting of Boende Health District in the DRC, to assess the baseline seroprevalence of antibodies against EBOV antigens among HCPs and frontline workers participants in the EBL2007 vaccine trial, to explore the HCPs-participants in the trial, trial staff, and local health authorities perception and experiences of the EBL2007, to Assess the effectiveness of the trial's information retention among participants over time, and to compile key challenges, mitigation strategies, and lessons learned from conducting the trial.

Methods

This doctoral thesis was centered on the EBL2007 vaccine trial, a joint initiative by the University of Antwerp and the University of Kinshasa, under the EU-IMI EBOVAC 3 project. Conducted in the Tshuapa province, DRC, from December 2019 to October 2022, the trial focused on vaccinating high-risk healthcare providers (HCPs) and frontline workers in Boende, a region previously affected by an Ebola outbreak in 2014. This setting provided a unique context to explore vaccine trial complexities in low- and middle-income countries (LMICs). The methodological approach was comprehensive, reflecting the diverse challenges of the Boende Health District. The methodologies were specifically tailored to address each research objective: iris Scanning as an biometric identification tool in the EBL2007 vaccine trial (Chapter 3): the study assessed the acceptability and accuracy of iris scanning as a biometric identification tool for HCPs using mixed methods, including focus group discussions and structured surveys. The system's performance was evaluated longitudinally, from enrollment

to follow-up. Long-term Experiences with Iris Scanning (Chapter 4): A qualitative study employing phenomenological methods captured the long-term experiences and perceptions of HCP-participants and staff regarding the iris scanning system,.The study employed thematic analysis, blending inductive and deductive methods to distill themes and insights from responses in French on iris scanning in the EBL2007 vaccine trial, later translating findings into English for broader accessibility. Challenges and Lessons Learned (Chapter 5): A narrative review methodology synthesized the challenges, mitigations, and lessons learned from the trial, aiming to guide future clinical trials in similar settings. Baseline Seroprevalence of EBOV Antibodies (Chapter 6): The study analyzed baseline serum samples using FANG ELISA and Luminex assays to assess the immunogenicity and safety of Ad26.ZEBOV and MVA-BN-Filo vaccines, including statistical analyses for cutoff determination and correlation of seropositivity. Trial's perception and experiences of participants (Chapter 7): Qualitative methods explored the trial's impact on healthcare providers, frontline workers, trial staff, and local health authorities. Data collection involved interviews and focus group discussions, analyzed using thematic approaches. Informed consent retention Assessment (Chapter 8): The trial's effectiveness in maintaining participants' understanding over time was evaluated using a Test of Understanding (TOU) at different intervals, with analysis conducted using statistical models. Documentation of challenges and mitigations in implementing the trial in a remote area of the DRC (Chapter 9): A narrative review compiled key challenges, mitigation strategies, and lessons from the trial, providing an in-depth look into the execution of the trial in a complex LMIC setting.

Results

A high acceptance rate of 99% was observed for iris scanning as an identification method among HCPs potential participant in the EBL2007 vaccine trial, before starting the recruitment. However, some participants expressed concerns about the potential physical harms of iris scan technology on eye. The technology accurately identified 93.1% of participants during follow-up visits, with the majority successfully identified on the first attempt and the scanning process taking 2 minutes or less. Over time, some participants reported a perception of diminished vision after using the iris scan, although no vision impairment was officially linked to the technology in the EBL2007 vaccine trial. Conducting vaccine trials in LMICs like the DRC faces numerous challenges, including complex regulatory environments, logistical and financial constraints, and the need for extensive international collaborations. Despite these obstacles, the EBOVAC 3 consortium facilitated high-quality trials in the area. The trial revealed a low seroreactivity to EBOV antigens among participants, with 1.4% showing reactivity to two EBOV-Mayinga antigens and 8.5% to GP-EBOV-Kikwit. Positive experiences were reported due to the trial's commitment to site improvement and volunteer training. However, issues like inadequate compensation for time and travel and concerns about frequent blood draws were highlighted. Participants' understanding of the trial, assessed through a TOU scores, showed a significant decline over time (year 1 and year 2), particularly among those with less education and older participants. The trial implementation in frame of a consortium faced several challenges, including cultural differences, language barriers, and regulatory issues, requiring clear communication and adaptability among international stakeholders. Despite these challenges, the trial achieved a

high participant retention rate of 92%, underscoring the importance of addressing participants' concerns, effective communication, and flexible, collaborative approaches in vaccine trial management in remote LMIC settings.

Conclusions

The EBL2007 vaccine trial in Boende Health District stands as a significant achievement in clinical research for resource-limited settings. Its use of iris scanning technology, accepted by 99% of HCPs, exemplifies the effectiveness of biometric tools in ensuring trial accuracy and integrity, with a notable 93.1% success rate in participant identification. Despite some concerns over potential physical impacts, like perceived vision changes, the trial emphasizes the necessity of ongoing community engagement and clear communication. Overcoming challenges such as complex regulations and logistical hurdles, the trial achieved a remarkable 92% participant retention, thanks to the EBOVAC 3 consortium's commitment to quality, responsive volunteer training, and addressing compensation and procedural concerns. The decline in participant understanding over time, as shown in TOU scores, underscores the importance of continual education, particularly for older and less-educated participants. The EBL2007 trial demonstrates the feasibility and importance of including diverse, socio-economically disadvantaged groups in clinical research, especially in LMICs, for effective EVD response. Strategic planning, anticipation of regulatory challenges, positive local collaborations, investment in local research infrastructure, and innovative identification tools like iris scanning are crucial for enhancing trial quality and contributing to global health progress, particularly in managing EVD epidemics.

Dutch Summary

Inleiding

Sinds de ontdekking van het Ebolavirus in 1976 heeft de Democratische Republiek Congo 15 uitbraken van ebolavirusziekte ervaren. Deze thesis onderzocht de uitdagingen van het uitvoeren van ebolavirusziekte (EVD) vaccinproeven in endemische en afgelegen gebieden, met focus op het Boende Gezondheidsdistrict in de Democratische Republiek Congo (DRC). De EBL2007 vaccinproef, onderdeel van het Innovatieve Geneesmiddeleninitiatief van de Europese Unie, had als doel een nieuw ebolavaccinregime te evalueren in een hoogrisicogroep van zorgverleners en eerstelijnswerkers. Dit onderzoek is cruciaal voor het verbeteren van de paraatheid tegen potentiële EVD-uitbraken, vooral in regio's met kwetsbare gezondheidssystemen en beperkte middelen.

Doelstellingen

De thesis werd geleid door de volgende doelstellingen: het evalueren van de aanvaardbaarheid, haalbaarheid en nauwkeurigheid van een irisscansysteem voor het identificeren van zorgverleners in de EBL2007 ebolavaccinproef, het onderzoeken van de langetermijnpercepties en ervaringen van zorgverlener-deelnemers en personeel met betrekking tot de irisscantechnologie, het identificeren en documenteren van de primaire uitdagingen en geleerde lessen van het opzetten van een ebolavaccinproef in een afgelegen omgeving van het Boende Gezondheidsdistrict in de DRC, het beoordelen van de basislijn seroprevalentie van antilichamen tegen EBOV-antigenen onder zorgverleners en eerstelijnswerkers deelnemers aan de EBL2007 vaccinproef, het verkennen van de perceptie en ervaringen van de deelnemers van de zorgverleners in de proef, proefpersoneel en lokale gezondheidsautoriteiten van de EBL2007, het beoordelen van de effectiviteit van het behoud van proefinformatie onder deelnemers na verloop van tijd, en het samenstellen van belangrijke uitdagingen, mitigerende strategieën en lessen geleerd van het uitvoeren van de proe.

Methoden

Deze doctoraalthesis was gecentreerd op de EBL2007-vaccinproef, een gezamenlijk initiatief van de Universiteit van Antwerpen en de Universiteit van Kinshasa, onder het EU-IMI EBOVAC 3-project. Uitgevoerd in de provincie Tshuapa, DRC, van december 2019 tot oktober 2022, richtte de proef zich op het vaccineren van hoogrisico gezondheidswerkers (HCP's) en eerstelijnswerkers in Boende, een regio die eerder getroffen was door een ebola-uitbraak in 2014. Deze setting bood een unieke context om de complexiteit van vaccinproeven in landen met lage en middeninkomens (LMIC's) te verkennen. De methodologische aanpak was veelomvattend en weerspiegelde de diverse uitdagingen van het Gezondheidsdistrict

Boende. De methodologieën waren specifiek aangepast om elk onderzoeksdoel aan te pakken: irisscannen als een biometrische identificatietool in de EBL2007-vaccinproef (Hoofdstuk 3): de studie beoordeelde de aanvaardbaarheid en nauwkeurigheid van irisscannen als een biometrische identificatietool voor HCP's met behulp van gemengde methoden, waaronder focusgroepdiscussies en gestructureerde enquêtes. De prestaties van het systeem werden longitudinaal geëvalueerd, van inschrijving tot opvolging. Langetermijnervaringen met irisscannen (Hoofdstuk 4): Een kwalitatieve studie met fenomenologische methoden legde de langetermijnervaringen en percepties van HCP-deelnemers en personeel vast met betrekking tot het irisscansysteem. De studie maakte gebruik van thematische analyse, waarbij inductieve en deductieve methoden werden gecombineerd om thema's en inzichten te destilleren uit reacties in het Frans over irisscanning in de EBL2007-vaccinproef, waarna de bevindingen werden vertaald naar het Engels voor een bredere toegankelijkheid.!. Uitdagingen en Geleerde Lessen (Hoofdstuk 5): Een narratieve reviewmethodologie synthetiseerde de uitdagingen, mitigaties en lessen uit de proef, met als doel toekomstige klinische proeven in vergelijkbare omgevingen te leiden. Basislijnseroprevalentie van EBOV-antilichamen (Hoofdstuk 6): De studie analyseerde basale serumstalen met behulp van FANG ELISA en Luminex assays om de immunogeniciteit en veiligheid van Ad26.ZEBOV en MVA-BN-Filo vaccins te beoordelen, inclusief statistische analyses voor afkapwaardebepaling en correlatie van seropositiviteit. Perceptie en Ervaringen van Proefdeelnemers (Hoofdstuk 7): Kwalitatieve methoden onderzochten de impact van de proef op gezondheidswerkers, eerstelijnswerkers, proefpersoneel en lokale gezondheidsautoriteiten. Gegevensverzameling omvatte interviews en focusgroepen, geanalyseerd met thematische benaderingen. Beoordeling van Behoud van Geïnformeerde Toestemming (Hoofdstuk 8): De effectiviteit van de proef in het behouden van het begrip van deelnemers over tijd werd geëvalueerd met een Test van Begrip (TOU) op verschillende intervallen, met analyse uitgevoerd met behulp van statistische modellen. Documentatie van Uitdagingen en Mitigaties bij de Implementatie van de Proef in een Afgelegen Gebied van de DRC (Hoofdstuk 9): Een narratieve review compileerde de belangrijkste uitdagingen, mitigatiestrategieën en lessen uit de proef, en biedt een diepgaande blik op de uitvoering van de proef in een complexe LMIC-omgeving.

Resultaten

Een hoge aanvaardingsgraad van 99% werd waargenomen voor irisscanning als identificatiemethode bij potentiële deelnemers onder HCP's in de EBL2007-vaccinproef, voor de start van de werving. Sommige deelnemers uitten echter bezorgdheid over de mogelijke fysieke schade van irisscantechnologie aan het oog. De technologie identificeerde 93,1% van de deelnemers nauwkeurig tijdens de vervolgbezoeken, waarbij de meerderheid met succes bij de eerste poging werd geïdentificeerd en het scanproces 2 minuten of minder in beslag nam. In de loop van de tijd meldden sommige deelnemers een perceptie van verminderd zicht na het gebruik van de irisscan, hoewel er geen visuele beperking officieel werd gekoppeld aan de technologie in de EBL2007-vaccinproef. Het uitvoeren van vaccinproeven in LMIC's zoals de DRC kent tal van uitdagingen, waaronder complexe regelgevende omgevingen, logistieke en financiële beperkingen, en de noodzaak voor uitgebreide internationale samenwerkingen. Ondanks deze obstakels vergemakkelijkte het EBOVAC 3-consortium hoogwaardige proeven

in het gebied. De proef onthulde een lage seroreactiviteit voor EBOV-antigenen onder deelnemers, waarbij 1,4% reactiviteit toonde voor twee EBOV-Mayinga-antigenen en 8,5% voor GP-EBOV-Kikwit. Positieve ervaringen werden gemeld dankzij de inzet van de proef voor verbetering van de locatie en training van vrijwilligers. Er werden echter problemen benadrukt zoals onvoldoende compensatie voor tijd en reizen en zorgen over frequente bloedafnames. Het begrip van de deelnemers over de proef, beoordeeld via de scores van de test van begrip (TOU), toonde een significante afname in de loop van de tijd (jaar 1 en jaar 2), vooral bij degenen met minder opleiding en oudere deelnemers. De uitvoering van de proef in het kader van een consortium kende verschillende uitdagingen, waaronder culturele verschillen, taalbarrières en regelgevingskwesties, die duidelijke communicatie en aanpassingsvermogen vereisten onder internationale belanghebbenden. Ondanks deze uitdagingen bereikte de proef een hoge deelnemersbehoud van 92%, wat het belang benadrukt van het aanpakken van deelnemerszorgen, effectieve communicatie en flexibele, collaboratieve benaderingen in het beheer van vaccinproeven in afgelegen LMIC-instellingen.

Conclusies

De EBL2007-vaccinproef in het Gezondheidsdistrict Boende staat als een belangrijke prestatie in klinisch onderzoek voor omgevingen met beperkte middelen. Het gebruik van irisscantechnologie, aanvaard door 99% van de HCP's, illustreert de effectiviteit van biometrische hulpmiddelen in het waarborgen van de nauwkeurigheid en integriteit van proeven, met een opmerkelijk succespercentage van 93,1% in deelnemersidentificatie. Ondanks enige bezorgdheid over mogelijke fysieke effecten, zoals waargenomen visuele veranderingen, benadrukt de proef de noodzaak van voortdurende betrokkenheid van de gemeenschap en duidelijke communicatie. Door uitdagingen zoals complexe regelgeving en logistieke hindernissen te overwinnen, bereikte de proef een opmerkelijke deelnemersbehoud van 92%, dankzij de inzet van het EBOVAC 3-consortium voor kwaliteit, responsieve vrijwilligerstraining en het aanpakken van compensatie- en procedurele zorgen. De afname van het begrip van de deelnemers in de loop van de tijd, zoals blijkt uit de TOU-scores, benadrukt het belang van voortdurende educatie, met name voor oudere en minder opgeleide deelnemers. De EBL2007-proef toont de haalbaarheid en het belang aan van het opnemen van diverse, sociaal-economisch achtergestelde groepen in klinisch onderzoek, vooral in LMIC's, voor een effectieve EVD-respons. Strategische planning, anticipatie op regelgevingsuitdagingen, positieve lokale samenwerkingen, investeringen in lokale onderzoek infrastructuur en innovatieve identificatietools zoals iris scannen zijn cruciaal voor het verbeteren van de kwaliteit van proeven en het bijdragen aan wereldwijde gezondheidsvooruitgang, met name in het beheer van EVD-epidemieën.

Chapter 1 General Introduction

1.1. Ebola outbreaks and Global Public Health Challenges

Ebola Virus Disease (EVD) is a serious and often fatal illness that affects humans and other primates, including monkeys, chimpanzees, and gorillas (1, 2). The disease is caused by a virus belonging to the Filoviridae family, characterized by non-segmented, negative-strand RNA ebolaviruses within the Ebolavirus genus. Due to their high fatality rate along with their high virulence, and potential for aerosol transmission in laboratory settings, filoviruses have been classified as Category A high-priority pathogens by the Strategic Planning Task Force of the Centers for Disease Control and Prevention (3, 4). The World Health Organization (WHO) also identifies EVD as a priority pathogen in its research and development blueprint for action to prevent epidemics (5).

The Ebola virus was first identified in 1976 at the Institute of Tropical Medicine in Antwerp, Belgium, following initial cases reported in Yambuku, Mongala Province, Democratic Republic of the Congo (DRC) (6). Simultaneously, it was recognized as the cause of outbreaks in Yambuku, DRC, and in Nzara and Maridi, southern Sudan (7, 8). Named "Ebola virus" to avoid stigmatizing Yambuku, subsequent findings revealed that the viruses from these outbreaks were of two distinct species: Zaire ebolavirus and Sudan ebolavirus (6).

To date, five ebolavirus species have been identified: Zaire ebolavirus (EBOV), Sudan ebolavirus (SUDV), Tai Forest ebolavirus (TAFV), Reston ebolavirus (REBOV), and Bundibugyo ebolavirus (BDBV) (9). Additionally, a related but unclassified virus, Bombali ebolavirus (BOMV), shares 55%-59% nucleotide identity with other known ebolaviruses (10). EBOV, SUDV, TAFV, and BDBV account for the majority of human cases, with EBOV recognized as the most dangerous pathogens globally due to its high mortality rates.

Since the emergence of EVD in 1976, most outbreaks were primarily localized to rural areas and contained through public health control measures (11). However, the 2014-2016 West Africa EVD epidemic, caused by the Makona variant of EBOV (12) and the largest since the virus‘ discovery, marked a significant escalation with 28,610 cases and 11,308 deaths worldwide (13). This outbreak was notable for nearly ten times the number of patients compared to all previous EVD outbreaks combined, and it included instances of infected individuals moving across borders into Africa, Europe, and North America, inadvertently initiating small transmission chains far from the outbreak's epicenter.

The second-largest EVD outbreak, caused by EBOV; occurred from 2018 to 2020 in the DRC's North Kivu, Ituri, and South Kivu provinces, as well as in Uganda. It resulted in 3,481 cases and 2,299 deaths (11)(Table 1). The DRC, being the most frequently affected, has experienced a total of 15 outbreaks by December 2023 (Table 2). From 2017 to 2022, there was at least one outbreak each year, with two in both 2021 and 2022. This increased frequency has highlighted

the urgent need for additional therapies, diagnostics, and vaccines. The WHO recommends several strategies to control Ebola transmission, including vaccination, robust surveillance, the use of laboratory services for confirmation, effective isolation and quarantine systems, safe burial practices, and community engagement.

However, the DRC's healthcare system, strained by various endemic diseases (COVID-19, cholera, measles, polio, yellow fever, Mpox, etc), often responds too slowly to epidemics (17, 18). The recurrent EVD outbreaks pose significant public health challenges, exacerbated by the nation's limited capacities for effective epidemic management (19). Preventive measures, including vaccination, are more cost-effective than reactive responses. For example, during the 10th Ebola outbreak in the DRC, the cost of treating a confirmed case averaged 1,464 USD, with a typical hospital stay of 14.3 days (20). Factors like poverty, community distrust of authorities, and political instability in regions like Ituri, North Kivu, and Equateur further impede disease surveillance efforts (19). Additionally, the remoteness and escalating insecurity in these areas challenge rapid and effective responses to new EVD outbreaks (21).

Table 1 A chronological list of the major outbreaks of Ebola virus disease in humans (Source: Centers for Disease Control and Prevention, History of Ebola virus disease outbreaks)

Year	Location	Strain	Confirmed case	Death	Epicentre	CFR (%)
1976	DRC	Zaire Ebolavirus	318	280	Yambuku, Province of Mongala	88
1976	South Sudan	Sudan Ebolavirus	284	151	Nzara and Yambio, Western Equatorial State	53
1977	DRC	Zaire Ebolavirus	1	1	Tandala, Province of Sud Ubangi	100
1979	South Sudan	Sudan Ebolavirus	34	22	Nzara and Yambio, Western Equatorial State	65
1994	Gabon	Zaire Ebolavirus	52	31	Mayibout, Ogooué-Ivindo Province	60
1994	Cote D'Ivoire	Tai Forest virus	1	0	Tai National Park	0
1995	DRC	Zaire Ebolavirus	315	254	Kikwit, province of Kwilu	79
1996	Gabon	Zaire Ebolavirus	60	45	Booué, Lopé Department	75
1996	Gabon	Zaire Ebolavirus	37	21	Mayibout 2	57

2000	Uganda	Zaire Ebolavirus	425	224	Gulu, Masindi and Mbarara	53
2001	Gabon	Zaire Ebolavirus	65	53	Mekambo, Ogooué-Ivindo Province	82
2001	Congo	Zaire Ebolavirus	57	43	Mbomo, Kellé, Cuvette Ouest Region	75
2002	Congo	Zaire Ebolavirus	143	128	Mbomo, Kellé, Cuvette Ouest Region	89
2003	Congo	Zaire Ebolavirus	35	29	Mbomo, Kellé, Cuvette Ouest Region	83
2004	Sudan	Zaire Ebolavirus	17	7	Yambio County, Western Equatorial State	41
2005	Congo	Zaire Ebolavirus	12	10	Etoumbi	83
2007	DRC	Zaire Ebolavirus	264	187	Luebo, Kasai Occidental Province	71
2007	Uganda	Bundibugyo Ebolavirus	149	37	Bundibugyo district.	25
2008	DRC	Zaire Ebolavirus	32	15	Mweka and Luebo, Kasai Province	47
2011	Uganda	Sudan Ebolavirus	1	1	Luwero district	100
2012	Uganda	Sudan Ebolavirus	11	4	Kibaale District.	36
2012	Uganda	Sudan Ebolavirus	6	3	Luwero, Jinja, and Nakasongola districts	50
2012	DRC	Bundibugyo Ebolavirus	38	13	Isiro, Haut Uele	36
2014	DRC	Zaire Ebolavirus	69	49	Ikanamongo Village, Tshuapa Province	71
2014	West Africa	Zaire Ebolavirus	28,646	11,323	Meliandou (Guéckédou Prefecture)	40
2017	DRC	Zaire Ebolavirus	8	4	Likati, province of Bas-Uele	50
2018	DRC	Zaire Ebolavirus	54	33	Bikoro, Equateur province	61

2018	DRC and Uganda	Zaire Ebolavirus	3470	2287	Mangina and Beni, North Kivu province	79
2020	DRC	Zaire Ebolavirus	130	55	Mbandaka, Equateur Province	30
2021	DRC	Zaire Ebolavirus	12	6	Beni, North Kivu Province	50
2021	DRC	Zaire Ebolavirus	11	9	Biena, North Kivu Province	81
2021	Guinea	Zaire Ebolavirus	23	12	N'Zérékoré Prefecture	52
2022	Uganda	Sudan Ebolavirus	164	55	Mubende District	33
2022	DRC	Zaire Ebolavirus	5	5	Beni, North Kivu Province	100

Table 2 Overview of Ebola outbreaks in the DRC. (Source: Centers for Disease Control and Prevention, History of Ebola virus disease outbreaks)

Outbreak	Year	Place (provinces)	No of fatalities	No of cases
1	1976	Mongala	280	318
2	1977	Sud Ubangi	1	1
3	1995	Kwilu	254	315
4	2007	Kasai	187	264
5	2008	Kasai	15	32
6	2012	Haut Uelé	13	38
7	**2014**	**Tshuapa**	**49**	**69**
8	2017	Bas Uelé	4	8
9	2018	Equateur	33	54
10	2018-2020	North Kivu, South Kivu and Ituri	2287	3470
11	2020	Equateur	55	130
12	2021 (Feb-May)	North Kivu	6	12
13	2021 (Oct-Dec)	North Kivu	6	11
14	2022	Equateur	5	5
15	2022	North Kivu	1	1

1.2. The Role of Vaccine Clinical Trials in LMICs

The most reliable method for preventing and/or controlling the epidemic is through a safe, effective, and affordable preventive vaccine tailored to the local context (16, 22, 23). Given the ongoing occurrence of EVD outbreaks, the pursuit of efficacious vaccines and treatments remains critical. Therefore, vaccine clinical trials implementation, which typically involve a larger number of human subjects than non-vaccine drugs, play a crucial role in introducing new vaccines.

Historically, most vaccine trials have been conducted in high-income countries (HICs), (27) (Figure 1), but in recent decades, there has been an increase in research centers and trial sites in Low and Middle-Income Countries (LMICs), primarily through international partnerships (27). This shift is relevant because the heaviest burden of infectious diseases lies in LMICs (28). There are notable differences in clinical trial outcomes between HICs and LMICs due to factors like diet, nutrition, genetic profiles, comorbidities, and co-infections, which suggest that results from one region may not always be generalizable to the other (29).

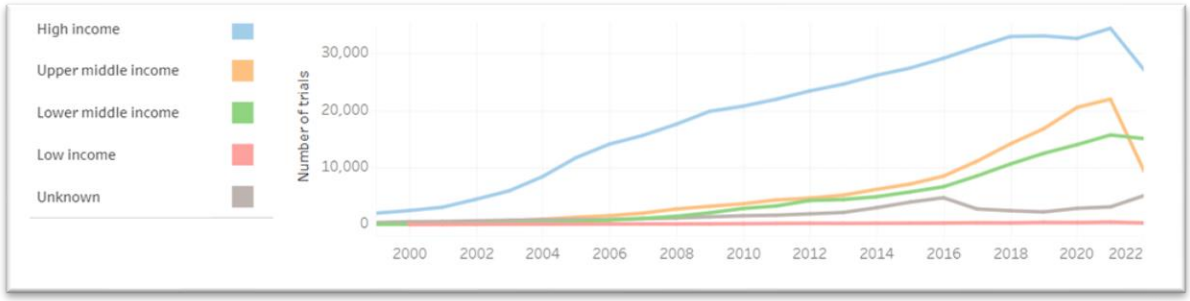

Figure 1 Number of trials by country based income (1999-2022)
(Source: WHO, https://www.who.int/observatories/global-observatory-on-health-research-and-development/monitoring/number-of-clinical-trials-by-year-country-who-region-and-income-group).

Additionally, volunteers in endemic settings are at a higher risk of exposure to infectious pathogens, making them potentially more likely to benefit from vaccination (28, 30). Conducting vaccine trials in regions where target diseases are endemic, like the DRC for Ebola, is scientifically valid and ensures that variations in population genetics and pre-existing immunity are accounted for (9, 29-31). It also enables a direct evaluation of the vaccine's ability to prevent disease, going beyond proxy measures like seroconversion and providing invaluable data on actual disease reduction in the population. This approach is crucial in developing effective vaccines for diseases like EVD (9).

All vaccine trials must adhere to stringent international standards Good Clinical Practice (GCP), clinical science, regulation, and ethics, to protect participants and ensure data integrity, regardless of geographical location.

However, countries like the DRC, characterized by underdeveloped research infrastructure, face unique challenges. Conducting trials in these environments often requires time, flexibility, and creativity, but it also presents an opportunity to elevate research standards and enhance public health. For instance, during the 2014 West-Africa EVD epidemic, the experimental use of the rVSVΔGZEBOV-GP Ebola vaccine, also known as Ervebo (Merck & Co.), provided crucial clinical efficacy data (32). This vaccine was rapidly deployed in the Equateur province during the 2018 outbreak, significantly aiding in controlling the spread of Ebola (33). The data from these studies contributed to its authorization by regulatory agencies like the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) in 2019 (34).

Past EVD outbreaks have revealed systemic challenges in effectively conducting vaccine trials in LMICs settings (35). To mitigate future outbreaks and improve preparedness, especially in countries with limited infrastructure and resources, initiative-taking measures are essential. This includes addressing research gaps and investing in necessary infrastructure and regulatory frameworks for trials implementation.

1.3. Healthcare System in the Democratic Republic of Congo and vaccine trials implementation

The DRC, the largest nation in sub-Saharan Africa, spans an area equivalent to Western Europe and exhibits significant provincial diversity in cultural, economic, and linguistic aspects. The geographic inaccessibility of many regions, reachable only by air or water, further amplifies these disparities. According to the World Bank (36,37), approximately 62% of the DRC's estimated 111 million population live on less than $2.15 daily, placing the DRC among the world's five most impoverished countries.

Only 20% of the country's population has access to electricity, leaving many towns and villages without this crucial service. Despite its abundant water resources, 59% of the DRC population has access to reliable drinking water sources, and basic handwashing facilities are available in only 22% of Congolese households. Furthermore, poor sanitation and hygiene are major health concerns and contribute to the high rate of chronic malnutrition, affecting 43% of children under five (37,38).

The COVID-19 pandemic has added to the strain on the healthcare infrastructure and society, with a total of 80,175 confirmed cases and 1,225 deaths as of January 11, 2022, in Kinshasa (38, 39). It is important to note that these figures might be underreported due to very limited testing capacities and limited access to care . Vaccine distribution faced challenges due to hesitancy and accessibility issues, and the pandemic has further exacerbated socioeconomic impacts, increasing poverty in 2020 and stressing the fragile healthcare system.

The DRC battles endemic diseases such as malaria, HIV/AIDS, tuberculosis, measles, cholera, Mpox, and filoviruses (like EVD and Marburg virus disease) (40). Vaccine-derived polio and meningitis outbreaks underscore the challenge of inadequate immunization coverage,

compounded by vaccine scarcity and regional inaccessibility (36).Additionally, these challenges are further exacerbated by increasing globalized vaccine hesitancy (41).

The governmental healthcare system in the DRC is structured in a pyramidal fashion, consisting of the central level (National Ministry of Health), the intermediate level (Provincial Health Division), and the operational level (Health Districts) (42, 43). Each Health District serves a population of 100,000 to 150,000 and is supported by a general referral hospital (HGR) offering comprehensive services (43). Health Centres at the base level provide basic care to approximately 5,000 to 10,000 residents, and are primarily staffed by nurses. Healthcare delivery is organized into a four-tiered level. Secondary care is available at district hospitals, while tertiary care is offered at provincial hospitals with specialized services. The highest level of care is provided by university and national hospitals (42, 43). Additionally, about 40% of healthcare is delivered by the private denominational and associative sector, while a lucrative private sector operates in urban areas, comprising medical and paramedical consultation and care practices (44).

Several key challenges hinder healthcare delivery in the DRC, including the absence of universal healthcare coverage, infrastructure deficits, and political instability. Barriers such as insufficient human resources, linguistic diversity, and limited health literacy exacerbate these challenges. The scarcity of trained healthcare professionals (HCPs) significantly impedes the advancement of the health sector. (45). Furthermore, poor data management and a lack of comprehensive health information systems hamper evidence-based decision-making.

In rural communities, health disparities are heightened by chronic diseases and limited access to clinical trials (47). Geographic isolation and scepticism towards research contribute to these disparities. Conducting targeted clinical trials in these regions is crucial to address the unique health needs and may contribute to mitigate some health-related inequities.

While clinical trials represent a 'drop in the ocean' in terms of their immediate reach and duration, their impact extends far beyond the direct involvement of participants. Firstly, the unique health profiles and challenges faced by rural communities often remain underrepresented in broader medical research. By conducting targeted clinical trials in rural areas, we gain invaluable insights into the specific health needs and responses of these populations. This not only aids in developing more effective and public health interventions for these communities but also enhances our overall understanding of disease processes in diverse populations.

Additionally, the skepticism towards research in rural areas may stems from a lack of engagement and representation in clinical trials. By actively including these communities, researchers not only address immediate health concerns through the trials themselves but also work towards building trust and understanding about the importance and relevance of medical research in these communities.

While clinical trials are indeed time-bound and limited in scope, the knowledge and advancements they yield have long-term implications. The results of these trials can inform healthcare policies, lead to the development of new treatment protocols, and ultimately contribute to stronger disease control and prevention strategies. Hence, the impact of clinical trials, transcends their immediate temporal and geographic limitations, in reducing health disparities and inequities, particularly in under-served regions.

1.4.EVD response and healthcare workforce challenges in the DRC

Outbreak response includes isolation of patients, treatment protocols, and supportive care for those infected (78). Rapid identification and isolation of EVD cases are crucial to prevent spread. Treatment involves managing symptoms and complications, providing fluids and electrolytes, maintaining oxygen status and blood pressure, and treating other infections if they occur (73, 78).

In regard to vaccination, there are two licensed and WHO-prequalified vaccines to prevent EVD. The Strategic Advisory Group of Experts on Immunization (SAGE) of WHO advocates for a ring vaccination strategy in response to EVD outbreaks (49). This approach involves vaccinating individuals who are most likely to be exposed to the virus, creating a 'ring' of immunized persons around each case to contain the spread. This strategy targets contacts of suspected EVD cases, HCPs, and frontline responders. In an outbreak scenario, SAGE advises that anyone who meets the criteria of being a contact of an EVD patient or contact of a contact should receive a dose of the rVSVΔGZEBOV-GP (Ervebo®, Merck & Co.), provided they haven't been vaccinated against EVD in the past six months. The rVSVΔGZEBOV-GP (Ervebo®, Merck & Co.) is optimal for rapid outbreak response, whereas the two-dose regimen Ad26.ZEBOV and MVA-BN-Filo (Zabdeno®, Mvabea®, Johnson & Johnson), requiring a minimum 56-day interval between doses, is less appropriate for immediate outbreaks but suitable for a proactive vaccination of national and international EVD response teams, including HCP and frontline workers (49, 50).

Given the limitations in vaccine availability and the indeterminate period of immunity, SAGE recommends not to broadly employ the rVSVΔGZEBOV-GP (Ervebo®, Merck & Co.), as well as the Ad26.ZEBOV (Zabdeno®), and MVA-BN-Filo (Mvabea®), for preventative purposes in non-outbreak contexts. However, proactive systematic vaccination of at-risk groups in EVD-endemic regions may preemptively curb epidemics (50). Nosocomial transmission has significantly fuelled most EVD outbreaks, highlighting the importance of HCPs in epidemic management.

The DRC features among the 55 countries on the WHO health workforce support and safeguards list, emphasizing the urgency for targeted support in its healthcare system (51). This inclusion highlights the significant challenges this country may face in achieving universal health coverage (UHC). WHO recommends a minimum threshold of 44.5 skilled healthcare workers (doctors, nurses, and midwives) per 10,000 population to deliver essential health services. The DRC's health workforce density was 26.67 per 10,000 population in 2018 (51) ,

falling below the global median of 49 per 10,000 inhabitants (45). Additionally, the DRC's UHC service coverage index remains below the defined threshold, further emphasizing the urgency for targeted support and interventions in its healthcare system.

Healthcare access in the DRC's rural regions is substantially compromised by the remoteness of numerous villages. A stark discrepancy exists in the distribution of medical professionals; rural areas suffer from a severe shortage of physicians, with an average ratio of 0.35 doctors per 10,000 inhabitants, as reported by a survey from South Kivu (52). Furthermore, there is a pronounced geographical imbalance in the allocation of HCPs resources within the country. Rural health District in the northern part of the country account for less than 3.0% of the nation's doctors (40). In contrast, Kinshasa, the capital, is home to 45% of all DRC's HCPs workforce, despite comprising only 30% of the total population.

Human resources form a cornerstone of the healthcare system, particularly in nations like the DRC where health sector funding is limited. The presence of dedicated health personnel is imperative for the optimal functioning of the healthcare system, especially in contexts of resource scarcity. However, the impact of a dedicated health workforce can be limited when it lacks necessary tools and infrastructure, which may lead to demotivation. To address this, it's important to recognize that dedicated staff alone can't solve all healthcare challenges. In such settings, cost-effective and preventive measures like vaccinations, health education, hygiene promotion, and task shifting are equally important. These strategies can help improve healthcare access and outcomes, even in areas with a shortage of HCPs.

For example, proactive vaccination efforts can prevent disease outbreaks in high risk settings, reducing the burden on the healthcare workforce. Task shifting, where non-physicians take on certain medical roles, can help address shortages in rural areas. By combining dedicated professionals with these measures, countries like the DRC can make progress in healthcare despite limited resources.

1.5.Ebola virus

The term "Ebolavirus" referred to the genus encompassing several virus species, including Ebola virus, whereas "Ebola virus" describes any member of this genus in a broader context. Recently, the genu name Ebolavirus has been updated to Orthoebolavirus. Bombali virus, Bundibugyo virus, Ebola virus, Reston virus, Sudan virus, and Taï Forest virus are orthoebolaviruses.

The Ebola virus possesses a sizable non-segmented, negative-strand RNA genome, approximately nineteen kilobases in length. This genome comprises seven genes arranged in a specific sequence. These genes include the 3' leader-nucleoprotein (NP), virion protein (VP) 35, matrix protein VP40, glycoprotein (GP), VP30, VP24, and the RNA-dependent RNA polymerase (L) followed by the 5' trailer (54, 55) (Figure 2). Each gene typically codes for a single protein, with the exception of the GP gene, which is responsible for encoding three different glycoproteins.

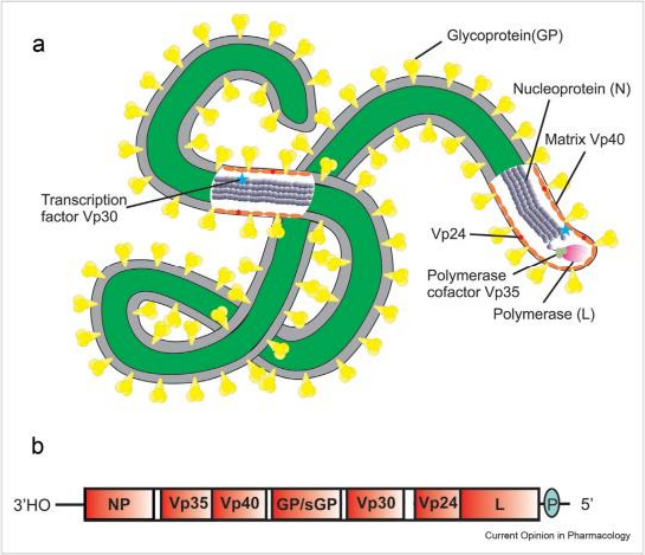

Figure 2 Schematic representation of structure of Ebola virus and its genomic organization

(a) The structure of Ebola virus. (b) The genome organization of Ebolavirus. NP = structural nucleoprotein; Vp35 = non-structural protein; Vp40 = matrix protein; GP/sGP = envelope glycoprotein; Vp30 = non-structural protein; Vp24 = matrix protein; L = RNA polymerase. (Source: Ghosh, Sanmitra, et al. "Genome structure and genetic diversity in the Ebola virus." Current Opinion in Pharmacology 60 (2021): 83-90.)

Prior to 2019, the term "Ebola Virus Disease" (EVD) encompassed all illnesses caused by viruses from the Filoviridae family (56). Due to ambiguities that led to confusion in communication among researchers and editors not well-versed in the distinctions between "Ebola virus" and "ebolavirus," or "Ebola Virus Disease due to Ebola virus infection" compared to "Ebola Virus Disease due to Bundibugyo virus infection," a new classification was adopted. According to the International Classification of Diseases 11th Revision (ICD-11), diseases are now more specifically categorized: Bundibugyo virus disease (BVD) for illnesses caused by the Bundibugyo virus (BDBV), Ebola virus disease (EVD) for those caused by the Ebola virus (EBOV), Sudan virus disease (SVD) for infections by the Sudan virus (SUDV), and Other specified Ebola disease (Atypical Ebola disease) for infections caused by other species, such as the Taï Forest virus (TAFV) (Figure 3).

New WHO-accepted filovirus disease classification

Main disease category: 1D60 Filovirus disease (FVD)

- First disease subcategory: 1D60.0 Ebola disease (EBOD)
  - Second disease subcategories: 1D60.00 Bundibugyo virus disease (BVD)[a]; 1D60.01 Ebola virus disease (EVD)[b]; 1D60.02 Sudan virus disease (SVD)[c]; 1D60.03 Atypical Ebola disease; 1D60.0Y Other specified Ebola disease[d]; 1D60.0Z Ebola disease, virus unspecified
- First disease subcategory: 1D60.1 Marburg disease (MARD)
  - Second disease subcategories: 1D60.10 Marburg virus disease (MVD)[e]; 1D60.11 Atypical Marburg disease; 1D60.1Y Other specified Marburg disease; 1D60.1Z Marburg disease, virus unspecified
- First disease subcategory: 1D60.Y Other specified filovirus disease
- First disease subcategory: 1D60.Z Filovirus disease, virus unspecified

ICD-11, The International Classification of Diseases Revision 11. [a]Caused by Bundibugyo virus (BDBV). [b]Caused by Ebola virus (EBOV). [c]Caused by Sudan virus (SUDV). [d]Caused by, for instance, Taï Forest virus (TAFV). [e]Caused by Marburg virus (MARV) or Ravn virus (RAVV).

Figure 3 New filovirus disease classification and nomenclature (WHO, ICD-11) (56)(57)).

1.5.1. Epidemiology of EVD

1.5.1.1. Host and reservoir

The natural reservoir of Ebola remains unknown, yet researchers suspect African fruit bats as the likely natural hosts for ebolaviruses. RNA of EBOV has been detected in four bat species: *Hypsignathus monstrosus*, *Epomops franqueti*, *Myonycteris torquata*, and *Miniopterus inflatus* (58).

These bats, often asymptomatic carriers of the virus, can transmit it to other forest-dwelling animals like porcupines and non-human primates including monkeys and apes (59, 60). African tropical forests, known for their rich animal biodiversity, have been identified as common ecosystems for the emergence of Orthoebolavirus.

1.5.1.2. Transmission among humans

Bushmeat is a major source of proteins for populations in tropical Africa, and hunting and consuming wild animals is a sociocultural practice in many African countries (61). Transmission of Orthoebolavirus to humans occurs near forested areas, primarily through consumption or handling of infected animals. The virus then spreads in human populations via direct contact with blood or body fluids of infected individuals or contaminated objects, including during sexual contact with infectious Ebola survivors.

The first EVD case in DRC in 1976 involved a teacher who had bought fresh and smoked antelope and monkey meat (11, 62). The 2014-2016 West-Africa EVD epidemic area in Guinea experienced extensive deforestation. Wars in Liberia and Sierra Leone and corruption in

Guinea led to poverty, migration for work, and facilitated virus spread (11). In addition to war, corruption, and deforestation, several other factors including the weak healthcare systems, relative high population density around the tropical forest region contributed to that EVD epidemic. Increasing Ebola outbreaks in DRC since 1995 have been linked to ecosystem changes from deforestation, displacing bats (11, 63, 64). In the past decade, there have been documented cases of EVD re-emergence linked to sexual contact, particularly noted during the 2014-2016 West-Africa EVD epidemic (26), In Sierra Leone (2015), Guinea (2016), and Liberia (2016), cases of Ebola EVD were documented. A physician developed uveitis nine weeks after clinical recovery during this outbreak, with Ebola virus isolated from the aqueous humor (22). Additionally, a nurse experienced a recurrence of neurological symptoms nine months after recovering from EVD (24). In the DRC between 2020 and 2023 (27), there were reports of recurring EVD cases, though specific details regarding sexual transmission or relapse were not provided. Notably, a documented case of acute EVD relapse occurred 149 days post-discharge (27).

Ebolaviruses are recognized for their ability to persist in certain immunologically privileged areas of the body even after the primary infection has been resolved. These areas include the testes, the interior of the eyes, the placenta, and the cerebrospinal fluid surrounding the brain (65). These immune-privileged sanctuaries serve as refuges where the virus is shielded from the host's immune responses, allowing it to persist even after being cleared from other areas of the body. The occurrence and endurance of ebolaviruses in these specific anatomical regions may vary among individuals who have recovered from Ebola. The length of time for which ebolaviruses remain in these physiological fluids after recovery remains a subject for further investigation (66-69). The persistence of the virus poses three risks: transmission to other asymptomatic individuals (e.g., through sexual contact), reactivation of the disease (posing a risk to the affected individual), and transmission from symptomatic individuals experiencing a resurgence of the disease (which may go unnoticed as recurrent/resurgent EVD) (Figure 1).

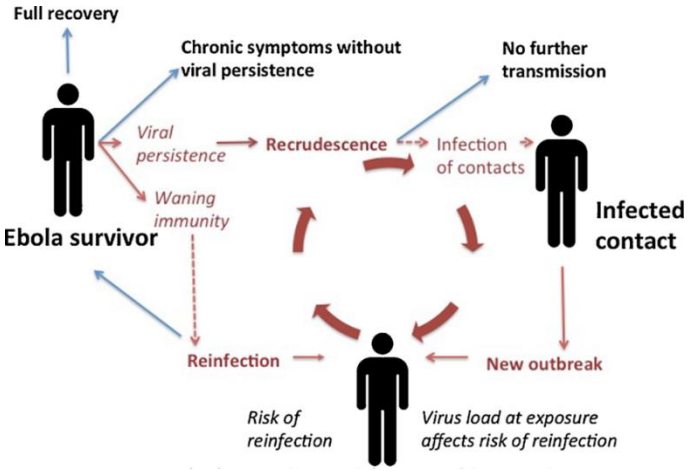

Figure 4 Ebola survivor with waned immunity (67)

1.5.2. Pathogenesis

During the initial phase of infection, viral dissemination is facilitated by mononuclear phagocytes and dendritic cells, which are primary targets of the Ebola Virus (EBOV). The substantial production of interleukins (IL), especially IL-1β, IL-6, and IL-8, along with tumor necrosis factor (TNF) in EBOV-infected cells, likely contributes to lymphocyte death. These factors are associated with disseminated intravascular coagulation and multiple organ dysfunction syndrome, characteristic of the latter stage of EVD (70). The clinical manifestations resemble those of other pathogens like malaria, typhus, and yellow fever, beginning with general discomfort, muscle pain, skin rash, and infection. Within 1 to 3 days after the onset of illness, patients develop nonspecific febrile illness, anorexia, and joint pains, followed by gastrointestinal symptoms including diarrhoea, nausea, and vomiting. Generally, the viral load of EBOV increases concurrently with the severity of clinical manifestations. A viral load exceeding 10 million genomic copies/mL in the blood is indicative of a poor prognosis (71). Higher mortality rates have been reported in patients co-infected with *Plasmodium falciparum* and plasmodia from other species (70, 72). Case fatality rates have fluctuated, ranging from 71% in outbreaks occurring between 1976 and 1986, to 62% in more recent years (11). The average fatality rate was around 50% during the 2014-2016 West Africa EVD epidemic and 66% in the 2018-2020 epidemic in the DRC (Table 1).

1.5.3. Diagnosis of EVD and Clinical manifestations

EVD diagnostics have evolved and improved, especially since the 2014-2016 West-African EVD epidemic. EVD confirmation has progressed from cell culture to more advanced technologies like serological assay [(Indirect Fluorescent Antibody Detection Test (IFAT) and Enzyme-linked Immunosorbent Assay (ELISA)] and molecular-based methods (Reverse Transcription-Polymerase Chain Reaction, Real-Time Polymerase Chain Reaction and the Real-Time (quantification), Reverse Transcription Polymerase Chain Reaction) (8). Genomic sequencing has been introduced to enhance surveillance and response efforts in the DRC, Guinea, Liberia and Sierra Leone.

Diagnostic approaches for EVD predominantly utilize reverse transcription polymerase chain reaction (RT-PCR) and antigen-capture enzyme-linked immunosorbent assay (ELISA). RT-PCR, known for its high sensitivity, is capable of identifying viral presence early in the infection, while ELISA, though less sensitive, is used to identify antibodies generated in response to the virus in blood or other bodily fluids (76). The WHO advises the use of automated or semi-automated nucleic acid tests for standard diagnostic procedures. For remote areas, rapid antigen detection tests are recommended (77, 78). In recent years, there has been the development of several fully integrated PCR platforms. These systems combine nucleic acid extraction, PCR amplification, and detection within 90 minutes or less and are suitable for decentralized healthcare environments, reducing manual labor and enhancing safety. The Xpert Ebola assay by Cepheid exemplifies this, being a cartridge-based, automated real-time RT-PCR system for detecting EBOV NP and GP genes in diverse samples like venipuncture whole blood and swabs from fingerstick blood or oral fluid. The process involves inserting the sample into a vial for processing in a GeneXpert instrument, requiring storage at temperatures of 2 to 28°C (78, 79). Prompt diagnosis of a disease relies on recognizing

individuals at risk. This involves assessing both a history of exposure and clinical symptoms indicative of illness, such as fever, headache, and muscle pain. Table 3.

Table 3. EVD Cases definition*

Case definition	
Suspect case	Any living or deceased person suffering or who has suffered from a sudden onset of high fever and who has been in contact with a suspected, probable, or confirmed case of Ebola or a dead or sick animal in the previous 21 days (for Ebola); OR
	Anyone with sudden onset of high fever and at least three of the following symptoms: headache, anorexia/loss of appetite, severe fatigue, muscle, or joint pain; OR
	Anyone with unexplained bleeding. OR
	Anyone with a sudden onset of high fever and at least three of the following symptoms: Headache; Anorexia/loss of appetite; Lethargy; Muscle or joint pain; Difficulty breathing; Vomiting; Diarrhea; Stomach pain; Difficulty swallowing; Hiccups
Confirmed case of Ebola	An Ebola case (living or deceased) is defined as a person who tests positive for Ebola by RT-PCR. A suspected case of MVE with laboratory confirmation (positive PCR using the GeneXpert Ebola test).

*Source: WHO (80)

1.5.4. Treatment of EVD

Supportive care, including oral or intravenous rehydration, along with managing specific symptoms, enhances survival rates. The U.S. FDA has approved two medications for treating EVD caused by EBOV. Inmazeb, the first of these, combines atoltivimab, maftivimab, and odesivimab - three monoclonal antibodies. This drug, suitable for both adult and pediatric patients, acts by targeting the Ebola virus' surface glycoproteins, crucial for the virus' entry into host cells. Inmazeb works by having its three antibodies simultaneously bind to the glycoprotein, thereby obstructing the virus' attachment and entry (78, 81). The second approved drug for use in adults and children is ansuvimab (mAb114), a human monoclonal antibody (73). Ansuvimab blocks the binding of the virus to cell receptors, preventing viral entry into the cell.

1.5.5. Vaccination

Vaccine development against the Ebola virus has progressed over the past decades. The first Ebola vaccine, the rVSVΔGZEBOV-GP (Ervebo®, Merck & Co.), developed from the vesicular stomatitis virus and effective against the EBOV strain, was approved in 2019. Administered as a single dose, Ervebo is the recommended vaccine by the Strategic Advisory Group of Experts on Immunization of WHO as part for epidemic response using the ring vaccination strategy in

which only direct and indirect contacts of probable and confirmed EVD cases, or frontline and HCPs and humanitarian staff are targeted. This vaccine does not provide protection against other species of Ebola virus (e.g., SUDV). Another vaccine approved since 2020 is a two-dose regimen, the Ad26.ZEBOV and MVA-BN-Filo Ebola vaccines named Zabdeno®, and Mvabea® respectively, developed by the Janssen Pharmaceutical Companies of Johnson & Johnson in collaboration with Bavarian Nordic A/S, suitable for individuals aged one year and older. Ad26.ZEBOV (Zabdeno®) is given first, followed by MVA-BN-Filo (Mvabea®) 56 days later. However, this two-dose prophylactic approach is less appropriate for epidemic response in areas requiring immediate protection. This vaccine has not yet been approved by the FDA for routine use.

Due to vaccine supply constraints and uncertain duration of protection, SAGE (49) advises against the widespread preventive use of the rVSVΔG-ZEBOV-GP vaccine or the Ad26.ZEBOV and MVA-BN-Filo Ebola vaccines outside of outbreak situations. However, during outbreaks, SAGE recommends these vaccines for individuals at some, but lower, risk of EVD, particularly health workers and frontline workers in neighboring areas and countries at risk of the outbreak spreading.

1.6. Rationale of the thesis

To augment preparedness for potential EVD outbreaks in regions endemic to EVD and susceptible to its resurgence, it is crucial to improve the healthcare system's fragility, especially in rural areas. The WHO forecasts a shortfall of 6.1 million HCPs in Africa by 2030, underscoring the urgency of this approach (51). Considering the sparse healthcare workforce in these regions, prioritizing EVD vaccination is a recommended intervention (21, 49). In 2014, the Tshuapa province of the DRC experienced an EVD outbreak, resulting in 66 cases and 49 deaths (82). As part of the European Union Innovative Medicines Initiative's (EU-IMI) EBOVAC 3 project, a collaborative effort between the University of Antwerp (UA) and the University of Kinshasa (UNIKIN), enrolled 700 HCPs from the Boende Health District in the Tshuapa Province (Figure 3) in a 2.5-year vaccine trial (EBL2007 vaccine trial) starting in December 2019. This trial aimed to evaluate a heterologous Ebola vaccine regimen candidate, involving an initial dose of Zabdeno (Ad26.ZEBOV) followed approximately 56 days later by Mvabea (MVA-BN-Filo). This project fits in a broader context of pro-active prevention of a potential reemergence of EVD in this region in vaccinating prophylactically a well-known cohort of HCPs and frontline workers at high risk of EVD exposure, including community healthcare workers, first-aiders workers, Health facility cleaners, nurses, doctors, midwives, nursing assistants, and pharmacists(83). This would enhance preparedness for future EVD outbreaks in this previously EVD affected and remote area. Secondly, implementing this trial aimed to enrich the existing database on the immunogenicity and safety of the heterologous vaccine regimen candidate.

Figure 5 Health District of Boende, Province of Tshuapa, DRC (83, 84)

The map of Africa shows the location of the Democratic Republic of Congo (DRC) on the left. On a detailed map of the DRC, the research area in Boende, located in the Tshuapa province, is specifically identified on the right, along with the delineation of other provinces in the country.

To successfully plan and execute this trial, it was imperative to surmount a range of challenges inherently associated with the DRC's healthcare system. This necessity was further compounded by some local constraints, particularly those stemming from the geographical remoteness of the Boende health district. The objectives of this doctoral thesis are built around the following context: to successfully plan and execute this trial, it was imperative to surmount a range of challenges inherently associated with the DRC's healthcare system. This necessity was further compounded by some local constraints, particularly those stemming from the geographical remoteness of the Boende health district.

The absence of a comprehensive census since 1984 in the DRC poses a significant challenge in legally identifying DRC's citizens and maintaining accurate demographic data. The voter register, used for general elections, remains the sole identification method available. This gap is particularly problematic in healthcare system, where distinguishing registered HCPs from others is impeded by the lack of reliable databases. We employed an iris scanner for participant identification from enrolment to trial completion in EBL2007, assessing its feasibility and cultural acceptability among healthcare providers.

Furthermore, establishing a baseline seroprevalence among trial participants is complicated by the absence of a known standardized assay for EVD seroprevalence studies. The FANG and Luminex ELISAs were jointly utilized to determine the exposure/protection threshold of

healthcare workers prior to vaccination using a statistically estimated cut-off. The FANG ELISA is a highly sensitive assay which specifically detects the EBOV-GP antibodies, while the Luminex Assay expands detection to at least two EBOV antigen enhancing specificity to 99%.

Additionally, ensuring that the EBL2007 vaccine trial adhered to ICH Good Clinical Practice in a precarious location in terms of infrastructure, equipment and skilled human resources was paramount. This entailed overcoming systemic weaknesses such as inadequate infrastructure and equipment in the Boende Health district, logistical challenges due to remoteness, and scarcity of qualified personnel for clinical trials. Attention was also given to how challenges related to trial- participant recruitments, study sources document handling, study vaccine storage, trial-participant's safety monitoring, financial management, involving multiple international partners, were mitigated to set up and implement the EBL2007 vaccine trial over a period of two years and half.

Understanding HCPs volunteering in the EBL2007 vaccine trial perceptions towards the participation in the trial and the study vaccine was essential. Their experiences and, expectations and views ending with follow-up were considered pivotal in influencing future trial participation in similar settings.

Maintaining trial-participant understanding of informed consent over the extended periods of two-years and half was challenging as well. We evaluated the degree to which participants retained and understood consent information over a two-year period post-enrolment.

Overall, detailing how we addressed the challenges encountered during the EBL2007 vaccine trial contributes to the body of knowledge on conducting vaccine trials in challenging environments. This offers valuable insights that could inform the design and implementation of future trials for high-priority pathogens such as Ebola and other filoviruses in similar contexts.

Hence, the structure of this thesis is informed by the following specific research questions:
1. How is the acceptability and accuracy of the iris scanning system evaluated as an individual identification method among HCPs in the EBL2007 Ebola vaccine clinical trial in the Tshuapa province of the DRC?
2. What are the long-term experiences and perceptions of healthcare provider participants and staff regarding the use of an iris scanning system as an identification tool during the EBL2007 Ebola vaccine trial?
3. What are the primary challenges and lessons learned from setting up an Ebola vaccine trial in a remote area of the DRC?
4. What is the prevalence of pre-existing antibodies against EBOV antigens in the baseline serum samples of healthcare providers and frontliners participating in the EBL2007 vaccine trial?
5. What are the experiences, perceptions, and impacts of the EBL2007 vaccine trial on healthcare providers, frontline workers, trial staff, and local health authorities?

6. How effectively do participants of the EBL2007 vaccine trial retain trial-related information over time, as measured by their performance on a TOU at baseline, one year, and two years following their inclusion in the trial?
7. What are the key challenges, mitigations, and lessons learned from conducting an Ebola vaccine trial involving 699 HCPs and frontliners in the remote city of Boende in the DRC ?

1.7. Thesis objectives

1.7.1. General objective of the thesis

This thesis aims to contribute to the body of knowledge on conducting vaccine trials in the challenging environments of LMICs, such as the DRC. It provides insights that could assist in the design and implementation of future vaccine trials for high-priority pathogens like the Ebola virus.

1.7.2. Specific objectives of the thesis

The thesis is structured around the following specific objectives, each addressing a critical aspect of the trial:

- To evaluate the acceptability and accuracy of the iris scanning system as an individual identification method among HCPs in the EBL2007 Ebola vaccine clinical trial.
- To investigate the long-term experiences and perceptions of healthcare provider participants and staff regarding the use of an iris scanning system for identification during the EBL2007 Ebola vaccine trial.
- To identify and analyse the primary challenges and lessons learned from setting up and executing an Ebola vaccine trial in a remote area of the DRC.
- To determine the prevalence of pre-existing antibodies against EBOV antigens in the baseline serum samples of healthcare providers and frontliners participating in the EBL2007 vaccine trial.
- To explore the experiences, perceptions, and impacts of the EBL2007 vaccine trial on healthcare providers, frontline workers, trial staff, and local health authorities.
- To assess trial-participants' retention of trial-related information over time, as indicated by their performance on a TOU at baseline, and then one- and two-years post-enrollment in the EBL2007 vaccine trial.
- To synthesize the key challenges, mitigation strategies, and lessons learned from conducting the Ebola vaccine trial with 699 HCPs and frontliners in the remote city of Boende, DRC.

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Chapter 2 Methodological framework and organisation of the thesis

2.1. Methodological framework

This doctoral thesis draws on the EBL2007 vaccine trial, a collaborative project between the University of Antwerp (UA) and the UNIKIN, under the EU-IMI EBOVAC 3 project. Conducted in the Tshuapa province of DRC from December 2019 to October 2022, the trial aimed to enhance preparedness for Ebola outbreaks by vaccinating high-risk HCPs and frontline workers in a region previously impacted by an Ebola outbreak in 2014. The trial's setting in Boende, a remote area of the DRC, presents a unique case study for exploring the complexities of vaccine trials in LMICs.

The methodological approach of this thesis is multifaceted, reflecting the diverse challenges encountered in the Boende Health District during the EBL2007 vaccine trial. Research activities were centralized in this district, which hosted the trial site at its general referral hospital (Hôpital Général de Référence, HGR Boende), providing a comprehensive setting for the study. The specific objectives guided the research, each addressed with tailored methodologies to yield a holistic understanding of the trial's intricacies:

1. To evaluate the acceptability, feasibility,and accuracy of the iris scanning system as an individual identification method among HCPs in the EBL2007 Ebola vaccine clinical trial **(chapter 3)**:

The acceptability of the iris scan as a biometric identification tool was evaluated through a mixed-methods study in two phases.

In the pre-EBL2007 vaccine trial phase (April 2019, eight months before the commencement of screening and recruitment), acceptability was assessed using a mixed-methods approach that incorporated FGDs with potential HCPs- participants (HCP-P) for the EBL2007 vaccine trial, and a structured questionnaire to gauge their willingness to be identified by the iris scanner if recruited for the trial. This approach enabled the collection of both qualitative and quantitative data.

In the intra-EBL2007 vaccine trial phase (December 2019-February 2020), acceptability was evaluated using only quantitative methods, specifically a spreadsheet that recorded whether enrolled HCP-participants of the EBL2007 vaccine trial accepted (consented) to be identified using the iris scanner in the trial (recruitment and subsequent schedulled visits of the trial). No qualitative methods were employed in this phase.

For the qualitative assessment of acceptability prior to the start of the trial, participants were selected through purposive, non-probability sampling. The selection criteria were deliberately chosen to ensure a diverse representation of perspectives related to the use of the iris scanner, as well as the probability of being exposed to Ebola for different professional categories in case of an outbreak. Profession categories were considered to capture a range of roles and responsibilities within the healthcare setting. Sex and work setting (e.g., reference hospital or health centers within the Boende Health District) were also factored in to ensure diversity in the sample.. At the time of conducting the study, a residential training seminar was organized by local health authorities, encompassing nurses head of health centers from all health districts within the Tshuapa province. This seminar was held in the Boende health district, the same location where our data collection activities were conducted. Thus, nurses were specifically included from health centers throughout Tshuapa province to represent a broader geographical area and to capture variations in healthcare delivery contexts. A total of 86 participants were enrolled across 12 focus group discussions (FGDs). Key informants from the following stakeholder groups were chosen to participate in the FGDs: nurses, community health workers, laboratory technicians, medical doctors, first-aid workers, midwives, and health facility cleaners. This purposive sampling approach was employed to gain in-depth insights into the acceptability of the iris scan as a biometric identification tool from a variety of healthcare provider perspectives. The study was conducted across five healthcare facilities within the Boende Health District. These included the Boende General Hospital, Boende Catholic Mission, N'sele Health Center, Motema Mosantu Health Center, and the Communauté des Disciples du Christ au Congo Health Center.

The feasibility and accuracy of the iris scanning system were assessed longitudinally during the EBL2007 vaccine trial (scheduled visits of day 1, day 56 and day 78), tracking the system's ability to consistently identify HCP-P at enrollment and during follow-up visits, specifically from the second to the third visit within the study's timeline. In this study, the accuracy of the iris scan as a biometric identification tool was assessed by evaluating the rate of successful recognition of study participants during their third visit (day 78). This involved comparing the iris scan results with the clinical trial identity card of each participant to ensure accurate identification at their scheduled visit. A mismatch was recorded when the system generated more than one possible identification record for a returning participant.

Feasibility was measured by examining the duration of the iris scanning process and the number of scanning attempts required. The time taken for the iris scanning device to recognize each study participant in the EBL2007 Ebola vaccine trial was recorded during the second and third visits (day 57 and day 78, respectively). The duration categories were defined as ≤1 minute, 1 minute 1 second to 1 minute 30 seconds, 1 minute 31 seconds to 2 minutes, 2 minutes 1 second to 2 minutes 30 seconds, or ≥2 minutes 30 seconds. Additionally, the number of attempts needed by the iris scan operator or the iris scan devices (including the tablet, scanner, server, and Wi-Fi connection between server and tablet) to successfully recognize a participant was also documented during these visits.

For the qualitive component (pre-EBL2007 vaccine trial phase), the lead qualitative researcher, GJ, a medical anthropologist, transcribed notes and reviewed audio files to

compile data for analysis and verification. Notes were typed in either English or French. A preliminary analysis of qualitative data was conducted throughout the data-collection process, employing an inductive thematic analysis approach (1). GJ was responsible for all thematic analysis of qualitative data, focusing on identifying dominant themes through a systematic review of FGD audio recordings and transcribed notes. The occurrence and recurrence of salient concepts were labeled throughout, and emerging trends were critically analyzed in relation to the research objectives and topic guide, adhering to the principles of inductive analysis. An appointed research member, TZM, was additionally responsible for maintaining the quantitative survey database, including the acceptance rate of iris scanning at the end of discussions. For the quantitative component (pre and intra EBL2007 vaccine trial), the dataset was meticulously reviewed for any inconsistencies, such as duplicate entries, and subsequently processed using Excel to summarize the results in terms of proportions. The normal approximation method (Z-test) for calculating 95% confidence intervals for proportions was applied in our analysis

2. To investigate the long-term experiences and perceptions of HCP-participants and staff regarding the use of an iris scanning system for identification during the EBL2007 Ebola vaccine trial **(chapter 4)**:

This qualitative study employed purposive sampling to collect data from trial participants and staff approaching the completion of the EBL2007 vaccine trial. The sample included trial participants who used iris scanning for identification, those who did not select iris scanning as their initial identification method, trial physician staff, and iris scan operators.

Data collection combined FGDs and individual in-depth interviews (IDIs). FGDs involved participants of the EBL2007 vaccine trial who had been using iris scanning as their identification method from the outset, representing diverse professional categories such as community healthcare workers, first-Aid workers, midwives, nurses, and facility cleaners. Considerations were given to sex and professional categories to foster open dialogue within each FGD.

IDIs were conducted with trial participants who initially opted against using iris scanning, as well as physicians involved as trial participants or staff, and iris scanning operators. The selection of participants who forwent iris scanning was based on documented refusals, with the predominant rationale being apprehensions regarding ocular safety.

The audio recordings of all conversations were meticulously transcribed and, if necessary, translated into French before being imported into NVIVO software (QSR International, Melbourne, Australia) for analysis. Each transcript was anonymized and assigned a unique identifier to ensure confidentiality. AP (a social scientist) and TZM (a doctoral student) engaged in regular collaborative meetings to ensure consistency in the coding process.

The data underwent thematic analysis approach, which commenced after an initial phase of familiarization with the dataset. The coding process combined inductive and deductive

approach (1, 2). Starting with the deductive approach, initial codes were derived directly from the two main themes predetermined in our interview guide: 1) the acceptability of the iris scan, and 2) knowledge, perception, and use of the iris scan. Initial codes that addressed similar themes were grouped into predetermined themes using a start list (3). As the analysis progressed, an inductive approach was adopted, enabling the identification of new codes that were not initially anticipated. The starting list was subsequently updated to incorporate these emergent codes. An iterative process was used to further refine and develop the sub-themes and themes, ensuring a coding process in line with the objective. The final themes were supported by significant quotations from the transcripts. The analysis was conducted in French to maintain the authenticity and nuance of participants' responses.

3. To identify and analyze the primary challenges and lessons learned from setting up and executing an Ebola vaccine trial in a remote area of DRC **(Chapter 5)**:

We employed a narrative review methodology to synthesize challenges, mitigations, and lessons learned from the EBL2007 vaccine trial, aiming to inform future clinical trials in similar LMIC settings, such as the Boende health district in the DRC. This review chronicled our own experiences and challenges faced during the preparation phase of the EBL2007 vaccine trial in the remote, endemic health district of Boende. The narrative review was chosen for its interpretive and discursive synthesis approach, allowing us to document the principal investigator's (UNIKIN) and the trial sponsor's (UA) perspectives, and providing directions for future research (3,4).

Information was gathered from the following sources: field notes, mission reports, summaries of activities, and minutes of weekly meetings. These sources covered the period from when the sponsor and the PI started working together in the preparation phase of the EBL2007 vaccine trial until the first participant was recruited. For Chapter 5, these documents were used as sources. For Chapter 9, the same documents were used, covering the timeframe from the recruitment of the first participant to the follow-up of the last participant in the trial. The PI, sponsor, and research team maintained comprehensive records throughout the setting up of the trial, documenting daily and weekly operations, logistical hurdles, and interactions with the local community, ethical, regulatory, health, and political authorities. Routine reports generated during the trial offered insights into the progress of activities related to site readiness, the training of local staff, renovations of the study site, acquisition of necessary approvals, purchase of cold chain and laboratory equipment, and the logistics of transporting these items to the site. Minutes from meetings involving all trial partners, including the PI, the sponsor, clinical research organization (CRO), data management company, medical monitors, and other consortium partners were considered as well. We carefully read these documents to identify the challenges, mitigations, and lessons learned in preparing the trial. The collected information was then integrated to build a table that summarized the main challenges, mitigations, and lessons learned in setting up the EBL2007 vaccine trial. Challenges were categorized based on an exploratory review of the literature on lessons learned in the field of vaccine trial implementation in LMICs (5-9). Then, we put together a narrative that combined these findings, showing the main ideas and trends that came out of the table."

4. To determine the prevalence of pre-existing antibodies against EBOV antigens in the baseline serum samples of healthcare providers and frontliners participating in the EBL2007 vaccine trial **(Chapter 6).**

The study collected baseline serum samples from healthy healthcare providers and frontliners in the Boende Health District, DRC, to assess immunogenicity and safety of Ad26.ZEBOV and MVA-BN-Filo vaccines using FANG ELISA and Luminex assays. Statistical analyses, including change point analysis (10) to determine cutoff values for seroreactivity and generalized linear models to correlate seropositivity with participant characteristics, aimed to evaluate pre-existing antibodies against EBOV antigens among the trial participants. Data were compiled and summarized using descriptive statistics for all participants enrolled in the EBL2007 vaccine trial using SPSS 28.0 IBM SPSS Statistics for Windows, version 28.0 and R 4.2.1 Statistical Software

5. To explore the experiences, perceptions, and impacts of the EBL2007 vaccine trial on healthcare providers, frontline workers, trial staff, and local health authorities **(chapter 7).**

This qualitative study employed FGDs and IDIs to explore the perspectives of HCPs, frontline workers, and local health authorities involved in the EBL2007 vaccine trial in Boende, DRC. A purposeful sampling strategy was utilized to select participants based on their roles in the trial, sex, and healthcare work categories, aiming to gain deep insights into their experiences and perceptions of the EBL2007 vaccine trial. Data collection continued until data saturation was achieved, which occurred after conducting 10 FGDs with a total of 85 participants and 15 in-depth interviews with 15 participants. This ensured that no new themes or insights emerged from additional interviews.

Data collection was facilitated using pre-tested, open-ended interview guides, with discussions conducted in French or Lingala according to participants' preferences. The EBL2007 vaccine trial site coordinator played a key role in extending invitations and ensuring a representative sample.

Prior to commencing each interview, whether FGDs or individual in-depth interviews, participants were reminded of the study's purpose. Each session lasted between 60 to 90 minutes, and participants' responses were recorded. Voice recordings in languages other than French were translated into French before being transcribed by two independent transcribers who were not involved in the trial. The accuracy and consistency of the transcripts were verified by TZM, who also coded all transcripts from both FGDs and in-depth interviews. AP and TZM collaboratively determined the codes and categories used to generate themes.

FGDs were facilitated by a social scientist (AP), who alternated between the roles of moderator and note-taker. Two doctoral students (TZM and FBB) also intermittently served as note-takers or moderators. TZM, one of the doctoral students, also acted as a sub-investigator of the EBL2007 vaccine trial, whereas FBB was not involved in trial activities. All

individual in-depth interviews were conducted by TZM, AP, or FBB in settings chosen for their tranquility and preferred by the interviewees. Each interview lasted approximately 50 minutes on average, allowing for thorough exploration of the topics discussed.

The analysis was conducted using a thematic approach, employing both inductive and deductive coding strategies (2). Recordings of discussions and interviews were transcribed and, if necessary, translated into French. The transcribed data were then imported into NVIVO software for analysis. AP (a social scientist) and TZM (a doctoral student) initially engaged in open coding to generate initial codes and organize them into potential themes through a collaborative and reflexive process.

Initial codes were derived from three predefined themes in the interview guide for the interviews and Focus Group Discussions (FGDs), specifically: 1. Understanding of the clinical trial in general and the EBL2007 trial protocol in Boende; 2. Experiences with the clinical trial in general and the EBL2007 protocol in Boende; and 3. Looking to the Future. These codes were grouped under the predetermined themes using a starting list (3). Subsequently, an inductive approach was adopted, enabling the identification of new codes that were not initially anticipated. The starting list was updated to incorporate emergent codes. An iterative process was employed to further refine and develop the sub-themes and themes, ensuring a coding process in line with the objective.The final themes were supported by significant quotations from the transcripts, providing a rich understanding of participants' experiences and perceptions.

6. To assess the effectiveness of participants' retention of trial-related information over time, as indicated by their performance on a TOU at baseline, and then one and two years post-enrollment in the EBL2007 vaccine trial **(chapter 8)**

The EBL2007 vaccine trial employed a TOU to assess participants' knowledge of the study vaccines, trial procedures, risks, and volunteerism, with the TOU conducted at baseline, one year, and two years post-enrolment. Data collection involved recording TOU scores and demographic information in a Redcap database, with quality assurance checks against the original paper questionnaires and additional data linked from the EBL2007 study database. Analysis of TOU scores over time utilized beta regression and generalized linear models, analysing the effect of time, age, sex, occupation, and specific question categories on participants' information retention, performed using various statistical packages in R.

7. To synthesize the key challenges, mitigation strategies, and lessons learned from conducting the Ebola vaccine trial with 699 healthcare providers and frontliners in the remote city of Boende, DRC (**Chapter 9**).

We conducted a narrative review based on our experiences and data collected during the implementation of the EBL2007 vaccine trial in Boende from December 2019 to September 2022, DRC, with the aim of informing future clinical trials in similar LMIC contexts. This review reported the challenges encountered during the execution phase of the EBL2007 vaccine trial

in the remote and endemic health district of Boende in DRC. The narrative review approach was chosen for its interpretative and discursive synthesis capabilities, allowing us to critically document and interpret the challenges encountered, how they were resolved, and the lessons learned during the trial execution period (4, 5). This approach thus provided deeper insights from the perspectives of the PI and the trial sponsor, with the aim of offering guidance for vaccine trials in similar contexts.

Information collection encompassed field notes from the authors, mission reports from the PI and sponsor, summaries of periodic activities, and minutes of weekly meetings from the enrollment of the first participant in the trial (December 2019) up to approximately the completion of follow-up by the last participant (September 2022). We carefully read these documents to identify the challenges, mitigations, and lessons learned in implementing the EBL2007 vaccine trial. The collected information was then integrated to build a table that summarized the main challenges, mitigations, and lessons learned in setting up the EBL2007 vaccine trial. Challenges were categorized based on an exploratory review of the literature on lessons learned in the field of vaccine trial implementation in LMICs (6-10) . Then, we put together a narrative that combined these findings, showing the main ideas and trends that came out of the table.

The dissertation culminates in a general discussion (**chapter 10**) and a concluding chapter **(Chapter 11)** that synthesize the insights gained from overcoming systemic and local challenges, establishing a seroprevalence baseline, assessing identification methods, and understanding the trial participants' experiences. This not only contributes to the knowledge of conducting vaccine trials in LMICs but also informs future designs and implementations of trials for high-priority pathogens like Ebola virus.

2.2. Organisation of the thesis

The thesis is organised into eleven chapters. The introduction chapter (Chapter 1) provides a comprehensive overview of the challenges and implications of EVD in global public health. It details the history and epidemiology of EVD, highlighting its emergence in 1976 and subsequent outbreaks, particularly in the DRC. The chapter discusses the development of vaccines as a crucial strategy in managing EVD outbreaks, emphasizing the role of vaccine clinical trials, especially in LMICs. It also examines the healthcare system in the DRC, focusing on the challenges of implementing vaccine trials in such environments. The chapter sets the stage for the thesis by presenting its objectives, which revolve around evaluating the EBL2007 vaccine trial's execution in the DRC. The Chapter 2 of this thesis outlines the multifaceted methodology applied in the EBL2007 vaccine trial and concludes by providing an overview of the entire thesis structure, detailing the content of each subsequent chapter and the overall organization of the thesis. The chapter 3 explores the acceptability, accuracy, and feasibility of iris scans in identifying participants in the EBL2007 vaccine trial in the Health District of Boende, DRC. Chapter 4 of the thesis examines the long-term experiences and perceptions of healthcare workers regarding iris scanning used for identity verification in the EBL2007 vaccine trial. Through focus group discussions and in-depth interviews, it reveals an initial wide acceptance of the technology, but notes a shift towards less favorable perceptions over

time, with concerns about potential vision impairment. The chapter emphasizes the importance of clear, continuous communication about the safety and function of iris scanning to alleviate concerns and dispel misunderstandings among trial participants. Chapter 6 presents a serosurvey conducted among healthcare providers in the DRC, assessing seroreactivity to Ebola virus antigens. The results show low seroprevalence, indicating the need for standardized antibody assays and cutoffs in Ebola virus seroprevalence studies. Chapter 7 explores the experiences of healthcare providers and frontline workers in an Ebola vaccine trial in the DRC. Interviews and discussions reveal positive trial experiences and the importance of considering participants' opinions and concerns throughout the trial. Chapter 8 investigates the long-term retention and understanding of trial information among participants of the EBL2007 vaccine trial. The study highlights a significant decrease in knowledge over time, emphasizing the need for regular information reinforcement for informed consent. Chapter 9 discusses the logistical, administrative, and ethical challenges of conducting a vaccine trial in a remote area of the DRC. The chapter underlines the importance of clear communication, collaboration, and flexibility in overcoming these challenges, contributing to high participant retention rates. Chapter 10 and 11 focus on the implications and recommendations for future clinical trials in remote and low-resource settings. They advocate for the integration of biometric technologies like iris scanning for accurate participant identification, standardization of baseline seroprevalence methods, rigorous adherence to GCP standards, and investment in local research infrastructure to enhance trial quality and credibility. The chapters also emphasize the importance of community engagement, ethical conduct, informed participation, and leveraging digital technologies for data management.

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Chapter 3 Use of iris scanning as a method of biometric recognition of healthy adults participating in an Ebola vaccine trial in the Democratic Republic of the Congo: a mixed-method study design

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3.1. Abstract

Background: A partnership between the University of Antwerp and the University of Kinshasa implemented the 'EBOVAC 3' clinical trial with an Ebola vaccine regimen administered to health care provider participants (HCP-P) in Tshuapa Province (Democratic Republic of the Congo). This RCT was part of an Ebola outbreak preparedness initiative financed, through an Innovative Medicines Initiative-European Union. The EBOVAC 3 clinical trial used iris scan technology to identify all HCP-P participating in the vaccine trial to ensure that the right participant received the right vaccine at the right visit.

Objective: We aimed to assess the acceptability, accuracy, and feasibility of iris scan technology as an identification method within a population of health care provider participants in a vaccine trial in a remote setting.

Methods: We used a mixed methods study. The acceptability was assessed prior to the trial through 12 focus group discussions (FGDs) and was assessed at enrollment. Feasibility and accuracy research was conducted using a longitudinal trial study design, where iris scanning was compared with the unique study ID card to identify health care provider participants at enrollment and at their follow-up visits.

Results: During the FGDs, health care provider participants were mainly concerned about the iris scan technology causing physical problems to their eyes or exposing them to spiritual problems through sorcery. However, 99% (85/86; 95% CI 97.1-100.0) of health care provider participants in the FGDs agreed to be identified by the iris scan. Also, at enrollment, 99.0% (692/699; 95% CI 98.2-99.7) of health care provider participants accepted to be identified by iris scan. Iris scan technology correctly identified 93.1% (636/683; 95% CI 91.2-95.0) of the participants returning for scheduled follow-up visits. The iris scanning operation lasted 2 minutes or less for 96.0% (656/683; 95% CI 94.6-97.5), and 1 attempt was enough to identify the majority of study participants (475/683, 69.5%; 95% CI 66.1-73.0).

Conclusions: Iris scans are highly acceptable as an identification tool in a clinical trial for health care provider participants in a remote setting. Its operationalization during the trial demonstrated a high level of accuracy that can reliably identify individuals. Iris scanning is found to be feasible in clinical trials but requires a trained operator to reduce the duration and the number of attempts to identify a participant.

Keywords: biometric identification; iris recognition; vaccine trial; participants' visits; acceptability; feasibility; Democratic Republic of the Congo; mixed methods; Ebola.

Trial Registration: ClinicalTrials.gov NCT04186000;
https://clinicaltrials.gov/ct2/show/NCT04186000

3.2. Introduction

Identification and recognition of study participants in a clinical trial – during the process of recruitment and follow-up visits – is a growing issue [1]. Conventional methods for the recognition of participants in health facilities may include patient name, date of birth, government identity card with photo, and phone number [2-5]. However, these methods are not always reliable or accurate [5]. For example, identity cards can be stolen or forgotten and there is a risk of assigning a participant's ID (intentionally or unintentionally) to another volunteer during a study visit. Some participants may give their ID card number to a family member, with a similar physical resemblance, if they are unable or unwilling to keep to their appointment time. In clinical trials, efficacy and safety data such as (serious) adverse events, are repeatedly assessed through anamneses, physical examinations and biological samples during different visits, possibly, over a long period of time. Thus, participant enrollment and identification are essential steps to ensure that all data collected is unique and neither the participant nor the visit has been misidentified [1,2]. A biometric identification method coupled with a unique participant ID number could mitigate the occurrence of mistakes made using conventional methods during initial and follow-up clinical trial visits [3].

Biometric technology confirms the physical presence of the person by assessing unique physical or behavioural characteristics that cannot be borrowed, stolen, or forgotten. Such technology uses matching algorithms or artificial intelligence for identifying the particular feature [3,6,7]. A number of biometric identifiers, including physical traits (e.g. fingerprint, face, palm, cornea, iris, thermogram of the body, face or ear, DNA) or behavioural traits (e.g. signature, voice, typing dynamics, smell and walk pattern) have demonstrated technical feasibility in various studies [6-10]. Biometric identification systems have many advantages over more conventional methods of identification such as easier fraud detection and being more accurate (than a photo) face recognition. Therefore, it is increasingly used worldwide in various fields to recognize individual persons and secure their data (e.g. during elections, at airports, for criminal detection) [6].

Irises are an ideal part of the body for biometric identification. The iris is flat and has a fine texture and geometric configuration determined randomly upon embryogenesis [3,10,11]. It is a unique, permanent and universal ‘biometric signature’ throughout a person’s lifespan which is covered by a highly transparent and sensitive membrane which makes it distinct from other biometric methods [1,2]. A human iris is always stable irrespective of age [10]. That is in contrast to the fingerprint structure - the most widespread biometric method of identification- that varies during childhood, and only becomes stable after many years [5,9]. Fingerprinting does carry additional risk such as spreading some infectious diseases as it requires the volunteer, and sometimes the operator, to come into physical contact with the fingerprint device. Identical twins (monozygotic) were found to have higher similarities of fingerprints patterns compared to non-identical twins in a study [4,5]. Iris scanning is feasible under most circumstances as it can be carried out from anywhere between 10 cm to a few meters away from the eye, and results are generally available within 30 seconds [7,12]. Even genetically

similar people have entirely independent irises thus the iris scanning recognition avoids misidentification of identical twins [3-5]. However, iris recognition may be challenging for people who suffer from diabetes or any other iris diseases [4, 5]. Moreover, the accuracy of the scanning devices can be affected by unusual light effects in comparison to fingerprinting [2,5].

Iris scans may offer one of the most secure strategies of authentication and recognition in clinical trials [7]. Iris-based biometric systems have demonstrated a promising performance during the process of recognition with an average time (during initial clinical trial enrollment) of less than two minutes, and a sensitivity of at least 86% [8,11,12]. In Kenya, an iris scan recognition sensitivity/accuracy of 95% was found in HIV and tuberculosis patients during routine hospital consultations [8]. This was better than fingerprint biometric recognition found in Ghana (68,7%) and in Uganda (75,5%) [11,12]. Thus, use of iris scan technology can substantially reduce the possibility for fraud and abuse within a clinical trial [3,9,10]. Lastly, it has a high acceptance rate with very low false match rate as well as rejection rates [1,2].

Despite its attractive design features, little is known about the acceptability of iris scan technology to the general public, especially how this varies across and within countries. Acceptability within a population may depend on many factors as positive perception, confidence and constraints presented against the use of iris scan. For example, one of the few studies available, a survey conducted in Australia on the willingness of the general population to use biometric security technologies, found that 61% of the population would accept fingerprints whereas only 41% would accept iris scan recognition [6]. In California, 72% of participants preferred an identification by fingerprint [9]. A remarkable acceptability rate of iris scanning itself (98.9%) was noted in a survey on an identification system of routine clinic services in Kenya [8].

As part of this ongoing Ebola vaccine clinical trial (EBOVAC 3, Study protocol number VAC52150EBL2007, clinicaltrials.gov identifier: NCT04186000), [13], we assessed acceptability, accuracy and feasibility of iris scan technology as a biometric identification method within a population of HCP-P in a remote setting.

3.3. Methods

3.3.1. Study design

A mixed-method study design assessed the acceptability, accuracy and feasibility of the iris scan as a biometric identification tool in the Ebola vaccine trial in Boende, Tshuapa province, Democratic Republic of the Congo. Acceptability was assessed through focus group discussions (FGDs) with volunteering HCP-P and via a survey with a structured questionnaire. Feasibility and accuracy research was conducted using a longitudinal study design where iris scanning was used to uniquely identify HCP-P at enrolment, and at their follow-up visits in the clinical trial. Accuracy and feasibility studies were conducted from December 2019 to April

2020 from the second participant visit (day 57) until the third participant visit to the study site (day 78) (Appendix 1).

3.3.2. Participants and recruitment procedures

For the qualitative acceptability assessment, study participants were selected using purposive, non-probability sampling. A total of 86 participants were enrolled in 12 focus FGDs (Appendix 1, Table 1). Key informants from the following stakeholder groups were selected for FGDs: nurses, community health workers, laboratory technicians, medical doctors, first-aid officers, birth attendants, and hospital cleaners. All recruited HCP-P worked at the reference hospital and/or health centers within Boende District (with the exception of nurses who worked in health centers throughout Tshuapa Province). Research activities occurred at five sites all located in the Boende Health Zone: Boende General Hospital (i.e. *Hôpital Général de Référence de Boende*), Boende Catholic Mission, N'sele Health Center (i.e. *Centre de* Santé *Boende II N'sele*), Motema Mosantu Health Center (i.e. *Centre de* Santé *Motema Mosantu*), and Communauté des Disciples du Christ au Congo Health Center (i.e. *Centre de* Santé *CDCC*).

For the quantitative study component (assessing acceptability, accuracy and feasibility), all HCP-P enrolled in the current clinical study (699 in total) were included. All participants were HCP-P working in Boende Health District. Their workstations were located between 0 kilometer and 50 kilometers away from Boende General Hospital.

3.3.3.Ethical approval

Research was conducted in line with the prevailing ethical principles of socio-behavioral studies with human populations to protect the rights and welfare of all participants. Permission to undertake the acceptability (qualitative) study was granted by the DRC National Ethics Committee for Health (Reference: n°93/CNES/BN/PMMF/2019), the Institute of Tropical Medicine, Belgium (Reference: 1293/19), and the University of Antwerp (UAntwerp), Belgium (Reference: 19/14/188). Permission for the accuracy and feasibility (quantitative) study, collected during the course of the ongoing clinical trial, was granted by the DRC Ethical National Committee (Reference: n°137/CNES/BN/PMMF/2019).

3.3.4. Data collection and informed consent

3.3.4.1. Pre-trial study

Based upon a literature review, a topic guide was developed highlighting potential key issues with regards to acceptance of new technologies among health care providers in DRC. This review formed the basis for the design of the FGDs tool which included questions and probes focusing on the background of HCP-P and their role in the community, their acceptance of new technologies and communication strategies, and their recommendations for appropriate identification and communication tools with trial participants (Appendix 2). UAntwerp and University of Kinshasa (UNIKIN) team members reviewed and refined the research tools prior to their finalization and implementation. Specific questions and probes were reviewed and refined during the research period in light of arising themes [(e.g. an on-going Monkeypox vaccine trial in Tshuapa at the time of data collection [14].

Key topics were addressed in each discussion in order to allow for generalization of themes across participant groups. The research was deliberately designed to facilitate input from multiple HCP-P stakeholders in a stepwise manner, so that issues raised by one group of participants were also discussed with other participant groups to assist with triangulation of data. At the start of each discussion, it was made clear to all potential participants that their involvement was optional and voluntary. The study's consent form was presented, explained in detail and all participants' questions were answered prior to beginning data collection. Informed consent was given verbally. All FGDs were conducted in either French or Lingala, depending on the linguistic preferences of participants. FGDs lasted for approximately 60-80 minutes. Audio recordings were made, along with field notes, which served as the basis for thematic analysis of data. Concurrent to FGDs, acceptability was (quantitatively) defined as the number of participants agreeing to iris scanning as a proportion of all the individuals approached. Reasons for declining iris scanning were elicited from participants.

3.3.4.2. Intra-trial study

Accuracy was measured by the rate of successful recognition of study participants (percentage of participants recognized by the iris scan) during the participants' third visit (day 78). This was achieved by cross-referencing the output of the iris scan with the clinical trial identity card of each participant to make sure that it was indeed the correct study participant returning on his/her corresponding scheduled visit date. A wrong matching was counted in the event a registered participant returned for his/her next visit and the system gave details of more than one possible identification record. Feasibility was measured by how long (i.e. duration of operation following these ranges, less or equal to 1 minute, 1'01" to 1'30'', 1'31'' to 2'00", 2'01" to 2'30''or more than 2'30''; Table 2) the iris scanning device took to recognize each study participant in the EBL2007 Ebola vaccine trial during the second and the third visit (i.e. day 57 and 78) and the number of scanning attempts that were required by the iris scan operator or by the iris scan devices (tablet, scanner, server, and Wi-Fi connection between server and tablet) during these same visits. The duration of operation included the time for the biometric tablet to capture iris image, identity photo, demographics, and the time it took to link this data with the local server. An assessment of time to recognize each study volunteer at their third visit was recorded by the operator. It is important to note that a problem was encountered during the first study visit (day 1) where all vaccinated participants who received their first vaccine dose on that day, should have had their demographic information and iris scan recognition registered on the server. However, this data was lost due to a manual error which occurred when attempting to save all of the data collected for this visit, resulting in the loss of participant demographic and biometric data. This error was corrected during the second visit (day 57) when all participant data was re-entered (Appendix 1).

3.3.5. Equipment and procedures

The iris scan operator, a trained and authorized study staff member, used an iris camera (Iritech, Irishield Monocular Fairfax, VA 22030, USA), and a tablet (Samsung Tab Active 2, Suwon, South Korea) connected via Wi-Fi to a local ruggedized server (Cincoze DX-1100, New Taipei City, Taiwan) located approximately 10 meters from his physical location. An external hard drive for backing up the iris scanning database was located nearby as well. A biometric

user interface running on the Samsung Tab Active 2 was designed by Janssen Pharmaceutica NV Beerse, Belgium. In addition to the iris scan, the operator captured demographic data on the biometric tablet such as gender, year of birth, participant ID, passport photo, contact telephone number, date, and time stamps of the iris scan. Activities performed on the tablet were captured in an audit trail with date and time stamps. The biometric tablet allowed the operator to assess whether administration of the second vaccine dose (administered on day 57) as well as the blood collection during the third visit (day 78) were in the predefined visit windows or not. To capture the irises, the operator was standing in the front of the study volunteer to identify, and he held a camera in his right hand and a tablet in his left hand. The volunteer was seated so that his/her head and body were vertically aligned. The distance between the iris and the camera could range from 3 to 10 cm.

3.3.6. Data analysis

3.3.6.1. Pre-trial study

At the conclusion of the research activities, the lead qualitative researcher, a medical anthropologist, transcribed notes alongside re-reviewing audio files in order to compile data for review and verification. Notes were typed in either English or French. Preliminary analysis of qualitative data was conducted throughout the data collection process. The lead researcher was responsible for all thematic analysis of qualitative data. Dominant themes were identified through the systematic review of FGD audio and transcribed notes. The occurrence and reoccurrence of salient concepts were labelled throughout, and emerging trends were critically analyzed according to the research objectives and topic guide. An appointed research member was additionally responsible for maintaining the quantitative survey database on the acceptance rate at the end of discussions.

3.3.6.2. Intra-trial study

Data with regards to the accuracy and feasibility study were collected on an Excel spreadsheet. The data set was checked for any inconsistencies such as duplicates and then processed using Excel to synthesize the results in terms of proportions.

3.4.Results

3.4.1. Pre-trial study: Acceptability and concerns about the iris scan

Data collection and in-country fieldwork was conducted in April 2019. Overall, acceptance (85/86; 99%) of the iris scan technology was widespread (95%CI: 97.1;100.0). As stated by one nurse, '*For me, I accept it* [iris scan] *because I know it is a process that is being used to cast away Ebola and we want this disease to leave*.' However, another research participant, also a nurse, refused to have her picture taken (while consenting to have her iris scanned) on the justification that having her picture taken may cause problems with her church superiors.

FGD participants voiced some primary concerns about the iris scan. The concern that the iris scan may cause physical problems to their eyes was widespread across all stakeholder groups and education levels. As stated by one community health worker, '*We are agreeing with what you say, but we are afraid with the use of the eye scan because we fear it may cause problems*

with our eyes.' Similarly, one birth attendant stated, '*We are asking because the eyes are the life of the people so after using the eye scan will there be some problems for us with our eyes?*'. Participants often associated the extended duration of (some) scans as harmful to their eyes due to the light emitted by the scanner. The research team often heard participants asking, '*Will the scanner disturb the eyes with the rays* [light] *in relation to the duration?*' and '*Are you sure that this scan will not hurt our eyes?*'. The pre-trial acceptability study therefore noted that there was a higher risk for the volunteers enrolled in the trial to link any vision loss to the iris scan. In fact, even if a participant consented to an eye scan at the time of the vaccination as indicated by the quantitative survey (i.e. '*we are agreeing with you'*), any problems pertaining to eyes (through naturally occurring means) could later be associated to the iris scan. This is illustrated by the following exchange with a laboratory technician, '*We are using the microscope and we are suffering from our eyes because of looking through the microscope so maybe we will have a problem in the long term with our eyes…our first thought will be that the technology caused this problem so this is why we need a very good explanation so that we know it is not the technology that is causing the problem*.'

A second concern was if *'iris scan will expose me to spiritual problems through sorcery?'* This was discussed by most stakeholder groups as primarily a problem for *'those who are not learned'* and/or those who belong to churches which reject vaccination ('*There are some churches here that are proving to the population that they should not receive a vaccination*'). For example, discussions with doctors, nurses and laboratory technicians regarding persons who may be concerned about the potential of the technology to open them up to witchcraft, often started with the phrase, '*For us, there is no problem* [with the iris scan]*, but other people will need to be sensitized to accept…the education level of the population is very low, if you use the eye scan they may think you are trying to make trouble through their eyes*.' By using the phrasing 'us', HCP-P are referencing persons such as themselves who are well-educated health professionals. This sentiment did not often extend to 'other' stakeholder groups (e.g. community health workers) with a lower-level of education. The following comment from a laboratory technician is illustrative, '*By using the eye scan, many people will be having a bad thinking, that the eye scan will cause trouble with the eyes and people will run away…Because if you are using the eye scanner, they will think you are putting something into their eyes*.'-The pre-trial acceptability study therefore noted that regardless of whether or not the HCP-P who are enrolled in the trial harbor suspicions about the technology with regards to witchcraft, they are embedded in the larger cultural and religious communities of Tshuapa who are likely to have such concerns. As such, trial organizers should be aware of (and have a communication plan prepared for) the potential myths and rumors to manifest in Tshuapa which associate the iris scan with the evil intentions of witchcraft.

Three types of identification were familiar and considered to adequately identify vaccine recipients: ID cards (containing the name, address, phone number, etc.), thumb/fingerprints, and facial photographs. Use of an ID card as a method of identification was used in the Monkeypox vaccine trial – which was still occurring in the area while the qualitative pre-EBOVAC 3 trial activities were ongoing – which used this as to identify the trial enrollees. Several doctors familiar with the Monkeypox vaccine trial felt it may be confusing for some

EBOVAC 3 participants to be requested to have their eye scanned as a method of identification given their familiarity with a different method as established by the recent Monkeypox vaccine trial. Participants also felt strongly that the use of a facial photograph by itself (without scanning both eyes) was a sufficient method to identify individual persons. As stated by one nurse, '*If the iris scan is just a picture of the eye, why not just take a picture of the person? This is also a positive way to identify them which does not take so much time*.' A community health worker similarly stated, '*I can change my clothes, but I can't change my face*.' In general, participants were confused as to why three pictures – one of their faces and one of each eye – were necessary as a method of identification. While the iris scan technology was not rejected by most participants, many favored the use of ID cards plus facial photographs as a positive method of identification. Preference for photos of their face rather than a scan of each eye was considered a less invasive, less time-consuming, yet equally positive way to identify an individual.

3.4.2. Intra-trial study: Acceptance, accuracy, and feasibility of iris scan identification technology

It was noted that of 699 participants enrolled, (692/699) [99.0 %(95%CI:98.2;99.7)] had given consent to be identified by the iris scan technology thus 7/699 [1.0 % (95%CI:0.3; 1.7%)] refused. Various reasons were given for refusing, but most of them argued more about the fear of seeing their visual acuity being altered over time (Table 1. Among the participants who agreed to have their iris scanned, 0.9% (95%CI:0.2;1.6) (6/692) did not return to the second and third visits. In addition, iris scan data of 0.4% (95%CI:0.0;0.9) (3/692) of the participants were not properly entered into the database at the second visit due to inattention by the iris scan operator during the registration process of inputting data into the server. As a result, the quantitative survey conducted for the accuracy and feasibility study was only possible for 683/692 participants who agreed to be identified by iris scan during their initial clinical trial visits.

Table 1. Participants in the EBL2007 clinical trial who refused to be identified by iris scan, Boende, Tshuapa Province, DRCongo

Gender	Iris scan performed	Reason
M	No	Fears the scanner will cause defective vision in the future

M	No	Participant had deteriorated vision prior to being enrolled in the clinical trial and feared the scanner would further damage their eyes
F	No	Fear of the iris scanning tools and devices
M	No	Fears the scanner will cause defective vision in the future
F	No	Fear of the iris scanning tools and devices; Fears the scanner will cause defective vision in the future
M	No	Fear of the iris scanning tools and devices
M	No	Fears the scanner will cause defective vision in the future

Capturing a successful and quick iris scan is a process requiring both a participant who is willing to follow operator instructions (e.g. face forward, chin down, etc.) and a skilled operator capable of balancing the tablet in one hand while successfully locating the iris with the scanning device in the other hand. It often took more than one attempt to receive feedback on the tablet screen that a participant's irises were correctly scanned in the iris scan server (Table 2).

Table 2. Characteristics of iris scan process in the EBL2007 clinical trial, Boende, Tshuapa Province, DRCongo

Duration of iris scanning operation to record subjects	Frequency (n)	Percentage (%)	CI95%	Cumulative percentage (%)
0 seconds to 1 minute	280	41.0	37.3-44.7	41.0
1 minute 1 second to 1 minute 30 seconds	332	48.6	44.9-52.4	89.6

1 minute 31 seconds to 2 minutes	44	6.5	4.6-8.3	96.1
2 minutes 1 second to 2 minutes 30 seconds	24	3.5	2.1-4.9	99.6
More than 2 minutes 30 seconds	3	0.4	0.0-0.9	100.0
Total	683	100.0		
Iris scanning attempts at the second visit	**Frequency (n)**	**Percentage (%)**	**CI95%**	**Cumulative percentage (%)**
Once	475	69.6	66.1-73.0	69.5
Twice	149	21.8	18.7-24.9	91.4
Three times	59	8.6	6.5-10.8	100.0
Total	683	100.00		
Duration of the operation at the third visit	**Frequency**	**Percent**	**CI95%**	**Cumulative Percent**
0 seconds to 1 minute	665	97.4	96.1-98.6	97.4
1 minute 1 second to 1 minute 30 seconds	1	0.1	0.0-0.4	97.5
1 minute 31 seconds to 2 minutes	9	1.3	0.5-2.1	98.8
2 minutes 1 second to 2 minutes 30 seconds	5	0.7	0.1-1.3	99.5
More than 2 minutes 30 seconds	3	0.5	0.0-1.0	100
Total	**683**	**100**		
Percentage of participants properly recognized by iris scan at the third visit	**Frequency (n)**	**Percentage (%)**	**CI95%**	**Cumulative percentage (%)**
Recognition by scanning of iris	636	93.1	91.2-95.0	93.1

Not recognized by the scanning of iris	47	6.9	5.0-8.8	100.0
Total	**683**	**100.0**		

During the process of re-recording each participant's iris scans for both the first and second visit of the clinical trial, the duration of the operation ranged from 1 minute 1 second to 1 minute 30 seconds for the majority of study participants (332/683 or 48.6%) (Table 2). Capturing a successful image of the iris often took several seconds and required multiple manipulations of participants face and body by the iris scan operator in order to obtain a successful reading (Table 2). This concern seemed to exacerbate participant conclusions that the eye scan was taking too long (and potentially causing long-term damage to their eyes). The process of recording study volunteers by scanning their iris, capturing a photo and entering their demographic data into the tablet, lasted 2 minutes or less for 96.0 (95%CI:94.6;97.5) of participants. At the third visit, it took less than 1 minute for 97.4% (95%CI: 96.1-98.6) of volunteers to be authenticated Overall, accuracy of the iris scan, calculated by the percentage of successful iris scanning recognition at the third visit, was 93.1%(95%CI: 91.2;95.0) (636/683) (Table 2).

3.5. Discussion

In general, iris scanning as a biometric technology for identifying volunteers in a clinical trial was acceptable, feasible and accurate. A high acceptability (99.1% pre-trial; 99.0% intra-trial) of biometric identification via iris scanning was noted among the HCP-P. This remarkable rate of acceptance was similar to the one found in the quantitative survey conducted prior to the implementation of this technology of the clinical trial.

Results from the quantitative survey should be interpreted with care as HCP-P may not be representative for the general population of Tshuapa province or elsewhere. The qualitative data presented here describes a more nuanced picture of technology acceptance (e.g. concerns over physical and/or spiritual problems as caused by the iris scan) than the reported quantitative survey results solely. Prior to starting the clinical trial, the quantitative survey conducted among potential trial participants found that less than 1.0% of them disagreed with having their irises scanned, preferring other identification methods such as simply capturing a photo on a participant's card, or registering fingerprints. This low refusal rate was confirmed during the implementation of the trial. That is an illustration of the need to anticipate risk perceptions in a community of potential clinical trial volunteers by a prior acceptability study in order to know in advance whether or not that community is ready to use an innovative biometric identification technology. To our knowledge, this is the first study in Sub-Saharan Africa demonstrating the use of iris recognition in a clinical trial involving an adult population. Our high acceptability (99.1% pre-trial; 99.0% intra-trial) are comparable to other observational studies using iris scan in Kenya and Brazil [8,15]. This is likely because potential volunteers were already briefed on the value of using iris scans in the trial prior to the start of

the study by holding a workshop in advance. The FGDs conducted during the pre-trial qualitative study helped clinical trial investigators to “empty all pockets of fear” with regards to use of this innovative technology. Further, demonstrations of the functioning of this tool as well as the explanations given during the qualitative survey – in addition to the ability to explore their potential fears and concerns about the technology through FGDs – likely had an influence on the willingness of HCP-P to accept the iris scan as an identification technique.

Various reasons were given by trial volunteers for potential refusal of the iris scan, but most were fearful of their visual acuity being altered over time. Fears associated with a new and unknown technology needed to be overcome not only by volunteers, but also by the iris scan operator who struggled at the start of the trial with using a new technology and making sure that all participant details were recorded quickly (to avoid participant fears) and accurately. That is, while implementing the iris scan in the clinical trial, several issues did arise with regards to the extended time to receive feedback that a good quality iris scan stamp was properly recorded before entering some participant's other demographic details. In addition, the use of the tablet to instantaneously capture an image of the participants face (prior to proceeding to scan the eye) caused participant to conclude that the eye scan was taking too long. Capturing an image of the participants face was always quickly, and immediately successful without any special posturing by the participant. However, capturing a successful image of the iris often took several seconds and required multiple manipulations of their face and body by the iris scan operator in order to obtain a successful reading. This sometimes caused whispering and fatigue in the queue of HCP-P who were often impatient to wait in line before being called for their turn, especially when the iris scan took 2 minutes or more to successfully capture one iris scan. The longer duration at the second visit was however due to data re-entering that had to be performed for both Visit 1 and Visit 2. That would likely not have been the case if the manual error had not occurred after the Day 1 visit, which could have saved time and speculations from the participants. During the third visit, things were easier for the iris scan operator as only one scan of an iris was enough for the system to give the picture and the appointment window of the participant. Continuous practice by the operator is therefore important for the success of using this technology.

Finally, quantitative research demonstrated that iris scanning technology can be used effectively in clinical trials in resource-poor countries. An accuracy rate of 93.1% in this study is better compared to the 85% reported in Brazil [15]. However, the accuracy rate in Kenya was even higher (95%)[8]. Throughout this appreciable accuracy rate, iris scan technology demonstrates the importance of being scaled up moving forward, for widespread use in clinical trials and for the automating of a subject’s identification process. The time duration required to capture the iris scan and other related information of HCP-P at enrollment is similar to that reported in Brazil (less than 2 minutes) [15]. This time is shorter than the average of 4 minutes in Kenya[8]. It is understood that this depends on the amount information needed for each person included in the study and that this is a factor that influences the recording time at recruitment. It should also be pointed out that the accuracy rate of 93.1%% may have been underestimated, given that a failed recognition was scored even when the correct matching profile was presented along with other possible matching

profiles upon completion of the iris recognition process. The training of the operator in order for him/her to know what they need to do if during the matching process multiple profiles are offered, is more important here and to a lesser degree an issue of practice.

Some weaknesses were found, that could be attributed either to the operator or to the iris scan system. With regard to the operator, if he did not scan both irises with equal precision (i.e. after each iris scan the biometric tool showed the iris scan precision with a green (high), orange (medium) or red (low) color code), the biometrical tool sometimes provided several possible participants matches as an output. Based on a photograph entered at the beginning of the trial, the operator could then select the correct participant. Similarly, an issue sometimes occurred when the operator did not correctly enter the study ID (which is the basis for the pop ups of the participant information on the tablet) of the participant in front of him/her. A loss of information is also possible (as was the case after the first visit of this study), if the operator and/or the site does not pay attention to the standard operating procedure of the system (e.g. how to save the recorded information). This would constitute a deadlock to identify participants for the future visits. In the event of a possible false match or a correct match, the identity of each participant was to be double checked using the profile picture and biographical data also entered into the biometric tool, as well as with a participant ID card. Yet, cross referencing output of a biometric tool to an ID card may present a risk in underestimating accuracy of the biometric tool. In fact, the use of the ID card (which can be tampered with) as reference can in such cases compromise the benefit of the biometric tool in detecting fraud [4] .

Nonetheless it is worth mentioning that during subsequent visits, the iris scan allowed detection of some cases of fraud attempts. For example, some people not enrolled in the trial, tried to come on a scheduled visit to replace a relative. In addition, a few study participants attempted to falsify their ID numbers in order to change the studies activities schedule (vaccination and/or blood sample collection). In these cases, the iris scanning system was able to catch the attempted fraud. Moreover, the Ebola vaccine trial has a quite long follow-up period. This highlights the relevance of using this technology to identify correctly the clinical trial participants, in order to make sure for example that a blood sample is collected from participants who actually received the study intervention and not from their relatives.

Recognizing the added value of a qualitative study including vaccine trial participants regarding their perception after being identified by iris scan, it seems important to consider such a study moving forward. This would provide a better understanding of the contours of this level of acceptability among HCP-P in a vaccine trial.

During the assessment of acceptability, accuracy and feasibility of the iris scan system, nearly no technical issue was encountered. The equipment that was used had the advantage of not being dependent of internet connection, as the device was connected to the server via a local Wi-Fi. In a few occasions where a technical problem occurred, it was troubleshooted automatically by rebooting or bringing the tablet closer to the server. Both the tablet and the server needed a power supply. Hence its implementation in a remote area should take this

into the account beforehand. In the Ebola vaccine trial in Boende, a generator was running permanently onsite and uninterrupted power supplies were available as back-up.

3.6. Conclusion

Identification through iris scanning is an innovative technology found to be acceptable, accurate and feasible in a HCP-P population in a remote setting. This biotechnical tool takes little additional time, can automate the process of identifying subjects in a clinical study and quickly recall relevant information in relation to trial appointments. Thus, it helps in guaranteeing the quality of data. Sensitization of both investigators and potential study participants and their communities is a necessary prerequisite to successfully introduce this promising technology in trials conducted in low and middle-incomes countries. This article will therefore hopefully spark the idea of proposing further explorations in the field of biometric identification technology. The difficulties encountered here could then find solutions to further leverage performance of the iris scan as fast and reliable biometric method to implement in clinical trials.

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Chapter 4 Long-term experiences of Healthcare providers using iris scanning as an identification tool in a vaccine trial in the Democratic Republic of the Congo: a qualitative study

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4.1. Abstract

Background: Iris scanning, as a means of human biometric recognition, has been increasingly deployed over the last decade and continues to improve and expand. To better understand the acceptability of this technology, we report the long-term experiences of healthcare provider (HCP) and frontline worker participants with iris scanning as an identification tool in an Ebola vaccine (EBL2007 vaccine trial) trial conducted in the Democratic Republic of the Congo (DRC). **Objective**: to document the long-term experiences with iris scanning for identity verification throughout this vaccine trial.
Methods: Two years after the start of the EBL2007 vaccine trial (February-March 2022), 69 trial participants, comprising of nurses, first-aid workers, midwives, and community health workers, were interviewed using focus group discussions. . Thirteen individual in-depth interviews were conducted with physicians partaking in the trial, iris scan operators, trial staff physicians, and trial participants who declined iris scanning. Qualitative content analysis was utilized to identify significant themes.
Results: Interviewees had initially widely accepted the iris scan and viewed this tool as a distinctive means to identify individuals participating in the EBL2007 vaccine trial. However, over time, this perception shifted to become less favourable. Some voiced concern that their vision diminished soon after using the tool, and that this continued until the end of the study. Others reported that the perception of diminished vision started long after the end of the clinical trial. However, no vision impairment had been reported as an adverse event or assessed in the trial as being associated with the use of the iris scan, which employs a previously certified safe infrared light for iris scanning.
Conclusions: Our findings suggest the relevance of continuous efforts to effectively disseminate and repeat information about the functioning and safety of the iris scan technology to potential users. Precise depiction of iris scanning as a harmless procedure could dispel misunderstandings, concerns, and perceived risks among its potential users in a vaccine trial.
Keywords: Iris scan, vaccine trial, iris, perception, experience, views, biometric identification, Democratic Republic of the Congo.

4.2. Introduction

In numerous low and middle-income countries (LMICs), the lack of dependable patient identification may render efficient routine medical care ineffective (1-3). In rural settings, the digitization of personal information and its availability in databases remains scarce. Additionally, the challenges encountered in identifying patients enrolled over an extended period of time (e.g., in longitudinal cohort studies) can give rise to misclassification and pose a relevant barrier to maintaining study data integrity. A recurrent issue at the time of a(n) (un)scheduled visit of study participants is that they do not present a consistent form of identification, either because they have misplaced it, forgotten to bring it, or it has deteriorated. Recently, biometric recognition has gained in importance as a tool for correctly identifying individuals in routine health information processes (4-6). Also in clinical trials, the deployment of human biometric recognition has expanded over the past decade, and its adoption is increasingly widespread due to the advantages it offers over traditional identification methods (4,7).

The term biometric recognition involves an operation relying on specific technical processing of data related to the physical, physiological, or behavioural aspects of the human body (including when in motion) for the purpose of authentication (8). In the realm of biometric recognition methods employed thus far, including fingerprints, facial recognition, iris scans, ear biometrics, and voice recognition, fingerprints are most prevalently used and have become the predominant form of biometric data (9). Nevertheless, unlike fingerprints, which lose legibility over time due to the widening gaps between ridges, the distinct patterns within the iris remain unchanged throughout one's lifespan, thereby bestowing iris scan identification with lifelong stability (2-4). Iris patterns, even those of each eye from the same person or of identical twins, are unique and therefore suitable as a proxy for identification (13). Additionally, iris scanning has the advantage of being a non-contact process, which prevents transmission of infectious diseases through direct contact. Hence, iris scanning may become an affordable, fast, and reliable identification tool in a wide range of contexts, including electoral voting, access control, and vaccine trials (4,5,14,15). The use of iris scan in clinical trials may also eliminate errors, fraudulent entries, and authenticate unique trial participant identification across (un)scheduled visits, thus protecting the integrity of trial results (5). However, there is limited prior experience in utilizing iris scanning as a means of identity verification in clinical trials.

Recently, the implementation of the iris scan tool demonstrated a high level of precision and acceptance among healthcare provider (HCP) and frontline worker participants, interviewed before and at enrollment, in an Ebola vaccine trial (further referred to as “EBL2007 vaccine trial”), conducted in a remote area of the Democratic Republic of the Congo (DRC) (4). This new qualitative research aimed to document the long-term experiences with iris scanning for identity verification throughout this vaccine trial.

4.3. Methods

4.3.1. Study design

The present study employed a qualitative methodology, specifically the phenomenological approach, to dive into the experiences associated with the long-term use of iris scanning as an identification tool at (un)scheduled visits throughout the EBL2007 vaccine trial.

4.3.2. Study setting and time

This qualitative iris scan perception study was nested within the EBL2007 vaccine trial, and was conducted in Boende, DRC. Trial recruitment began in December 2019 and the last patient visit took place in October 2022 (Figure 1). At screening, potential vaccine trial participants were invited to opt-in to an innovative method for participant identification; an iris scan that captures biometric data from the iris to identify a person. They were informed that this tool was safe and would allow for more accurate recognition of each participant at (un)scheduled trial visits. This qualitative study took place between February and March 2022, corresponding with the second follow-up period in the EBL2007 vaccine trial, where most participants had already completed their last scheduled visit.

Long-term experiences entail the operational viability of the iris scan as perceived by its users, following a prolonged duration (two years) of systematic utilization within the EBL2007 vaccine.

trial. Further details regarding the vaccine trial are reported elsewhere (16).

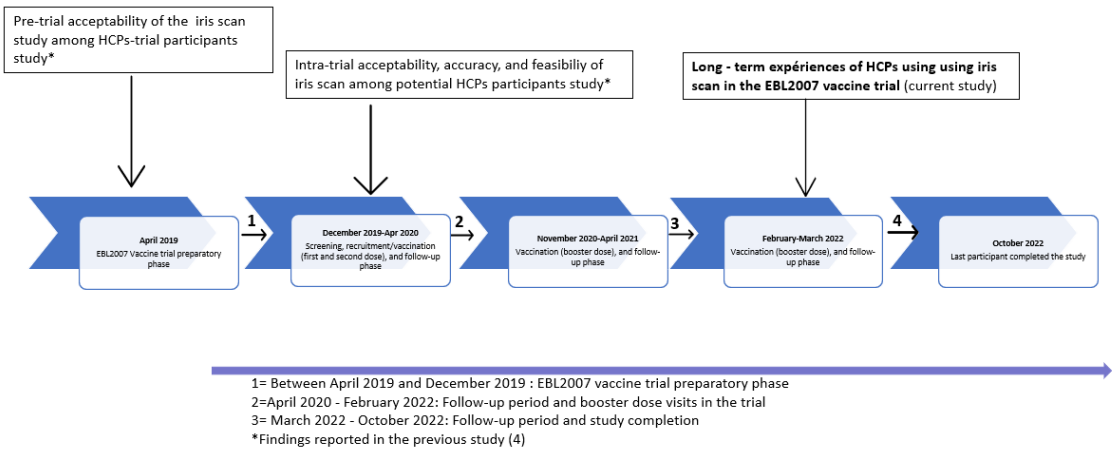

Figure 1 Chronology of events in the EBL2007 vaccine trial and data collection period for the current qualitative iris scan perception study.

4.3.3. Participants recruitment and sampling

This present qualitative study employed a purposive sampling to collect data from trial participants and staff approaching the completion of the EBL2007 vaccine trial. Included were trial participants who employed iris scanning for identification, those who did not select iris scanning as their initial identification method, trial's physician staff, and iris scan operators.

A combination of focus group discussions (FGDs) and individual in-depth interviews (IDIs) was applied to collect data among these participants.

Participants in the trial who had been using iris scanning as their identification method from the outset were invited to participate in Focus Group Discussions (FGDs). These participants represented a diverse array of professional categories, including Community Healthcare Workers1, First-Aid Workers, Midwives, Nurses, and Facility Cleaners. To foster a conducive environment for open dialogue within each FGD, considerations were given to both sex and professional categories of the participants.

Furthermore, In-Depth Interviews (IDIs) were utilized to extract insights from those trial participants who initially opted against using iris scanning for identification in the trial. Notably, physicians involved as trial-participants, and iris scanning operators were also extended invitations to engage in these IDIs.

Selection of trial participants who forwent iris scanning was based on documented refusals, as established in prior research (4). The predominant rationale for the refusal of iris scanning among these participants was attributed to apprehensions regarding ocular safety.

4.3.4.Procedure

For both a semi-structured questionnaire was used (Supplement). FGDs were conducted in groups of 6-10 participants. Before commencing the IDIs or FGDs, interviewees were reminded of the study's purpose. Each interview lasted between 60 to 90 minutes, and the conversations were recorded after obtaining the interviewees' consent. Voice recordings in languages other than French were translated into French an then into English before being transcribed by two independent individuals . The accuracy and coherence of the transcriptions were thoroughly verified by AP and TZM. TZM coded all transcripts derived from both FGDs and individual IDIs. AP and TZM reached a consensus on the coding system and categories utilized to generate thematic analysis.

4.3.5. Analysis

The audio recordings of all the conversations were transcribed, translated into French (if necessary), and imported into NVIVO software (QSR International, Melbourne, Australia) for analysis. Each transcript was anonymised and given a unique identifier. AP and TZM held

[1] [1] A Community health worker is a community member who acts as intermediary between health services and the local population. He plays a crucial role in disseminating information, providing health education, identifying early cases of diseases, promoting positive health practices, and encouraging individuals to seek healthcare from professionals when needed.

regular meetings aimed at aligning and harmonizing the coding process. An inductive thematic approach was employed to analyse the data, aiming to identify emerging themes and extract meaningful quotations that align with the study's objectives.

4.3.6. Iris scan equipment and procedures

The operator responsible for the iris scanning procedure was a trained and authorized EBL2007 vaccine trial staff member. The iris scan was conducted using an iris camera (Iritech, Irishield Monocular Fairfax, VA 22030, United States) in conjunction with a tablet (Samsung Tab Active 2, Suwon, South Korea) connected to a local ruggedized server via Wi-Fi. Further information regarding the procedures are described elsewhere (4).

The iris scanner used in the EBL2007 vaccine trial was certified as safe for use with infrared light under all operating conditions according to the international standard (International Electrotechnical Commission, IEC 62471:2006-07) (17). In addition, the irradiance was less than 2% of the Eye Safety Standard Regulation and tested for photobiological safety.

At enrolment, a list of trial participants that consented to the use of the iris scanning tool as a means of identification during trial visits was developed. Based on this list, the iris scan operator was instructed not to perform iris scans on trial participants who had not provided consent for the use of the tool in the trial. For these participants, an alternative identification method was applied. The participant's identification number (ID), assigned during their trial enrolment, was recorded, and only their demographic data and identification photo was taken at the time of inclusion with the iris scanning tool tablet. Entering their ID number into the tablet allowed for the recognition of the participant based on the photograph during subsequent (un)scheduled visits. For consenting individuals, the operator collected demographic data, took an identification photo, and scanned both irises (left and right eye) as the main method of identification.

4.3.7. Ethical aspects

This research was conducted in line with the prevailing ethical principles to protect the rights and welfare of all participants. Permission to undertake the research was granted by the National Ethics Committee of Health of the DRC (Reference number: 368/CNES/BN/PMMF/2022).

4.4. Results

Interviews were concluded once data saturation was achieved, with a total of 82 trial participants and staff included in the study. Data saturation was reached when no new themes emerged from subsequent conversations. In total, 69 trial participants took part in FGD, representing community healthcare workers, first-aid workers, midwives, nurses, and cleaners (Table 1). Additionally, IDIs were conducted with six trial participants that refused iris scanning, with five medical doctors in charge of safety monitoring in the trial, and with two iris scanning operators.

Participants had an average age of 51 years old (SD=11). At least one representative from each main professional category enrolled in the EBL2007 vaccine trial was involved in the IDIs or FGDs. The study included 51.2% female EBL2007 vaccine trial participants and staff (Table 1).

Three themes relevant to the objective of this qualitative study were identified from the collected data, which encompassed: (1) long-term experiences of using iris scan as an identification tool in the EBL2007 vaccine trial, (2) the use of iris scanning in future vaccine trials; and (3) comparison of the iris scan to previously known identification tools. Subthemes and categories were further identified within each theme.

4.4.1. Long-term experiences of HCPs using iris scan as an identification tool in the EBL2007 vaccine trial

4.4.1.1. Purpose-of-use understanding of trial participants who opted to use iris scan

Some respondents demonstrated a clear understanding of why the iris scan was used, as indicated by the following statements:

It helped us because it brought out the whole face [FGD, Midwife, trial participant, Woman]

It was for identification purposes [FGD, Midwife, Trial participant, Woman]

However, a few respondents had a different understanding of the iris scan tool, which they regarded as a means for vaccine trial investigators to detect disease or the impact of the experimental vaccine in the eyes.

Me, I believed they're going to find the disease in the eyes, and they're going to tell us, but they haven't told us, and we haven't asked. [FGD, Midwife, Trial participant, Woman].

Table 1 Data collection activities for the long-term experiences of HCPs using iris scanning in the EBL2007 vaccine trial

	Method	Interviewees occupation	Male	Female	Total (N)
Trial-participants					
	2 FGD	Nurses	10	8	18
	2 FGD	First aid workers worker	9	8	17
	2 FGD	Community health workers	8	7	15
	1 FGD	Mid-wives	-	9	9
	1 FGD	Cleaners	2	8	10
	3 Int*	Physician	3		3
	6 In	Entered the trial but refused iris scan	6		6

Staff in the trial					
	2 In	Physician monitoring safety	2	-	1
	2 In	Iris scan operator	2		2
Total			42	40	82

*In-depth interview

4.4.1.2. Acceptability of the iris scan

In general, HCPs and frontline workers volunteering in the EBL2007 vaccine trial widely accepted the use of iris scan technology, as was consistent with findings at the start of the trial (4). One Community health worker mentioned:

[…] I haven't come across anyone who would tell me that they didn't accept it. You see, I haven't encountered any group of people or any individual who would refuse to be examined by this device. [FGD, Community health worker, Trial participant, Man].

4.4.1.3. Reasons for accepting the iris scan

The primary reason given by most interviewees for accepting iris scan was the willingness to receive the study vaccine. Confidence in the trial staff motivated them to also trust the procedures proposed in the trial.

We accepted it because we were looking at our study vaccine, we were looking at the advantage of being enrolled in the Ebola vaccine trial. [FGD, Nurse, trial participant, Man].

However, some of the respondents stated that they had adopted the iris scan because they understood that it was the appropriate identification tool to correctly recognize the participants enrolled in the trial.

We have observed that it is a valuable tool for identification, which is why it was accepted. [FGD, First aid worker, Trial participant, Woman].

4.4.1.4. Reasons for not accepting the iris scan

A handful of interviewees indicated that they did not feel comfortable with this tool due to fear of retention of their demographic data recorded by this tool:
Yes, for a psychological reason, for example, we might take this photo and put it in a book, in a documentary, so that people can see you. So that's why I refused. But as I didn't have any information, I couldn't accept. [FGD, Community health worker, Trial participant, Man]

Due to the condition of their eyes prior to the use of the iris scan, some interviewees expressed a fear of vision loss associated with the use of this tool, in combination with the fear of seeing the iris scan tool for the first time.

I refused because I'm sick. My eye hurts, especially my left eye, which has been bothering me since 2012. I've been suffering for four years. It's in this sense that I refused because the way

it was flashing, it may burn my eye. Especially when she filmed it, it scared me. That's why I refused. [Interview, Trial-participant B who refused iris scan]

4.4.1.5. Purpose-of-use understanding of trial participants who declined the use iris scan

Some interviewees who declined iris scan identification reported that they were unaware of the rationale behind the use of this tool in the vaccine trial.

Well, I don't know the importance of this. [Interview, Trial participant A who refused iris scan]

I still have doubts, but I've seen the people who have had iris scans, and I don't see the point in continuing to doubt, given that there are friends who have accepted, and they're still here. [Interview E, Trial participant who refused iris scan].

"In my opinion, regarding the importance of iris scan, when they captured us, I explained that my eye is diseased, but they told me they would capture the other eye, and I refused. But I don't know the significance of it." [Interview, Trial participant F who refused iris scan].

Some of the interviewees reported having understood that this scanning tool was intended for identification purposes during visits, to anticipate possible fraud.

[…] We mentioned that we would use it to confirm the person's identity. If someone else tries to pretend to be me, when they put the iris scan in their eye, it will show that it is not them. That is what I remember about the iris scan. [Interview, Trial participant E who refused iris scan]

4.4.1.6. The perceived accuracy of iris scan in identifying participants

Most interviewees reported that they found the iris scan tool in the trial to be very accurate because with the help of this tool, cases of fraud were avoided among participants. They gave some illustrations of fraud attempts to describe the effectiveness and accuracy of this tool. An example is the following statement:

[…] There was an incident where I arrived for a scheduled visit and the person sitting close to me presented his father's identification card, claiming to be him. Upon verification, the operator discovered that the person in front of him was not who he claimed to be. The photo in the system of iris scan did not match his appearance. He eventually admitted that his father had passed away. The individual conducting the check informed him that we had not been informed of the father's passing, and upon further investigation, it was revealed that the father was traveling and had authorized his son to represent him. This incident revealed potentially fraudulent activity, as the person attempted to manipulate the results of the vaccine trial by assuming someone else's identity. [FGD, Community healthcare worker, Trial participant, Woman]

4.4.1.7. Perceived risk of iris scan identification

Some participants reported a perceived association between the use of the iris scan and the loss of vision in their eyes. Additionally, there were concerns expressed by most participants that vision loss may occur at a later stage because of the amount of light this device produced.

Certain interviewees alluded to the eclipse phenomenon or to sunlight to compare how much light was produced by the iris scan, which may have contributed to the vision loss.

We were scared, there was the light and there was uncertainty as to whether the eye would crack or not […] [FGD, Community healthcare worker, Trial participant, Man]
Thank you, now after the iris scan, I have noticed that there is a reduction of vision, especially for reading, so we have to use glasses now. [FGD, First aid volunteer worker, Trial participant, Man].
Certain interviewees mentioned that they did not observe any unusual occurrences during the scanning process and expressed no apprehension regarding the safety of their eyes in the future, as they did not perceive iris scan as a hazardous practice.

There was no reaction. They just tell you to stare like this and then they tell you, it's okay. There wasn't really any direct reaction like that. [FGD, Nurse, trial participant, Woman]
I was just afraid for my eyes, but it is not dangerous. Even if it comes back to my village, we will make people aware of this device. It is for identifying people. [Interview, Trial participant E who refused iris scan]

4.4.1.8. Rumours and reactions from the surroundings

Certain interviewees and FDG expressed worries about the well-being of their eyes based on conversations. Nevertheless, most of them emphasized that they had not heard rumours associated with the iris scan. Instead, rumours primarily focused on the experimental vaccine and other study procedures, such as blood sampling.

They said your eye is sick if you use this device, your eye will be completely damaged, which is why I was afraid. Otherwise, there wasn't much to it. [Interview, Trial participant B who refused iris scan].
No, in the neighbourhoods there hadn't been any rumours, but it was about the vaccine and blood sampling that people were talking nonsense about, not about the iris scan. [FGD, Red cross, Trial participant, Man]
The people around us didn't know that we were having the iris scan in the study, they only knew that we were selling our blood and getting vaccinated, period, but concerning the Iris scan, nothing was said, it was only us, trial participants, who knew about the Iris scan, but not the community, they didn't know anything about it. In the neighbourhood, we were nicknamed blood sellers. [FGD, Cleaner, Trial participant, Woman]

4.4.2. Use of iris scans in future vaccines trials or other public health activities

4.4.2.1. Acceptability of iris scan in the wider community

Because the iris scan tool is part of a vaccination monitoring system that collects additional data (i.e. demographics, photo of the face) to what is recorded for routine vaccinations (i.e. demographics, previous vaccination, name of the vaccine administered, its lot number and expiry date), certain participants believed that iris scan tool would not be accepted nor feasible

in the context of wider vaccination activities with the general population. Participants alluded to a yellow fever vaccination campaign that had taken place in Boende. During this campaign, people agreed to receive the vaccine, but many of them were not willing to give full identities and demographic data. Therefore, some interviewees and FGD-participants suggested that it would be preferable to employ a different tool, such as fingerprint, instead.

I wanted to say that for the population, it's going to be a bit difficult, because we've noticed here that with yellow fever, we only recorded the name on the card and then gave the vaccine directly. It was also difficult to get someone to agree to give their full identity so that they could be vaccinated, so it would also be very difficult with the iris scan. It's better even with the fingerprint, maybe it will be all right. With the iris scan, it will be a bit difficult with this population. [FGD, Nurse, trial participant, Woman].

Some interviewees underscored that iris scanning could be an acceptable, effective, and reliable tool for uniquely identifying individuals who might volunteer in future clinical trials. However, this should be accompanied by researchers conducting a robust awareness campaign to disseminate and repeat sufficient information about the tool's safety.

At first, people will refuse, but after awareness-raising and testimonials from those who have experienced the tool, they will accept. [Interview, trial participant A who refused iris scan].

4.4.2.2. Recommendations from interviewees and FGD-participants

When it comes to implementing iris scanning in vaccine trials, particularly in remote areas like Boende, some interviewees and FGD-participants recommended to consider the availability of ophthalmic specialists. As per their statements, the iris scan operator who used the iris scan tool in the EBL2007 vaccine trial was not fully aware of the risks involved in scanning the eyes, and unable to provide clear explanations related to the safety of trial participants' eyes.

[…] when you come to scan people's eyes, come with the eye specialist. […]. All those who have handled our eyes are not specialists. […] They are photographers, so you should to come with eye specialists. An ophthalmologist because it's a sensitive organ. [FGD, First aid worker trial participant, trial participant, Man]

Few of those who participated in the FGDs voiced their concern about not having seen the vaccine trial investigators having their eyes scanned.

[…] Until now we haven't seen the staff being vaccinated, or scanned the eyes with iris scan. We haven't seen; they haven't scanned themselves. [FGD, First aid worker, Trial participant, Man]

Some other participants in this research recommended to consider reducing the amount of light used during scanning, widening the distance between the eye and the scanner, and carrying out demonstrations during the screening/consent process.

I think that, as my colleague the Community health worker just said, the distance from the iris scan is too close. Isn't there some way of finding ways of making it even bigger? […]. [FGD, Community health worker, Trial-participant, Woman]

4.4.3. Comparison of iris scan to previous known identification tools

By making reference to the available and known means of identification previously utilized, most interviewees voiced that the iris scan would be the best for uniquely identifying volunteers in the trial.

With the experience that I have, with the age that I have…I believe that the only method of escaping fraud is scanning […] [FGD, Community health worker, Trial participant, Woman].

[…] So, with today's technology, we may easily modify the photo by taking someone's face and putting it on another body to make it look like it's me, but it's not. But with the iris scan, it's easy to see that it's not me, it's just someone else's face. So, with the iris scan, it's hard to commit fraud. [FGD, Community health worker, Trial participant, Woman].

Some interviewees suggested to use the traditional fingerprint biometric tool to minimize the risk of compromising their eyes with iris scanning tools or deploy other methods such as the use of names, date of birth, and identification number recorded in a computer. Others proposed more innovative identification tools, such as collecting identity data using the laser thermometer used to measure temperature at entry points or using blood samples already collected at the first visit.

I'm going to recommend the fingerprint because the signature may be imitated, but your fingerprint, your own blood, will reveal all your data. [FGD, Community health worker, trial participant, Man].

[…] instead of using this device, there was no way of using our fingerprints. Because.... Isn't it possible to use a fingerprint? [FGD, Community health worker, Trial participant, Man].

[…] As we've received all the doses of vaccine as well as blood samples were taken, it's the computer that will indicate that for such and such a participant, he's finished his doses, these appointments are over, so that's it. The computer is a method. [FGD, Cleaner, Trial participant, Woman].

[…] We may have a thermometer that records all the identity as well as the blood pressure and everything, so as not to have any problems with the use of that iris scan laser. [FGD, Community health worker, Trial participant, Man].

4.5. Discussion

4.5.1. Principal Results

The current qualitative study aimed to document the long-term experiences of the EBL2007 vaccine trial participants and staff regarding the use of an innovative iris scan biometric. In general, the tool was found to be acceptable, accurate and able to verify the identity of participants throughout the trial, avoiding fraud or errors.

Though clearly explained during the consent procedure that the iris scan was non-compulsory, it remains possible that some participants feared that they would not be enrolled if they declined the iris scan. Similarly, the safety of iris scan had been well-explained to trial personnel at the start of the trial. Despite these efforts, some interviewees and FGD participants still felt that scanning their eyes posed safety problems, or that their eyes might pose a problem in the future.

It is important to highlight the expected motivational benefit of participation in the EBL2007 vaccine trial, as some interviewees may have only accepted the iris scan in view of receiving an Ebola vaccine regimen since they reside in an area at risk of an outbreak (18) and/or they wished to receive the vaccine trial travel cost and time offered reimbursement (19). Furthermore, interviewees' concern about the safety of their eyes after using this tool should not be overlooked. Various vision problems were perceived to be associated with the iris scan.

In studies conducted elsewhere, similar reasons for hesitance i.e., general safety concerns and anxiety about the physical effects of biometric scanning, have been reported (20-22). However, it is important to note that vision impairment was not reported as an adverse event or assessed as being associated with the use of iris scanning during the trial. The issue related to collecting and safeguarding additional personal data following the iris scan was raised as well. It is important to highlight that the collection of such information among our trial participants was new in the remote area of Boende (trial site location), where the most common practice (e.g. during checks within the public administration for payroll purposes or when applying for passports or voter cards) remains the use of fingerprinting as biometric tool (23). This may have influenced their comfort with fingerprints compared to iris scanning, and raised some security concerns about iris scanning, even though they have generally accepted it and found it accurate.

It seems likely that what was perceived as vision disorders associated with iris scanning in the EBL2007 vaccine trial may have other causes. Some trial participants may have had pre-existing eye conditions. For example, while the mean age of trial participants was 45 years old (23), it is well-known that the incidence of vision impairment increases from middle age onwards (24,25). Additionally, at the time of the EBL2007 vaccine trial conduct, there was no ophthalmological care in Boende. Hence, participants' vision or ophthalmological complaints might not have been treated at the time of enrolment in the trial. Promotional and preventive activities aimed at improving eye health may also be necessary, as some studies have shown that the burden of visual impairment is high in populations living in remote, resource-constrained areas due to a lack of access to quality healthcare services (26). Yet, implementing such activities may bring additional costs to researchers. Given the increasing spread of the culture of digital legislation and democracy in Africa (27), it is possible that the above mentioned concerns would gradually diminish in the event of a wider use of the iris scan tool. This entails that even if research participants do develop vision problems, probably as a result of ageing or some other reason, they will not associate them with iris scanning because of the widespread use of digital technology.

Given that some of those interviewed said that they found the iris scanner operator to look more like a photographer than someone who could properly explain the tool and its safety information, the iris scan tool and purpose do not seem to have been sufficiently explained at inclusion and during follow up visits in the trial. As a lesson learned, it is therefore crucial to

provide a more detailed training to the iris scan operator so that he or she is able to answer more specific questions from research participants.

It is also worth stressing that the timing of conducting this qualitative study (i.e., two years after the start of the trial) may explain why some of the iris scan tool information, provided during the consent process at the beginning of the trial, was gradually forgotten by interviewees and FGD participants. Previous studies have highlighted the extent to which trial volunteers no longer or remember less of the information they were given at the time of enrolment (28,29). It entails that in long-term studies, the contents of the informed consent form need to be re-explained to participants.

This study showed that the use of iris scan in vaccine trials in resource-poor settings has valuable potential and is generally accepted to identify participants. The iris scan acceptability complies with properties required of a high-performance biometric tool, such as universality, uniqueness, permanence, collectability and circumvention (27,30). However, during the EBL2007 vaccine trial the process of iris scanning became tiresome when identifying the iris was not possible because a participant failed to comply with the instructions of the operator. In order to facilitate rapid identification through iris scanning, it is imperative that the individual being identified remains attentive and adheres strictly to the instructions provided by the operator conducting the identification process. Hence, iris scanning technology may appear challenging in vaccine trials involving younger infants (less than one year) for example. Though, infants are the population most in need of vaccines, they cannot follow detailed instructions (for example, looking into a camera) to enable iris recognition (15). Other approaches, such as iris scanning of the adult accompanying the child (proxy ID), ear-based or palm-based automatic recognition may be more applicable in an infant population or other populations with dependency (31,32).

4.5.2. Limitations

This study has some limitations. First, our findings are drawn from long-term experiences (i.e., multiple iris scanning moments over a 2-year study period) of a population of HCPs and frontline workers who likely have a higher level of understanding of health-related phenomenon. These findings may thus not be extrapolatable to the general population. Second, some of the researchers who performed FGD and interviews, despite not being directly involved in the medical aspects of the vaccine trial, could be perceived by the interviewees as representing the vaccine trial team. This might result in a desirability bias in the IDIs and FGDs. Finally, some participants working at the general referral Boende hospital, also the study site of the trial, may have hesitated to express negative concerns about the iris scan because of the location where the interviews took place (i.e., at the general referral hospital), and/or because a trial investigator conducted some of the interviews. Nevertheless, the findings of this qualitative research on iris scanning, report the a posteriori experiences of the participants and staff over time and are complementary to the qualitative research conducted on iris scanning acceptability. This study, combined with the previously mentioned acceptability of the biometric identity verification tool study, are, to our knowledge, the only studies providing a broader understanding of both the initial acceptability, followed by the actual experiences with iris scanning in the same trial population (4). Insights provided can help implement a broader use of the iris scan tool in vaccine trials or other long-term longitudinal research.

4.6. Conclusions

The findings of this qualitative research underline the continuous acceptability and perceived high accuracy of the iris scan tool for unique recognition of adult participants in a vaccine trial over time. When the iris scan functionality is not well-understood or memorized by the users, few concerns may rise, such as the perceived risks to long-term vision, the use of data retained by users, as well as its ability to rapidly ascertain information, regardless of age, education level or health condition. Further efforts should be made to provide clear information to users and to dispel misconceptions about the fears and perceived risks of the iris scan tool, prior to a wider implementation in vaccine trials or other research.

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4.10. Supplements

Table 1 Focus group discussion with HCPs potential participants in the EBL2007 vaccine trial for the qualitative acceptability assessment prior the trial*

	Method	Interviewees occupation	Male	Female	Total (N)
	4 FGD	Nurses	22	4	26
	1 FGD	First aid workers worker	6	2	8
	3 FGD	Community health workers	20	4	24
	1 FGD	Mid-wives	-	7	7
	1 FGD	Cleaners	1	6	7
	1 FGD	Physician	7	1	8
	1 FGD	Lab technicians	5	1	6
Total			39	41	86

* Matuvanga, Z., Trésor, et al. J. Med. Internet Res. 23.8 (2021): e28573.

Semi structured questionnaires

I. Thematic discussion guide for Focus Groups Discussions with Trial-participants in the EBL2007 vaccine trial

Theme 1: Knowledge and perception of iris scanning technology

1. What is the purpose of iris scanning technology?
- Why was the iris scanning tool used in this study?
2. How does the iris scanner work?
3. How was the iris scanning tool explained to you?
- Who explained it to you and how?
- What information reassured you the most? What information worried you the most?
4. How did you feel during identification using the iris scanner?
5. How did you like the iris scanning tool used by the project (study EBL2007 vaccine trial) to identify you on the day of your various study appointments?
- After trying it out, do you still have any questions or fears about using this tool? Please describe them.
6. After using the iris scanning tool, was it worth using it in this project, in your opinion?
7. How reliable do you think this method of identification is? What do you think are the reasons for this high/low reliability?
8. How do you think volunteers/participants like you in a clinical trial should be identified during visits to ensure that it is the right person who is in the clinical trial in the future?
- Do you know of any other secure identification tools/means used in other clinical trials?
- Which identification tool would you recommend for future clinical trials? Why or why not?
- If you had the choice between iris scan tool, fingerprints, photo ID or other methods of identification at the start of the EBL2007 vaccine trial, which method would you choose?

Theme 2: Acceptability of the iris scan tool

9. Of those who decided not to be identified using the iris scanning tool, what do you think were the reasons for their decision?
10. What reactions did you experience from your family/community members after being identified with the iris scan tool as a clinical trial participant? Why or why not?
11. Have you heard of any participants being stigmatised here in Boende because of their identification with the iris scan tool?
- How are they stigmatised?
- By whom?
- By whom?
Have there been any rumors in your circle about the iris scanner? What is being said about this tool in the context of the current study?
12. Do you think that other healthcare professionals or future participants in other studies, here or elsewhere, will accept the iris scanning tool as a means of identification? Why or why not?
13. In your opinion, what could be some potential considerations for researchers who plan to use the iris scan tool in future vaccine trials?
- Do you think it will be different or similar for studies conducted in another region of the DRC?
II. In-depth Interview guide for semi-structured EIAs with participants who refused to be identified using the iris scanning tool
Theme 1: Knowledge and perception of iris scanning technology
1. What is the purpose of iris scanning technology?
- Why was the iris scanning tool used in this study?
2. How does the iris scanner work?
3. How was the iris scanning tool explained to you?
- Who explained it to you and how?
- What information reassured you the most? What information worried you the most?
4. What were your reasons for refusing identification using the iris scanner?
5. After observing the use of the iris scanner, do you still have the same questions or fears about using this tool?
- If not, what made you change your mind?
6. After observing the use of the iris scanning tool, was it worth using it, in your opinion?
7. Do you think that this technology made it possible to detect cases of fraud that would have been missed using other means usually used to identify people (vaccination card, identity document, etc.)? How and thanks to what would this have been possible?
8. How do you think volunteers/participants like you in a clinical trial should be identified during visits to ensure that it is the right person who is in the clinical trial in the future?
- Do you know of any other secure identification tools/means used in other clinical trials?
- Which identification tool would you recommend for future clinical trials? Why or why not?
Topic 2: Acceptability of the iris scanning tool
9. What reactions would you have experienced from your family/community members if you had been identified with the iris scan tool as a clinical trial participant? Why or why not?
10. Have you heard of any participants being stigmatised here in Boende because of their identification with the iris scan tool?
- How are they stigmatised?
- By whom?
- Why or why not?

11. What is being said about this tool in the context of the current study?
12. Do you think that other healthcare providers or future participants in other studies, here or elsewhere, will accept the iris scanning tool as a means of identification? Why or why not?
13. In your opinion, what could be some potential considerations for researchers who plan to use the iris scan tool in future vaccine trials?
- Do you think it will be different or similar for studies conducted in another region of the DRC?
III. Interview guide for In-depth individual interview with physicians staff in the trial
Theme 1: Knowledge and perception of iris scanning technology
1. What is the purpose of iris scanning technology?
- Why was the iris scanning tool used in this study?
2. How does the iris scanner work?
3. How was the iris scanning tool explained to you?
- Who explained it to you and how?
- What information reassured you the most? What information worried you the most?
4. How did you assess the iris scanning tool used by the project (EBL2007 vaccine trial) to identify you on the day of your various study appointments? How did you feel during the identification process using the iris scanner?
- What are your thoughts or feelings about using this tool after having tried it out?
5. After using the iris scanner, what do you really think of it personally? Do you think it was worth using?
6. Do you think that this technology has made it possible to detect cases of fraud that would have been missed using other means usually used to identify people (vaccination card, identity document, etc.)? How and why would this have been possible?
7. How do you think volunteers/participants like you in a clinical trial should be identified during visits to ensure that it is the right person who is in the clinical trial in the future?
- Do you know of any other secure identification tools/means used in other clinical trials?
- Which identification tool would you recommend for future clinical trials? Why or why not?
Theme 2: Acceptability of the iris scanning tool
8. Of those who have decided not to be identified using the iris scanner, what do you think are the reasons for their decision?
9. Did you hear any participants complain about the use of the iris scan tool as being the cause of a problem with the health of their eyes or another part of the body?
- If so, what problem did the participants mention? How was this understood by the participant?
10. In the day-to-day management of patients, did you identify any eye health problems that could be explained using the iris scanning tool in a participant in this clinical trial?
- If so, what was the reaction of those concerned? How do you understand this?
11. What reactions did you experience from your family/community members after being identified with the iris scan tool as a clinical trial participant? Why or why not?
12. Have you heard of any participants being stigmatised here in Boende because of their identification with the iris scan tool?
- How are they stigmatised?
- By whom?
- Why or why not?
13. Have there been any rumours in your circle about the iris scanner? What is being said about this tool in the context of the current study?

14. Do you think that other healthcare professionals or future participants in other studies, here or elsewhere, will accept the iris scanning tool as a means of identification? Why or why not?
15. What are your thoughts on the potential impact of using the iris scan tool in organizing future vaccine trials?
- Do you think these challenges and difficulties will be different or similar for studies conducted in another region of the DRC?
IV. Interview guide for In-depth individual interview with operators who have handled the iris scanning tool in the trial
Theme 1: Knowledge, perception and use of iris scanning technology
1. What is the purpose of iris scanning technology?
- Why was the iris scanning tool used in this study?
2. How are volunteers in this clinical study identified using the iris scanning tool?
3. How was the operation of the iris scanning tool explained to you?
- Who explained it to you and how?
- What information reassured you the most? What information worried you the most?
4. From the point of view of handling, what do you think of this tool?
- What was easy and what was difficult during handling?
- What would need to be improved to make this tool easier to use?
- What do you think of its effectiveness in identifying study volunteers? Do you think that this technology made it possible to detect cases of fraud that would have been missed using other means usually used to identify people (vaccination card, identity document, etc.)?
- What aspects could be improved to make this tool more effective?
5. Could you describe your experience when using the tool, including any feelings or reactions you had?
6. How did the participants react when their irises were scanned?
- What questions did participants ask you most often?
- What were their fears?
- Were there any differences in reaction between the first and second visits?
- Which reaction impressed you the most?
7. How do you think volunteers/participants like you in a clinical trial should be identified at future visits to ensure that it is the right person who is in the clinical trial?
- Do you know of any other secure identification tools/means used in other clinical trials?
- Which identification tool would you recommend for future clinical trials? Why or why not?
8. After experimenting with the tool, do you still have questions or fears related to its use? Please describe them.
9. After using the iris scanner, what do you really think of it personally? Do you think it was worth using?
Theme 2: Acceptability of the iris scanning tool
10. Of those who decided not to be identified using the iris scanner, what do you think were the reasons for their decision?
11. Have you heard of any participants being stigmatised here in Boende because of their identification with the iris scanning?
- How are they stigmatised?
- By whom?
- Why or why not?

12. What is being said about this tool in the context of the current study?
13. Do you think that other healthcare professionals or future participants in other studies, here or elsewhere, will accept the iris scanning tool as a means of identification? Why or why not?
14. What are your thoughts on the potential impact of using the iris scan tool in organizing future vaccine trials?
- How might the challenges and difficulties of conducting studies vary across different regions of the DRC, in your opinion?

Chapter 5 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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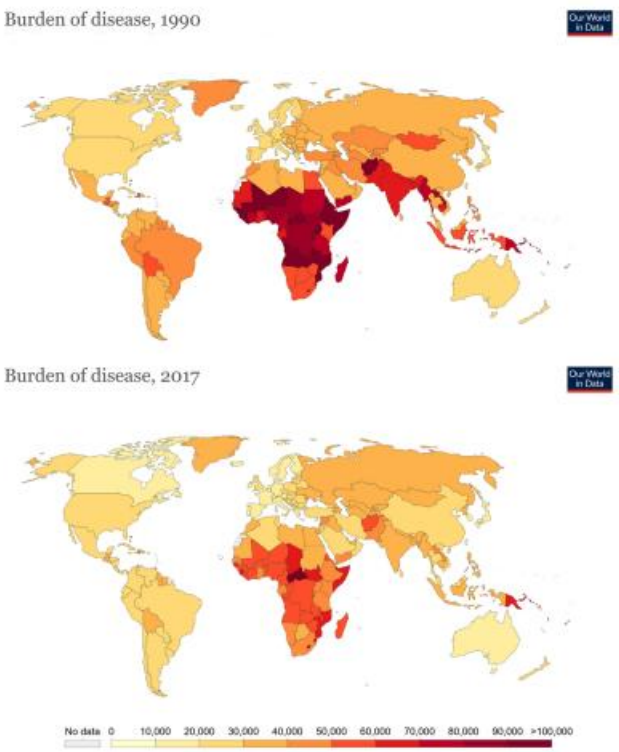
5.1. Abstract

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 EBOVAC 3 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.

Keywords: Challenges; Democratic Republic of the Congo; Ebola virus disease; Endemic; Experiences; Health care providers; Lessons learned; Mitigations; Past activities; Vaccine trial.

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 (Figure 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 (Figure 1) (3), a minority of clinical trials are performed in low- and middle-income countries (LMICs) (Figure 2) (2).



Note: to allow comparison between countries and over time, this metric is age-standardized

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 of life lost due to premature death and years lived with a disability. One DALY equals one lost year of healthy life [2]. Ourworldindata.org/burden-of-disease. Source: IHME, Global Burden of Diseases. https://ourworldindata.org/burden-of-disease

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).

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 where 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 EBOVAC 3 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 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. Ensure staff capable of explaining essential documents in both official and local languages.	Foresee essential documents that need to be completed by participants in the country's official language (e.g. French).

		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.
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. 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.
	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.	

	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.
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.	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. 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 electricity	Foresee generators (including fuel) and solar panels.	
	Lack of water access	Construct a bore hole, foresee water tanks and plumbing.	
	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.	

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.	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.
Limited expertise in the area for the setup and maintenance of the trial equipment	Foresee maintenance contract with companies in Kinshasa. Foresee the maintenance at each start of activities on the site by the key persons from Kinshasa. Foresee back-up generator for electricity/contingencies. Foresee back up refrigerator/freezers 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 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.	Assess staff fears and how to mitigate these before starting a vaccine trial to ensure good team spirit and motivation.

	Ship the vaccines and serum samples in two separate shipments.	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.

	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.	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 available workforce for trial site renovations in Boende	Hire workforce in Mbandaka to do renovation works (e.g. borehole construction) in Boende	
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

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.	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.
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).

During the 10th Ebola epidemic 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 front-line 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 the 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 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 kilometers 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 active study activity) 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 to potential participants. 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 (HCP) were questioned about the acceptability of the identification tool and the feasibility of the mobile messaging system (5). 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 meters in height) did not allow transport by air as the only domestic aircraft to Boende measured 1.5 meters 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 25km from the site) and 2) participants traveling more than 6 hours (approximately more than 25km 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 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.

5.5. Funding

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5.6. Conflicts of interest

Authors have no conflicts to declare.

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Chapter 6 Low seroprevalence of Ebola virus in health care providers in an endemic region (Tshuapa province) of the Democratic Republic of the Congo

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6.1. Abstract

Introduction: A serosurvey among health care providers (HCPs) and frontliners of an area previously affected by Ebola virus disease (EVD) in the Democratic Republic of the Congo (DRC) was conducted to assess the seroreactivity to Ebola virus antigens.

Methods: Serum samples were collected in a cohort of HCPs and frontliners (n=698) participants in the EBL2007 vaccine trial (December 2019 to October 2022). Specimens seroreactive for EBOV were confirmed using either the Filovirus Animal Nonclinical Group (FANG) ELISA or a Luminex multiplex assay.

Results: The seroreactivity to at least two EBOV-Mayinga (m) antigens was found in 10 (1.4%: 95% CI, 0.7- 2.6) samples for GP-EBOV-m + VP40-EBOV-m, and 2 (0.3%: 95% CI, 0.0 - 1.0) samples for VP40-EBOV-m + NP-EBOV-m using the Luminex assay. Seroreactivity to GP-EBOV-Kikwit (k) was observed in 59 (8.5%: 95%CI, 6.5-10.9) samples using FANG ELISA.

Conclusion: In contrast to previous serosurveys, a low seroprevalence was found in the HCP and frontline population participating in the EBL2007 Ebola vaccine trial in Boende, DRC. This underscores the high need for standardized antibody assays and cutoffs in EBOV serosurveys to avoid the broad range of reported EBOV seroprevalence rates in EBOV endemic areas.

6.2. Introduction

Ebola virus disease (EVD) was first observed during two simultaneous epidemics in 1976 in South Sudan and the Democratic Republic of the Congo (DRC) (1,2). Since then, there were fifteen epidemics throughout the DRC, including one in the Boende area, province of Tshuapa in 2014 (3,4). The frequency of EVD epidemics in the DRC increased tremendously over the past five years with seven epidemics occurring between 2017 and 2022. Mathematical models predict at least one epidemic each year (5). An improved surveillance system and better diagnostic tools can partly explain the increasing trend. Further, villagers are more likely to come into contact with the natural reservoir of Ebolavirus Zaire (EBOV), as pristine habitats in the Congo Basin are transformed into farmland and cut at an unprecedented rate to provide wood for industries (6). Human encroachment into these new habitats results in increased bushmeat hunting and a higher level of exposure to the virus, which is most likely spilled over from bats or monkeys (7). Furthermore, flare-ups of EVD epidemics might also result from chronically infected patients, which was noted recently in Guinea where a survivor passed the virus on to his partner via semen more than 500 days after contracting (5,8,9).

While spillover from animals to humans is considered to be rare (10), epidemics are primarily the result of direct person-to-person transmission via body fluids or indirect transmission via contaminated materials (11). Due to occupational exposure, healthcare providers (HCPs) are more at risk during an outbreak than others in the general community and become a potential source of transmission themselves (12). For example, during the seventh EVD outbreak in the DRC, which occurred in Boende Health District (2014), three HCPs were identified as potential super-spreaders of community-level disease transmission (13). Similarly, health facilities may facilitate transmission to the community as infected patients, visitors, and the general public come together there (14,15). For example, the EBOV epidemic in 1995 was mainly driven by nosocomial transmission at Kikwit General Hospital of DRC (16).

While epidemics are typically monitored through PCR-confirmed active cases, serosurveillance data represents the accumulative number of infections and may detect several undiagnosed cases. Indeed, EBOV infections may remain asymptomatic or paucisymptomatic after exposure to the pathogen (17). This has been observed in recent studies where EBOV antigen seroreactivity is increasingly reported (8,18,19). In unaffected areas, seroreactivity to EBOV-GP was reported in urban areas of Cameroon (1.3%), and DRC in Kinshasa (2%) and Kasaï Oriental (3.5%) (8,18,20,21). In a resident pygmy population including traditional hunters in Watsa locality (Haut-Uele province, DRC) a seroprevalence of 18.7% was reported (8). A study including HCP and frontliners, regardless of their self-reported history of EVD, found 3.4% of EBOV antigens seroreactivity in Kabondo - Dianda (southeastern DRC and forest-savannah area) (22). A serosurvey conducted at the end of the 2014-2016 epidemic in Sierra Leone showed a seroreactivity of 8% among apparently healthy participants volunteering for an Ebola vaccine trial, with no self-reported history of EVD (23). Serosurveys in the DRC obtained highly variable seroprevalence estimates depending on the region and the target group. While the EBOV seroprevalence in Boende after the previous epidemic of 2014 was high (28.1%) among healthy HCP never reporting an infection (24), the seroprevalence estimate was much lower in another study conducted in the same area (7%) (22). A serosurvey conducted on blood samples collected from clinically suspected EVD cases that were sent home after testing negative in two consecutive EBOV RT-PCR during the tenth EBOV outbreak in DRC Ituri, Nord Kivu and Sud Kivu provinces, 2018-2020), reported an EBOV antigen seroreactivity of 2.3% (25) (Table 1).

Table 1. EBOV seroprevalence estimates using different Assays in DRC

Area of DRC	Year	*EBOV Seroprevalence (%)	Assay	CI	Population	Sample size (N)	Studies
Kikwit	1995	2.2	ELISA	0.3 – 4.0	Forest and City Workers	414	Busico et al. *Journal of Infectious Diseases* 1999, 79: S102-S107.
Watsa	2002	18.7	ELISA	14.4 – 23.5	General popualtion (pygmy)	300	Mulangu et al. *BMC infectious diseases* 2016, 16.1: 1-6
Sankuru	2007	11.0	ELISA	9.9 – 12.7	General population	3415	Mulangu et al. *The Journal of Infectious Diseases* 2018, 217.4: 529-537
Kinshasa	2011 – 2012	2.0	Luciferase immunoprecipitation system + neutralization	0.7 – 5.1	Blood donors	752	Imke et al., *Emerging Infectious Diseases*. 25 (5) 2019
Boende	2015	22.5	ELISA	19.2 – 25.9	Healthcare workers	611	Doshi et al. *The Journal of Infectious Diseases* (2020).
Boende	2015	28.1	ELISA, Luciferase immunoprecipitation system + neutralization	24.4 – 31.4	Healthcare workers	565	Hoff et al. *The Journal of infectious diseases*, 2019, 219.4: 517-525
Boende	2015 – 2017	7.0	ELISA	5.0 – 8.8	General population	687	Bratcher et al. *PLoS Neglected Tropical Diseases*, 2021, 15.8: e0009566.

Beni, Butembo, Katwa, and Mabalako	2018-2020	2.3	Luminex assay	1.1–4.0	Suspected cases of the tenth DRC epidemic of Ebola	600	Nkuba-Ndaye et al. *J Infect Dis*. 2022;226(2):352-356

*Seroprevalence based on the GP-EBOV antigen seroreactivity

However, despite many studies assessing the GP-EBOV antigen seroreactivity in different populations and different locations/countries, the interpretation of this seroprevalence data is challenging given the variation of the assays employed and diversity of cutoff algorithms used ((8,12,18,26–28). Seroreactivity to a single EBOV antigen may not be sufficient to demonstrate prior exposure to EBOV, especially in asymptomatically infected persons (29,30). Despite the broad range of EBOV seroprevalence rates in the EBOV endemic areas, previous serological surveys may have overestimated seroprevalence rates due to cross-reactivity against other infectious diseases (i.e. low specificity) (10,28). The use of more specific assays to determine the seroreactivity based on at least two antigens may therefore provide a better understanding of the baseline seroprevalence before a vaccine immunogenicity assessment (31,32).

The study presented here, combines (1) the seroresults of baseline blood samples collected among HCP and frontliners participating in the EBL2007 vaccine trial which evaluates the safety and immunogenicity of the two-dose Ad26.ZEBOV, MVA-BN-Filo Ebola virus vaccine regimen (ClinicalTrials.gov identifier: NCT04186000) with (2) the results from an ecological survey to determine information related to the current and past residence and work locations of a cohort of HCP included in the EBL2007 vaccine trial (33). On the baseline blood samples collected, pre-existing antibodies against EBOV among the participants were assessed using both FANG ELISA and Luminex assay. While the first assay only targets IgG antibodies against the glycoprotein (GP) of EBOV, the second assay also targets the nucleocapsid (NP) and the viral matrix protein 40 (VP40) which increases its specificity to 99% (34). This manuscript reports the baseline seroprevalence of Ebolavirus Zaire (EBOV) among HCP and frontliners participants in the EBL2007 trial conducted in the health district of Boende in DRC.

6.3. Materials and Methods

6.3.1. Origin of samples

Baseline serum samples were collected before vaccination in an open-label, monocentric, phase 2, randomized trial to evaluate the immunogenicity and safety of Ad26.ZEBOV and MVA-BN-Filo in healthy HCP and frontliners in Boende Health District of DRC (EBL2007 trial, ClinicalTrials.Gov: NCT04186000) (33). The trial site was located in the Boende General Hospital of Tshuapa province at approximately 750 km north-west of the capital city of Kinshasa in DRC. Blood samples were collected from healthy participants with no reported history of EVD or previous EBOV vaccination. During the first visit of the EBL2007 trial serum samples were collected for baseline determination of IgG GP-EBOV by the means of FANG ELISA and Luminex assay. At one year after inclusion of participants in the EBL2007 vaccine trial, a survey nested within the EBL2007 vaccine trial collected information related to where HCPs and frontliners lived and worked in the past, and their previous contacts with EVD cases.

6.3.2.Operational definition

The HCP term in the EBL2007 vaccine trial included medical doctors, nurses, midwives, laboratory staff, pharmacy staff, hygienists, health facility cleaners, and nursing assistants working in a hospital, Health Center, Health Post, or Health District office. Frontliners encompassed community health workers, first aiders, and those working in the Health District office and or the Provincial Division of Health. Direct contact was defined as any HCP and frontliners who may have had direct interaction with patients infected with EVD at a hospital or treatment center during an outbreak. Indirect contact was considered the work of frontliners and other HCPs whose jobs did not bring them into direct interaction with sick patients but could bring them in contact with contaminated material.

6.3.3. Serological testing

The study was performed according to the good clinical laboratory practice guidelines of the Division of Acquired Immunodeficiency Syndrome and WHO (35,36) to ensure high quality, reliable, and reproducible data at Q Squared Solutions (San Juan Capistrano, CA, US) Vaccine Testing Laboratory for FANG ELISA and Institut National de Recherche Biomédicale (INRB) in DRC for the Luminex Assay.

Considering only the seroreactivity to GP EBOV antigen, a higher specificity (95.4% : IC95% 89.6-98.0) and similar sensitivity (96.8% : IC95 91.3-98.9) to that of commercial ELISA assays was reported in a study comparing Luminex to the commercial ELISA kits more commonly used in previous serological surveys (32). Using the FANG ELISA was shown to be greater accurate and precise than a commercial alternative for assessing immune response after Ebola vaccination (37).

6.3.3.1. LUMINEX Assay technology

The serology testing was performed with Luminex Magpix® technology (Luminex Corp., Austin, TX) as per the previously published protocol (17,32). Four recombinant commercially available EBOV antigens were coated onto magnetic beads: two glycoproteins, GP-EBOV-kis (Kissidougou/Makona 2014 strain) and GP-EBOV-m (Mayinga 1976 strain); 1 nucleoprotein, NP-EBOV-m (Mayinga 1976 strain); and 1 40-kDa viral protein (VP40-EBOV-m, Mayinga 1976 strain). The bead-coupled antigens were mixed with the patient sample (1:1000 sample to dilution buffer), and the signal from the response for anti-EBOV immunoglobulin G (anti-IgG) was read and stored on Bio-Plex 200 hardware (Bio-Rad, Marnes-la-Coquette, France). All results were reported as the median fluorescence intensity (MFI). Based on the serological responses, a participant was deemed to bear the pre-existing antibodies against EBOV antigens when the sample was reactive above the cutoff for at least two different EBOV antigens.

6.3.3.2. FANG ELISA

The methods used to perform the FANG ELISA have been described in previous studies (37). Before the addition of test samples, 96-well microplates were coated with 100 µL of recombinant GP-EBOV-Kikwit (k) and incubated at 4°C in the absence of light. In addition to this, a standard obtained from one or more serially diluted vaccinated donors had been added. Incubation was performed by adding horseradish peroxidase conjugate from goat anti-human IgG to each well. The substrate 3, 3', 5, 5'-tetramethylbenzidine was then incorporated into each well. The addition of sulfuric acid solution stopped the enzymatic reaction. The color change was then observed with a plate reader. The plate reader was used to report the quality controls as well as the concentrations of the added samples. The concentrations of these

samples were based on the standard curve calculated using a 4-parameter logistic curve (4PL) and are expressed as ELISA units/ml (EU/ml). Final titers were determined based on a cutoff optical density (OD) value and were reported as the reciprocal of the highest dilution with a positive OD value.

6.3.3.3. Sample Size and Statistical Analysis

The number of participants eligible for the EBL2007 trial with available aliquots (n=698) at the inclusion visit predetermined the number of enrolled subjects in the serosurvey. Subjects reacting to EBOV antigens (GP, NP, and VP40) were summarized using proportions with 95% confidence interval. Demographic and ecological data were compiled and summarized using descriptive statistics for all participants enrolled in the EBL2007 vaccine trial using SPSS 28.0 IBM SPSS Statistics for Windows, version 28.0 and R 4.2.1 Statistical Software.

Both the FANG ELISA and Luminex assay do not have an established cutoff to distinguish individuals with seroreactivity to an EBOV antigen. In the absence of a represented control panel to estimate a cutoff, we calculated cutoff values by change point analysis (38) using R (39). In the supplementary information, we also provide seroprevalence estimates based on cutoff values obtained from literature.

To further investigate if the signal of the antibody assay represents true past exposure to EBOV, we tested if participants from the EBOV risk groups (based on age, sex, direct or indirect contact with patients in general, working in a hospital or elsewhere, previous contact with Ebola patients or experienced an outbreak at a location where you lived) were significantly more likely to be antibody positive. We used a generalized linear model with binomial link function. For each individual antigen, the participant's seropositivity status was included as response variable and the participant characteristics as explanatory variables. Only combinations of the Luminex GP-EBOV-m+VP40-EBOV were considered, as the sample size of the positive group was too small for all other combinations. P-values were considered significant below a value of 0.05.

6.3.4. Ethics statements

Ethics Committee of the University Hospital of Antwerp/University of Antwerp (approval reference n°19/14/177) and the National Ethics Committee of the DRC Ministry of Health approved the study protocol of EBL2007 (approval reference n°121/CNES/BN/PMMF/2019). The National Ethics Committee of the DRC Ministry Health under approval reference n ° 212/CNES/BN/PMMF/2020 approved the ecological survey nested in the EBL2007 Vaccine trial. For both the EBL2007 trial and the ecological survey participants provided written informed consent.

6.4.Results

6.4.1. Participants characteristics

A total of 720 HCPs and frontliners were screened for inclusion in the EBL2007 trial, of which 699 (96.9%) agreed to participate in the baseline seroprevalence study. However, one participant withdrew consent prior to blood collection. Thus, blood samples were available for 698 (99.9%) participants with a mean age of 45 years (standard deviation=12.0) and 534 (76.5%) were male (**Table 2**). The FANG ELISA results for five samples were indeterminate. Nearly two-thirds of the HCPs and frontliners [492 (70.5%)] worked in a health facility in the Boende Health District and 410 (59.0%) were HCPs working in direct contact with patients.

Forty-three (6.2%) of them reported a direct contact with patients during a previous Ebola outbreak in Boende or elsewhere. From a minority (3.5%) we are not sure if they ever had contact with infectious patients during an Ebola outbreak.

Table 2. Participants characteristics

Characteristic	N=698	%	Mean (SD)	Min	Max
Age (year)			45.0 (12.0)	19	75
Sex					
Female	164	23.5			
Male	534	76.5			
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			
Medical Doctor	13	1.9			
Health Facility Cleaner	10	1.4			
Care Giver	7	1.0			
Other	3	0.4			
Laboratory Technician	2	0.3			
Pharmacist Assistant	2	0.3			
Place of work in Boende					
Health Facility (Hôpital, Centre de Santé, Poste de Santé)	492	70.5			
Health District Office (Bureau central Zone de Santé)	8	1.1			
Croix-Rouge Boende	177	25.4			
Inspection Provinciale de la Santé	1	0.1			
Aire de Santé	10	1.4			

Division Provinciale de la Santé Tshuapa	9	1.3
Programme Elargi de Vaccination Boende	1	0.1

6.4.2. Seroreativity to EBOV proteins using FANG ELISA and or Luminex

When considering antibody responses against EBOV antigens individually, we found that 8.5% (60/698; 95% CI: 6.5 -10.7) of samples tested positive on the Luminex for GP-EBOV-m, 9.4% (66/698; 95% CI: 7.5 -11.8) for GP- EBOV-kis, 9.4% (87/698; 95% CI: 10.3-14.9) for VP40-EBOV-m, and 1.3% (9/698;95% CI: 0.6-2.6) for NP-EBOV-m (Table 3). The seroreactivity to at least two EBOV antigens using Luminex was encountered in 1.4% (10/698; 95% CI: 0.7-2.6) and 0.3% (2/698;95% CI: 0.0-1.0) of sera for VP40-EBOV-m + GP-EBOV-m and VP40-EBOV-m + NP-EBOV-m respectively. No sera tested positive for NP-EBOV-m+GP-EBOV-m.

Table 3: Seroprevalence for different (combinations of) antibodies against Ebola virus antigens as measured by the Luminex or FANG ELISA in Health care providers from Boende, DRC.

	Antigen	Cutoff	Positives n (N)	Seroprevalence % (95% conf. Int.)	Age /year	M vs F	Direct Contact with patients: Direct vs indirect	Working Hospital vs elsewhere	Experienced Ebola outbreak/patients vs others
					p-value	p-value	p-value	p-value	p-value
FANG ELISA	**GP-EBOV-k**	526 EU/ml	49 (693)	7.0 (6.5, 10.9)	0.89	0.44	0.52	0.93	0.94
Luminex	**GP-EBOV-m**	669 MFI/100 beads	60 (698)	8.6 (6.5,10.7)	**0.03**	0.93	0.05	0.63	0.09
	GP-EBOV-kis	670 MFI/100 beads	66 (698)	9.4 (7.5,11.8)	0.05	0.99	0.05	0.52	**0.005**
	VP40-EBOV-m	441 MFI/100 beads	87 (698)	12.4 (10.3,14.9)	0.11	0.07	0.31	0.74	0.46
	NP-EBOV-m	602 MFI/100 beads	9 (698)	1.3 (0.6,2.6)	0.41	0.40	0.13	0.26	0.07
	GP-EBOV-m+NP-EBOV-m	C1	0 (698)	0					

	GP-EBOV-m+VP40-EBOV-m	C2	10 (698)	1.4 (0.7,2.6)	0.75	0.02	0.39	0.68	0.47
	NP-EBOV-m+VP40-EBOV-m	C3	2 (698)	0.3 (0.0,1.0)					
Luminex and FANG ELISA	**GP-EBOV-m + GP-EBOV-k**	C4	6 (693)	0.8 (0.1,1.5)					

C1= 669MFI/100 beads for GP-EBOV-m and 602 MFI/100 beads for NP-EBOV-m
C2= 669MFI/100 beads for GP-EBOV-m and 441 MFI/100beads for VP40-EBOV-m
C3= 602 MFI/100 beads for NP-EBOV-m and 441 MFI/100 beads for VP40-EBOV-m
C4= 669 MFI/100 beads for GP-EBOV-m using Luminex and 526 EU/mL for GP-EBOV-m using FANG ELISA

GP-EBOV-k seroreactivity on the FANG ELISA was found in 7 % (49/693; 95% CI: 6.5-10.9) of participants' sera. Looking at participants whose GP-EBOV seroreactivity was identified in both Luminex and FANG ELISA, 0.8% (6/693; 95% CI: 0.1-1.5) of the tested samples were positive by a combination of the Luminex and FANG ELISA assays. We performed seroreactivity analyses using cutoffs determined in the literature and found similar results as depicted in the Supplementary material.

A weak correlation between the FANG ELISA and Luminex was shown (k=0.2) (Figure 1).

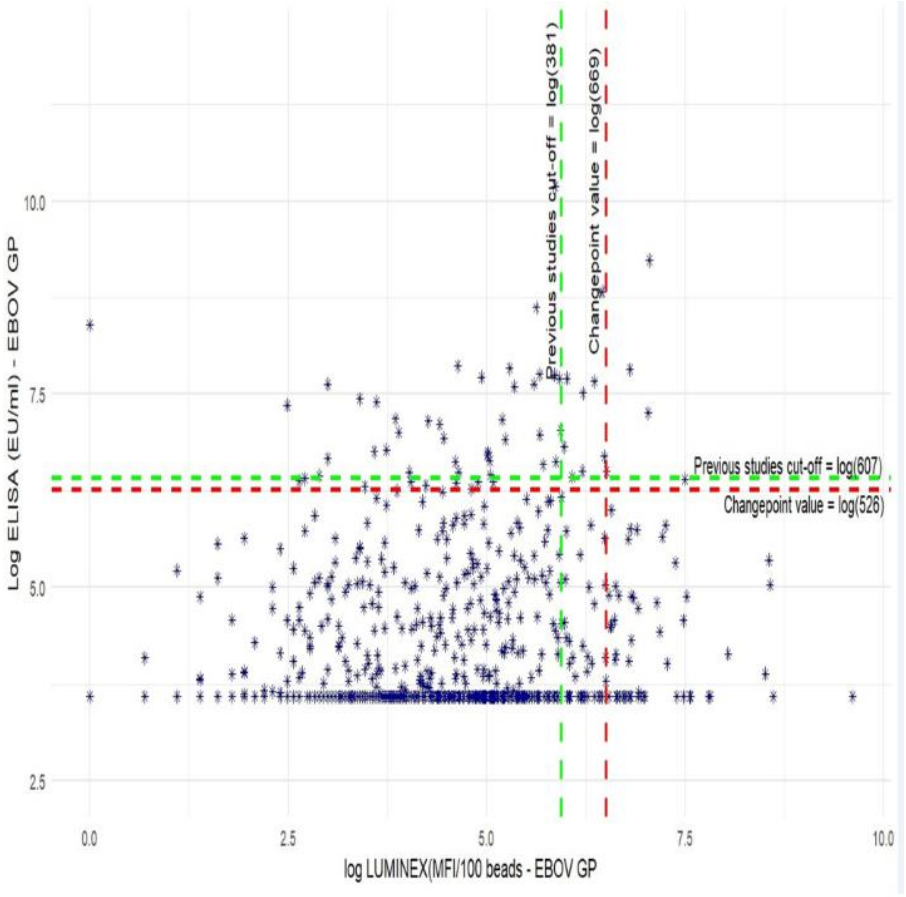

Figure 1. Pearson Correlation between FANG ELISA and Luminex

Seroreactivity against the glycoprotein (GP) of Ebola virus in health care providers and frontliners from Boende. (A) The X-axis reports the log values of the antibody titers (IgG) as measured by Luminex in MFI/100 beads. (B) Y-axis represents antibody titers as measured by FANG ELISA in EU/ml. (C) The vertical dashed line in red represents the cutoff of the changepoint analysis and the dashed horizontal line in green represents the cutoff obtained from previous studies.

In seeking which participant characteristics influenced seropositivity, we observed significant differences in the seropositivity rate between HCPs and frontliners who previously made direct contact with an Ebola patient or experienced an outbreak in their hometown. When looking at the GP-EBOV-k antigen, HCPs and frontliners who previously became into contact with Ebola were significantly less likely to be seropositive compared to HCPs and frontliners who never experienced an Ebola outbreak (estimate=-1.22, std. error = 0.52, P=0.02). When looking at the GP-EBOV-m, seropositivity status significantly decreased with age (estimate = -0.02, est. error = 0.01, P=0.04).

6.5.Discussion

We report the baseline seroreactivity to EBOV-m antigens in apparently healthy HCPs and frontliners enrolled in the EBL2007 vaccine trial.

Based on seroreactivity in two different assay formats (FANG ELISA and Luminex), only a minority (0.8%) of HCPs and frontliners blood samples seroreacted to the GP-EBOV-m and GP-EBOV-k surface antigen. Similarly, a minority of participants sera tested positive to at least two antigens on the Luminex (0.3% for NP+VP40 EBOV-m and 1.4 for GP-EBOV-m+VP40-EBOV-m). None of the participants sera tested positive to GP-EBOV-m+NP-EBOV-m.

Additionally, when we investigated whether seropositivity correlated with participants' prior exposure to EBOV-m, we did not observe a relevant positive correlation. This suggests that the majority of seropositive participants implied based on the single antigen using FANG or Luminex assays analysis are in fact false positives.

Unexpectedly, HCPs and frontliners participants who made previous contact with an Ebola case were less likely to be EBOV-seropositive than those who never became into contact. This result also suggests that the FANG ELISA is less suitable for seroepidemiological studies in African populations. Indeed, while the detection limit of the FANG ELISA (36-11 EU/mL) was established based on non-African samples, the limit needs to be increased in the African population. In this context, the Luminex multiplex assay might be much more suitable due to the use of multiple antigens that increase the specificity (40).

Overall, these results suggest that the baseline seroprevalence against EBOV-m in HCPs and frontliners in Boende is very low. Our seroprevalence estimates are much lower compared to previous serosurveys conducted after the EVD outbreak of 2014 in HCP of Boende Health District (22.5% and 28%) (GP-EBOV-m seroreactivity using ELISA) (12,27). Our estimates are also lower than the one previously reported in Boende Health District among the general population (7%) (GP-EBOV-m seroreactivity using ELISA) a year after the 2014 Ebola epidemic (6). The seroprevalence based on one EBOV-m antigen (GP-EBOV-m) found in this study using either ELISA or Luminex is lower than other previously reported in the Watsa Pygmy

population of DRC in 2002 (18.7%) (GP-EBOV-m seroreactivity using ELISA) and the Sankuru rural population in 2007 (11%) (GP-EBOV-m seroreactivity using ELISA) (8,21).
By employing this approach, our seroprevalence estimates became comparable to those of previous serological surveys conducted in Kikwit (2.2%) (GP-EBOV-m seroreactivity by ELISA) and Kinshasa (2%) (GP-EBOV-m seroreactivity by luciferase immunoprecipitation system + neutralization) (18,41).
It is worth noting that LUMINEX built on an approach of simultaneously targeting multiple EBOV antigens, demonstrated a specificity (99.1%) and a sensitivity (95.7%) similar to higher than, respectively, the specificity (100%) and sensitivity (92.5%) of the commercial ELISA in a study (32). The FANG ELISA was developed and validated to quantify Filovirus anti-EBOV-GP immunoglobulin G (IgG) binding antibodies in human and non-human primate serum sample to enable bridging of immunogenicity data between humans and animal models in vaccine trials (37).
The higher seroprevalences found in other serosurveys conducted in Boende or elsewhere in the DRC may be explained by the fact that different assays were used in the different studies, other cutoff algorithms were used, and the definition of reactivity discrimination (one or two EBOV antigens) may have decreased the specificity of these assays. This could have led to overestimation of EBOV antigen seropositivity. On the other hand, it cannot be ruled out that more people were indeed infected during the 2014 outbreak in Boende and that antibody titers waned over time or at least dropped below the detection threshold, explaining the low seroprevalence that we observed. However, a number of other studies have shown IgG positivity typically prolonged to more than 10 years after an EBOV declared epidemic in an area (42,43). It is unclear if the high compliance with infection prevention and control measures may have led to the low seroprevalence of the majority of HCPs and frontliners participants in the EBL2007 vaccine trial and serosurvey during the 2014 epidemic. This would have kept them free of EBOV exposure and might explain the low seroprevalence.
Likewise, this low prevalence may reflect a rare incidence of asymptomatic EBOV infection among HCP and frontliner population from the Boende Health District. The previous scenario may reflect a susceptibility to future outbreaks of EBOV. Yet, negative antibody titers do not rule out other types of immunity, such as T-cell immunity (44).
FANG ELISA or Luminex are assays that can only detect binding antibodies and are unable to differentiate them from neutralizing antibodies (37). The latter are typically detected using neutralization assays, which are still considered the gold standard for serological testing (45,46). However, such testing involves infectious cells, are labour intensive and time consuming (47). For viruses such as EBOV, all experiments should be performed under a biosafety laboratory (BSL)-4 conditions, which are limited in availability and expensive to operate (48). Thus, it is beneficial to use alternative neutralization assays that do not require viruses or live cells, and that can be performed in BSL-2 laboratories to assess neutralizing antibody capacity (47,49). These alternative assays should conclude if a person with high binding antibodies against EBOV (based on FANG ELISA or Luminex) was indeed infected with the virus (although some level of cross-reactivity can never be ruled out) (29). The challenge of comparing different serosurveys that have assessed the EBOV seroprevalence makes the implementation of international standardization of units for EBOV antibody detection and quantification of paramount importance (50).
The poor linear relationship between the two assays used (FANG ELISA and Luminex) in this serosurvey confirms that both assays likely contain many false positive results, when using

single antigens. Hence, the reported seropositivity could be an effect of other filoviruses or infectious microbes, which may cause cross-reactions (51).
Limitations of our study are the lack of positive and negative control samples to determine the positive cutoff and relative long timing since the outbreak (6 years). However, in the absence of a standard serological assay for EBOV seroreactivity, Luminex can still be employed in serosurveys due to its ability to detect seroreactivity to combinations of different EBOV antigens (32).

The strength of this survey resides in the use of high cutoffs to determine the EBOV seropositivity that aligns with recommendations in EBOV serosurveys generally applied to Congolese cohorts (18,28,52). Thus, the combination of FANG ELISA and Luminex results can be considered a starting point, showing how previous serological surveys may have overestimated the seroprevalence of EBOV in a non-exempt area. Like the recent index case of the fourteenth outbreak in DRC (Mbandaka, 2022), whose symptoms began three weeks after returning from Boende with no notion of contact with an Ebola survivor (5). The next step could be the use of a neutralization assay for assessment of neutralizing antibodies among this population of HCP and frontliners participants in the EBL2007 trial, to further document whether or not this population of HCP is naive to EBOV exposure. Alternatively, an assessment of EBOV seroprevalence in a different population cohort closer to the time of an EVD outbreak, using negative controls, may provide insight into the utility of using Luminex or other multiple assays as the gold standard in EBOV seropositivity investigations.
The low baseline seroreactivity to EBOV antigens observed in HCP and frontliner population of Boende suggests that the majority of this population never came into contact with the virus, despite the fact the many HCP and frontliners worked during previous EBV outbreak in 2014. In the event of a future epidemic, mathematical models suggested that the vaccination rate of HCP in an infected area should be at least (30%) to prevent a major epidemic (53). Therefore it is clear that HCP in endemic regions should be primary targets for vaccination in the frame of the Ebola epidemic preparedness plan in DRC (53).

6.6. Conclusion

In contrast to previous studies that observed high seroreactivity against EBOV-m in Boende, our results show that the baseline seroprevalence of HCP and frontliners that reported no previous EBOV infections is low. This suggests that asymptomatic infections are unlikely to occur or that antibodies rapidly wane after infection (or at least drop below the cutoff of detection). Irrespective of the cause, it means that the majority of HCPs in the area are likely susceptible to EVD despite the history of outbreaks in and the area of Boende. Given the high variance between seroprevalence estimates by different studies in the same region, we highlight the need for more uniform antibody assays. Neutralizing antibody quantification methods, which are inexpensive in terms of resources, are likely to be crucial for improving EVD surveillance in this region, given the high background of concomitant parasitic disease burden that can be expected to be found in the serum of this population. Low resources affordable approaches to quantifying neutralizing antibodies are likely to be crucial in enhancing surveillance of EVD disease in this region.

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Supplement

Table 1 Seroprevalence with cut off obtained from literature for different (combinations of) antibodies against Ebola virus antigens as measured by the Luminex or FANG ELISA in Health care providers from Boende, DRC.

	Antigen	Cutoff	Positives n (N)	Seroprevalence % (95% conf. Int.)	Age /year	M *vs* F	Direct Contact with patients: Direct vs indirect	Working Hospital vs elsewhere	Experienced Ebola outbreak/patients vs others
					p-value	p-value	(p-value)	(p-value)	p-value
FANG ELISA	**GP-EBOV-m**	607 EU/ml	49 (693)	7	0.73	0.35	0.24	0.67	0.61

Luminex	**GP-EBOV-m**	381 MFI/100 beads	104 (698)	14	**0.005**	0.22	0.05	0.81	0.24
	GP-EBOV-k	501 MFI/100 beads	89 (698)	13	**0.02**	0.81	0.05	0.99	0.45
	VP40-EBOV-m	580 MFI/100 beads	69 (698)	10	0.14	0.11	0.41	0.35	0.16
	NP-EBOV-m	950 MFI/100 beads	8 (698)	1	0.54	0.57	0.31	0.16	0.08
	GP-EBOV-m+NP-EBOV-m	C1	0 (698)	0					
	GP-EBOV-m+VP40-EBOV-m	C2	19 (698)	3	0.92	0.37	0.91	0.38	0.13
	NP-EBOV-m+VP40-EBOV-m	C3	2 (698)	0.2					
Luminex and FANG ELISA	**GP-EBOV-m + GP-EBOV-k**	C4	6 (693)	0.8					

*cutoff (C) represent values obtained from literature and previous studies
C1= 381 MFI/100 beads for GP-EBOV-m and 580MFI/100 beads for NP-EBOV-m
C2=381 MFI/100 beads for GP-EBOV-m and 950 MFI/100 beads for VP40-EBOV-m
C3= 950 MFI/100 beads for NP-EBOV-m and 580 MFI/100 beads for VP40-EBOV-m
C4= 607 EU/ml and 381MFI/100beads for GP-EBOV-k and GP-EBOV-m

Chapter 7 Healthcare Providers' and Frontline workers' experiences of an Ebola vaccine trial in the Boende Health District of the Democratic Republic of the Congo

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7.1. Abstract

This study explored the experiences of healthcare providers (HCP) and frontliner workers who were involved in an Ebola vaccine trial in the Democratic Republic of the Congo. The researchers interviewed a total of 99 participants (HCP and frontline workers) living and working in the Boende health district during the period of the study, from February to March 2022. These individuals included a mix of trial participants and non-trial participants (staff of the trial, local health authorities, and head nurses of health centers). In-depth individual interviews, as well as focus group discussions (FGDs), were employed to understand interviewees' experiences and perceptions. The data were analyzed to identify the main themes. The findings unveiled a multitude of positive experiences among interviewees/FGD-participants. The commitment of the trial investigators to improve the study site and to equip the volunteers with necessary skills and knowledge greatly contributed to a positive trial experience. However, some interviewees felt that the reimbursement for time and travel expenses during their trial visits was insufficient compared to their expectations. Additionally, there were expressions of worry about the frequency of blood draws during scheduled trial visits. Our findings emphasize the critical importance of addressing and continuously considering the perspectives and concerns of trial participants before designing and implementing vaccine trials. By actively incorporating their inputs, researchers can mitigate concerns, and tailor communication strategies, potentially enhancing the overall success and impact of the vaccine trial.

Keywords: Ebola vaccine, clinical trial, experiences, perception, acceptance, motivation, trial participants, Healthcare providers..

7.2. Introduction

The Ebola virus disease (EVD) is prevalent in the Democratic Republic of the Congo (DRC), and there remains a risk of re-emergence from an animal reservoir or through relapse among survivors(1-3). The rise in the occurrence of EVD outbreaks observed from 2020 to 2023 in DRC is rooted in previous outbreaks due to relapses among survivors (3, 4). At least one outbreak has occurred each year from 2017 to 2022 in both the Equateur and North Kivu provinces in the DRC.

Prophylactic vaccination against EVD is not yet part of the standard prevention strategy (at the time of writing this manuscript). However, the proximity of successive outbreaks in recent years in the same regions of the DRC, particularly in remote areas, underscores the need to continue efforts to provide prophylactic vaccination to specific at-risk populations, including health care providers (HCPs) and frontline workers. These individuals are at a much greater risk due to their frequent and close contact with patients who may be infected with the virus (5). It is worth highlighting that even minimal deaths among HCPs population in a remote area of the DRC can have disastrous effects on a health care system already weakened by several endemic diseases, including malaria, and the recurrence of epidemics such as measles and monkeypox, adding strain to an already fragile healthcare system (6). Prophylactic vaccination of HCPs and frontline workers before the start of an EVD epidemic has great potential to significantly reduce the number of EVD cases and the death rates, and to mitigate its impact on the health system (6, 7).

In 2014, the Boende Health District experienced an outbreak resulting in 66 cases and 49 deaths. Furthermore, in 2022, the initial case of the 14th EVD outbreak in Mbandaka, located in the neighbouring Equateur province, was traced back to an individual who had recently returned from a vacation in the Boende Health District, a month prior to experiencing symptoms. Notably, this individual was a medical student who had recently concluded his vacation internship at the general referral hospital of Boende. No contact with an EVD survivor was reported. Given the persistent zoonotic exposure in this region, it is challenging to accurately forecast the occurrence of an epidemic, implying that the risk of such exposures may endure indefinitely.

Within the framework of the European Union's "EBOVAC3" project (IMI-EU), the University of Antwerp and the University of Kinshasa conducted an Ebola vaccine trial known as “EBL2007” (NCT04186000). The EBL2007 vaccine trial – an open-label, randomized, Phase 2 study – evaluated the immunogenicity and safety of the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen and an Ad26.ZEBOV booster dose in 699 registered HCPs and frontline workers (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) between 2019 and 2022. We considered HCPs who work in a health facility and could come into contact with infected patients in this facility (e.g., doctor, nurse, midwife, lab technician, health facility cleaner, etc.) and frontliners those with a profession leading to early potential EBOV exposure in the community (e.g., first aid workers, community health care workers, stretcher bearers, care givers, etc.)(8). It is crucial to highlight the following aspects concerning volunteering for the EBL2007 vaccine trial. First, to enhance potential participants' understanding of the trial, a Test of Understanding (TOU) was carried out after the study protocol had been explained. The TOU consisted of a pre-tested, structured questionnaire (comprising closed-ended questions) devised by the EBL2007 trial investigators to evaluate the potential participants' grasp of the study's essential

information and requirements. This TOU was conducted prior to the signing of the consent form during the screening visit. Successful completion (≥9/10; 3 attempts possible) was a prerequisite for signing the informed consent and an inclusion criterium for enrolment the trial. Second, regarding reimbursement for time and travel expenses, participants who traveled for less than 6 hours were provided with a fixed amount of 20 USD to cover transportation costs while those who traveled for more than 6 hours received a reimbursement of 40 USD, which was allocated to cover expenses associated with food and accommodation (9).

While the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen has been approved by the European Commission for the prevention of EVD since July 2020, the findings of this study are anticipated to generate supplementary data from a new population, thereby contributing to a more comprehensive understanding of this vaccine regimen (10). Numerous challenges were encountered pertaining to the issue of mistrust surrounding a different Ebola vaccine (rVSV-ZEBOV vaccine), during another vaccine trial conducted amidst the tenth EVD epidemic in the eastern provinces of the DRC (North Kivu and Ituri) (11, 12). Some Ebola patients and Ebola contacts (suspected cases) actively rejected vaccination and going to the proposed EVD treatment center because they did not believe in the existence of the Ebola virus (13-15). This contributed to a prolonged epidemic, the most widespread and long-lasting in the recorded history of EVD to this day in the DRC (2018-2020). Additionally, results from other studies on the acceptance of Ebola vaccines among HCPs suggest that it is not easy to predict the acceptability and perception of a non-approved vaccine (11, 14). Aware of these forms of hesitancy and resistance, we carried out a qualitative investigation during the follow-up period of the EBL2007 trial to understand how it was perceived by participants, trial staff and health authorities.

7.3. Methods

This qualitative study was nested within the above-mentioned vaccine trial conducted in the Boende Health District, a remote area in the DRC (8).

7.3.1. Study setting

The Boende Health District, one of twelve health districts in the Tshuapa province in DRC encompassing 6 territories and the capital city of Boende (Figure 1), is home to about 296,253 residents(16). This district is characterized by the presence of 2 ethnic groups (Bantu and Pygmies), with the Bantu ethnic group being predominant. The languages mostly spoken across the province are Lomongo, Lingala, Longando, Topoke and French, the latter being used in administration and education. The most practiced religions include Catholicism (with over half of the population in the DRC identifying as Roman Catholic), and minorities practicing Protestantism, Kimbanguism, Islam, Revivalism, Brahmanism, Jehovah's Witnesses, Banga Nzambe, Kitawalism, etc. (17). In spite of that, the traditional religious beliefs heavily influence local beliefs and practices (18). The main way of travelling to other cities for the local people is the improvised boat, commonly called as a whaleboat (Appendix 1, Figure 1), which poses a frequent risk of crashes and overturning, resulting in the loss of human lives, as well as motorcycles and biking. Travelling by plane is also possible, but not a common mode of transportation for most residents.

Figure 1.Tshuapa and the Health Districts limits (25)

The provision of healthcare services within the province is ensured through governmental structures such as the Health Center (Centre de Santé), Referral Health Center (Centre de Santé de Référence), and HGR, as well as other private facilities including healthcare services offered by non-governmental organizations and religious organizations. Traditional medicine represents a significant portion of the health system as visiting traditional healers represents a significant portion of health-seeking behaviour; either due to the financial cost associated with seeking treatment in health facilities, or for treating specific conditions such as fractures, mental disorders, etc., which are not considered curable or can only be partially cured by the modern medicine available in this remote location.

7.3.2. Participant's recruitment and sampling

A purposeful sampling technique was used, and interviewees were invited per their role in the EBL2007 vaccine trial, gender, and healthcare work categories. Facilitated by the EBL2007 vaccine trial site coordinator, the researchers extended invitations to a total of 117 HCPs and frontline workers who either participated or discontinued participation in, or did not participate in the EBL2007 vaccine trial, and local health authorities of the province of Tshuapa.

From a literature review(9, 20-24), methodological tools were developed, including a topic guide, outlining key issues, and serving as a basis for designing a discussion framework for the focus groups and in-depth interviews (Supplement 1- 4).

Perceptions related to the EBL2007 clinical trial were discussed with trial participants, those withdrawn from the trial, and non-participants (, trial staff, head nurses of health centers and local health authorities). More specifically, their experiences, perspectives on the services rendered and/or obtained throughout the EBL2007 vaccine trial; the challenges faced by trial staff/trial participants and how they were overcome; internal, and external collaboration, and communication (between trial staff and trial-participants, between trial staff and local health

authorities); the acceptability of the study vaccine; the motivation for participating in the trial; and the expectations and hopes for the future of the study site, and Ebola vaccine research.

7.3.3. Data collection methods

Study data were collected during focus group discussions (FDG) and in-depth individual interviews (or one-to-one interview), using adapted, open-ended, pre-tested interview guides (supplement 2).

7.3.3.1. Focus Group Discussion

To gather firsthand accounts from HCP and frontline worker participants in the EBL2007 vaccine trial, FGDs were conducted. For the FGDs, trial participants were grouped according to their gender, and occupation. In each FGD, 6 to ten participants participated. One FGD assembled registered Heads of health centers (*infirmiers titulaires*) that were not part of the trial. All the FGDs followed the same interview guide, ensuring a systematic approach to data collection (Supplement 1). The questions in the guide were pre-tested, reviewed, and refined during the fieldwork based on emerging themes from the discussions. The FGDs encompassed the following themes: general understanding of the EBL2007 vaccine trial; motives behind the decision to participate; experiences as participants throughout the trial and its finalization; expectations and hopes for the trial; perceptions of the trial's impact on participants' daily lives and the community; and perspectives on the future of the trial site and Ebola vaccine research following their participation. Discussions were conducted in either French or Lingala, depending on the group's preference.

7.3.3.2. Individual In-depth interviews

The interviews utilized a semi-structured grid (Supplement 2, 3) to capture testimonies regarding the EBL2007 vaccine trial. The following areas were covered: difficulties encountered and strategies for overcoming them; psychological well-being during the trial; collaboration and communication with other medical staff and stakeholders; perceptions of the vaccine trial in Boende and relationships with the community; perspectives on the future of the trial site and capacity building received during the trial; and prospects for career trajectory after the trial concludes.

The narratives of participants who withdrew consent encompassed several key aspects, including their overall comprehension of vaccine trials, and specifically the EBL2007 trial protocol in Boende. Additionally, they shared insights into the factors that influenced their decision to withdraw consent.

Insights from HCP and frontline worker participants in the trial or staff, as well as local authorities such as the provincial Minister of Health, the Chief of provincial health division (*Chef de la Division Provinciale de la Santé*) of Tshuapa, and the Head of the Boende Health District were captured using a pretested semi-structured questionnaire (Supplement 4).

7.3.4. Procedures

Prior to starting an interview (FGD or Individual In-depth interviews), participants were reminded of the purpose of the study. Each conversation lasted 60 to 90 minutes, , and participants' statements were audiotaped. The collected voice recordings in a language other than French were translated into French prior to being transcribed by 2 independent transcribers not involved in the trial. The correctness and consistency of transcripts were double-checked by TZM; TZM coded all transcripts obtained from all FGDs and in-depth interviews. AP and TZM agreed on the codes and categories used to create themes.

The FGD were facilitated by a postdoctoral researcher (AP) who acted sometimes as the moderator or notes-taker, while 2 doctoral students (TZM and FBB) who intermittently also

acted as note-takers or moderator. One of the doctoral students, TZM, was acting also as a sub-investigator of the EBL2007 vaccine trial, while FBB was not involved in the trial activities. All individual In-depth interviews were conducted by either by TZM, AP or FBB, in a carefully selected and tranquil setting preferred by the interviewees. Each interview had an average duration of approximately 50 minutes, allowing for in-depth exploration of the topics discussed.

7.3.5. Analysis

The recordings of the discussions and interviews were transcribed and/or translated into French (if necessary) and imported into the NVIVO software for analysis. Each transcript was anonymized and attributed a unique identifier. Using the NVIVO software, AP and TZM held regular meetings to harmonize the coding. An inductive thematic approach was used to analyze, identify emerging themes, and extract significant quotations from them according to the objectives of the study.

7.3.6. Ethical considerations

The National Ethics Committee of the DRC Ministry of Health gave its approval before the study commenced (Avis n°368/CNES/BN/PMMF/2022). At the start of each discussion, it was made clear to all potential participants that their involvement was optional and voluntary. Informed consent was given through verbal consent of all those participating (in either French or Lingala depending on participant preference) before the interviews or FGDs.

7.4. Results

A total of 10 focus group discussions (FGDs) and 15 in-depth interviews were conducted to explore the perspectives of healthcare professionals (HCPs), frontline workers, and local health authorities involved in the EBL2007 vaccine trial. The sample comprised 99 participants, including trial participants, non-trial participants, those withdrawn from the trial, and local health authorities. Participants were selected using a purposeful sampling strategy to ensure a diverse range of experiences and perspectives. Data saturation was achieved after conducting the FGDs and in-depth interviews with these 99 willing respondents. The demographic characteristics of the participants are presented in Table 1.

Table 1. Interviewees by data collection methodsFGD-participants and characteristics

Relation to the EBL2007 vaccine trial (participants 'category)	Occupation	Data collection method	Male	Female	Total (N)
Trial Participants	Nurses	2 FGDs	10	8	18
	First aids	2 FGDs	9	8	17
	Community Health Workers	2 FGD	8	7	15
	Midwives	1 FGD	-	9	9
	Health Facility Cleaners	1 FGD	2	8	10
	Medical doctors	1 FGD	6	-	6
Non-trial participants	Head nurses of health centers (Centre de Santé)	1 FGD	10	-	10
Total		10 FDGs	45	40	**85**

Staff in the trial	Laboratory technician	2 In-depth individual Interviews	1	1	2
Staff in the trial	Nurse	2 In-depth individual Interviews	1	1	2
Staff in the trial	Sentinel (watchman)	1 In-depth individual Interview	1	-	1
Staff in the trial	Cleaning Lady	1 In-depth individual Interview	-	1	1
Withdrawn from trial	Anonymous	4 In-depth individual Interviews	2	2	4
Local health authority	Provincial Minister of health	1 In-depth individual Interview	-	1	1
Local health authority	Head of the health division of Tshuapa	1 In-depth individual Interview	1	-	1
Local health authority	Chief of the Health District	1 In-depth individual Interview	1	-	1
Local health authority	directorate of the general referral hospital of Boende	1 In-depth individual Interview	1	-	1
Total		15 In-depth individual interviews	8	6	**14**

Prior to presenting our research findings, it is crucial to provide a contextual framework to better understand the responses provided by the interviewees. The recruitment phase of the EBL2007 vaccine trial commenced in December 2019, marked as the day 1 visit and the vaccine regimen under investigation had not yet received approval for the use in the European Union (EU) at that time. Study participants received the study vaccines at 56-day intervals between December 2019 and April 2020. During the initial visit, participants were randomized into 2 groups, with one group scheduled to receive a booster vaccination after one year (2020-2021) and the other group after 2 years (2021-2022) following the day 1 visit. Consequently, the year 2 visit served as the final scheduled visit for the first group, while the second group was expected to continue with safety follow-ups for 6 more months.

Our research was conducted shortly after the year 2 visit, coinciding with the completion of all visits for the first group. It is worth noting that the recruitment and follow-up period of this trial coincided with the occurrence of 5 EVD outbreaks in the provinces of Equateur and North Kivu (see appendix 2, Table 1), and it is important to highlight that no EVD epidemic was officially declared in Boende during the entire duration of the trial activities.

Upon analysis of the data, the experiences of both participating and non-participating HCPs) and frontline workers, trial staff, and local health authorities were categorized into 3 major themes: overall perceptions of the trial experience, appreciations of the trial, and perspectives on future Ebola/Ebola vaccine research. The themes, including identified sub-themes and categories are described below.

7.4.1. Overall perceptions of the trial

7.4.1.1. Aims of the trial

The objective of the EBL2007 was perceived by some interviewees and FGD-participants as an initiative/project to assess whether the investigational vaccine confers protection, and to describe any adverse reactions associated with vaccination during the trial's inclusion period. However, the participants correctly recalled that that receiving the vaccine did not necessarily mean they would be protected against Ebola, or that they could be exposed to Ebola risk practices without contracting Ebola:

"The primary objective of our research was not centered around personal protection against Ebola virus disease, but only to study it. The vaccine was not brought to protect someone so that someone may say: 'not as I am vaccinated with EBOVAC […]. I see someone who has the Ebola virus who must bite me because I am protected' […]. It doesn't mean that we are sure that we are protected or immune […]. " (FGD, Nurse participant in the trial, Woman).

" […].. We observed all these people who received the vaccine, and that's the objective, to see how people will react to the Ebola immunization during a certain period". (Interview, Local health authority, Man).

7.4.1.2. Achievement of objectives in terms of expectations

Some respondents expressed a mix of frustration, anger, and sadness as the trial neared its end. They felt this ending was unexpected, particularly because the promised results (which were not yet available when data was being collected) had not been shared with them yet. These participants believed that the disclosure of the vaccine study results or the feedback on the collected blood samples would be perceived to acknowledge and value their altruistic contributions to the science.

"Well! What they said: 'We took the samples' and then they left. As such Gwen[2] had said if it is after 6 months that they are going to come back with the results… So, on that point I don't know if it will be like that." (Interview, trial staff, nurse, Woman).

" […], given the experience of Lokolia[3], we told ourselves: 'even if we are taken as guinea pigs, we may sacrifice ourselves for the others', but otherwise we were waiting, until today, we are waiting for the results of the study that was conducted here [...]" (FGD, trial participant, medical doctor, Man).

However, the conclusion of the trial was generally regarded as a major triumph by the trial staff, as it marked the culmination of a 2.5-year endeavor in a remote area of the DRC. Notably, the study demonstrated an impressive retention rate of over 90%, further highlighting the success and dedication of the teams and participants involved. Several interviewees emphasized that the objectives of the trial exceeded their initial expectations. This sentiment arose from the recognition that, alongside the trial vaccine, additional components were incorporated such as trainings of HCPs and frontline workers volunteering in the trial, from which the area of Boende would inevitably benefit to improve health care delivery.

"[…] Although we initiated the study with a considerable number of participants, it is important to acknowledge that a small proportion was lost to follow-up. However, it is noteworthy that most of these losses did not significantly impact the overall participant cohort. The study commenced with an initial cohort of 700 participants. However, the attrition rate remained relatively low, as we did not experience a loss of more than 100 participants …, I don't have the count on my head, but we have about 600 and some participants like that " (Interview, Medical Doctor, trial staff, Man)

" […] So in the study, even if it was not stated in the protocol… but other things we did with the participants, it strengthened their capacity to deliver and even in what they do, especially the first aid worker, they had a lot of training sessions, and they were very happy that they themselves were starting to train their participants " (Interview, Local health authority, Man).

Few respondents expressed a concern regarding the large population who was not part of the project, which they expected to expand in the province and to the entire population, as in the following statements:

"In my opinion, the goal is achieved for some, but not for others. You did the injustice, why you called some and you left others?" (Interview, Health Facility Cleaner, trial staff, Woman)

"When a disease emerges, it does not discriminate based on occupation or whether one works in the healthcare sector. The disease affects the entire community. Personally, I strongly advocate for larger vaccination among all community members." (Interview, trial-participant who withdrew consent, Man).

7.4.1.3. Informed Consent

A significant number of interviewees and FGD-participants perceived volunteering in the EBL2007 vaccine trial as establishment of a contractual relationship with the research team.

[2] A Sponsor staff member of the research project (EBL2007 vaccine trial)

[3] Lokolia is the health area where the index case of the 2014 epidemic occurring in the Boende Health District was observed.

This was to result in a long-term plan to address any harm related to the experimental product. Given the contractual perception of the study consent document among trial participants, some of them expected consistent financial benefits in relation to the risk and time spent in the trial accordingly. This concern caused some to leave the study during the follow-up:

"My question was the following; a community healthcare worker asked me: '[…] Were we paid in the risk that we had taken of testing this vaccine in our body?' I told them, I also want to ask the study coordinator. The community healthcare workers are going to go home empty-handed?" (FGD, Community healthcare worker, trial participant, Man).

Some of the interviewees who withdrew from the trial expressed that the expected money offered for travel costs and time spent in the trial was deemed insignificant.

"I believed that I would have consistent payment for travel cost and time offered when entering this trial. That is what motivated me to enter, but it was insignificant. That's what made me feel discouraged even if I quit… there was not much…" (Interview, trial-participant who withdrew consent, Woman).

"I did not encounter difficulties during the study; everything was going well except for the payment that had discouraged me (Interview, trial-participant who withdrew consent, Woman)".

7.4.1.4. Motivations for discontinuation

A considerable number of interviewees voiced concerns regarding the frequency of blood withdrawals during each scheduled visit in the trial, as well as safety issues associated with the study vaccine. These concerns ultimately led to their decision to discontinue their participation. As far as blood is concerned, few participants found it unclear why the collected blood samples were being sent to laboratories located overseas. The blood collection is perceived as not permissible according to certain religious beliefs of some interviewees like Jehovah witnesses, who highlighted that blood should not be distributed or tampered with.

"No, blood is something that is in someone's organism. My blood is my blood, yours is yours. I may not take my blood away to give you, if I have germs, it will happen to you. Don't you think it's bad? Even the Bible doesn't want it that way… We fear God, we don't fear anyone… (Interview, trial-participant who withdrew consent, Man)".

Some health issues following vaccination in the trial were perceived by some respondents as being caused by the investigational vaccine, as indicated in the following statements.

"Upon initiation of the trial, I began experiencing pain, which initially affected my entire body. However, over time, the pain has become localized to my feet. Furthermore, during the time I participated in the trial, I also developed oedema in my lower limbs". (Interview, trial-participant who withdrew consent, Woman)

"No, I declined participation solely due to observed changes in my body, but it was not due to any negative experiences" (Interview, trial-participant who withdrew consent, Woman).

During the interviews, it became evident that a portion of the respondents had no recollection whatsoever of the information presented to them during the informed consent process, which had taken place 2 years prior. Their memory seemed to have failed regarding the reasons behind their voluntary participation in the vaccine trial for example, as stated by one interviewee:

"I was sitting somewhere...Well, we were invited, and I didn't know the procedures of the study and I didn't know that they were going to draw blood. The first time I got there I didn't know, the second time it was the same, when the third time came, my conscience was worked on. I'm a Christian, Jehovah's Witness, for us the blood… They take the blood and put it on you, it's a sin, and it was the conscience that worked on me, and I made the decision to stop." (Interview, trial-participant who withdrew consent, Man).

7.4.1.5. Study vaccine acceptability and motivations

The firsthand experience of the 2014 Ebola virus disease epidemic and the prevailing concern regarding a potential outbreak in the foreseeable future played a fundamental role in the widespread acceptance of the study vaccine among interviewees and FGDs-participants.

"What pushed me to accept, was that the vaccine in question, if we accept it, it will help us for the next epidemic… if the virus catches you and if you were vaccinated, the disease severity will not be fatal (Interview, trial-participant who withdrew consent, Woman)".

Furthermore, the provision of ancillary care (i.e., the care provided to participants that goes beyond the research aims or intervention), along with the reimbursement of travel expenses and offered compensation for the time dedicated to each scheduled visit in the trial, emerged as predominant motivating factors for other respondents.

"I was interested in this study because of the money I was given for transportation [...] It helped me to buy things to eat" (FGD, Nurse, trial-participant, Woman).

"[...] What pushed me is that I am a community health worker… Well the community health worker works on a volunteer basis but they have a little motivation for the… It depends on the agencies or the NGO, but when EBOVAC came, I decided to join because I heard that there was also the motivation of transportation reimbursement" (Interview, trial-participant who withdrew consent, Man).

"When we joined the study, it was good. For instance, if you fell ill, we would support your medical care until you were discharged from the hospital. If you were only hospitalized, we would continue your care until you were discharged. And if you came solely for medical treatment, we would prescribe medication and provide it to you free of charge. It was truly for our benefit". (FGD, Midwife, trial-participant, Woman).

7.4.1.6. Vaccine safety

Many interviewees and FGD-participants reported safety concerns about study vaccine as they experienced events such as abortions, onset of diabetes, high blood pressure, back pains, gastritis, which suddenly occurred after the vaccination in the study and were perceived as related to the study vaccine.

Since the study vaccine was not recommended in pregnant women and those intending to become pregnant within 3 months, few participants questioned the relationship between the study vaccine and pregnancy. The administration of the vaccine was even perceived by a few respondents as facilitating to their ability to conceive and become pregnant, while for those who got pregnant after vaccination and experienced pregnancy loss, these miscarriages were perceived as being caused by the vaccine. Those who experienced pregnancy loss expressed this as a concern about the acceptability of this experimental vaccine when expanding the immunization efforts to the broader community beyond the HCPs and frontline workers population. Nevertheless, the reimbursement of expenses following a medical incident played a role in bolstering the level of confidence among the most trial participant respondents (31).

"Some people say: 'since when I have received the vaccine, I am too sick'. Some other people say: 'since when I have received the vaccine, I am like before'. Other people say: 'since when I have received the vaccine, I am fine'… there is a mother who told us: 'I never got pregnant; after receiving the vaccine, now I am pregnant', she is very happy, for her, the vaccine has done something for her" (Interview, Nurse, Trial staff, Woman).

"Some of the trial participants asked me to say this: "Doctor, since when I have been in this trial, since I got the vaccine, I noticed that when I conceive, 2 months or 3 months later, I lose conception, menstruation comes back, appears. I wonder if it's not related to the vaccine" (Interview, Nurse, Trial staff, Woman)".

7.4.1.7. Acceptability of the study site and target population

When contemplating the suitability of the study site, most interviewees and FGD-participants indicated that the trial should have been conducted in the vicinity of Lokolia and its

surrounding health areas, where the previous 2014 Ebola outbreak happened. Additionally, some respondents questioned the representativeness of the HCPs, as those residing in proximity to the 2014 outbreak area were not enlisted or invited to for screening procedures in the study.

"This study should normally be carried out in Lokolia. Where there was an epidemic, where there is the community, the people who lived it. As Lokolia is a health area in the Boende Health District, it's not bad, but the sample [...] You should have had to recruit a lot of people from Lokolia, but in the 700, if you observe, there are only... Even the head nurse [of Lokolia] was not involved" (FGD, Nurse head of health center, non-trial-participant, Man).

7.4.1.8. Communication

7.4.1.8.1. Interactions between trial participants, non-participants, and local authorities

Some local hospital authorities raised concerns about the employment of some staff of the HGR of Boende, along with the use of some hospital's premises. This potentially diverted resources among the HCP staff most involved in the EBL2007 vaccine trial. To reach an agreement, the investigators had to preserve transparent communication indicating that trial conduct would neither interfere with the medical care of patients nor affect the hired hospital staff's workload. Therefore, in this regard, each HCP employed in the trial had to ensure that he/she had a backup so as not to interrupt general care services. Additionally, the amount of invited HCP and frontline worker volunteers per visit was limited, per professional categories, in order not to leave some health care services empty. Finally, certain local health authorities expressed a desire to establish permanent training programs for HCPs working in the Boende Health District, via a partnership with international sponsors of the trial, to ensure a lasting impact and knowledge transfer in this remote area.

"Indeed, there were instances where interactions with the staff, particularly at the hospital, were challenging, primarily involving the hospital director. Initially, there were concerns as a portion of the department was relocated and merged with other units to accommodate the implementation of the EBOVAC project [...] (Interview, Local health authority, Man).

7.4.1.8.2. Rumors

Circulating rumors suggested that the vaccine trial was perceived as an established or prearranged agreement between the research team and the vaccine manufacturer. The alleged purpose of this agreement was either to intentionally cause delayed mortality in the vaccine recipients or to reduce their life expectancy. Consequently, the act of collecting blood samples during scheduled visits in the trial was perceived as a mechanism to seal this alleged deal. These rumors reflect the concerns and speculations surrounding the motivations and intentions behind the vaccine trial.

"We were told that you came to kill us: 'they take blood to go and kill people and said that since the whites are smart people, they will reduce our years by using our blood', people criticized in one way or another" (FDG, Community health worker, trial-participant, Woman).

"And one thing doctor, if I may add, you have selected only the people who are not working at the study. Why didn't you use those who are staff of the trial? That is also a question. As you are there, you are not vaccinated, even the woman, even the man. However, we are vaccinated… However, you, the driving forces the trial, you are not vaccinated. Why are you not vaccinated? That is, there is something hidden behind it, or we are sacrificed." (FGD, Medical doctor trial-participant, Man).

7.4.2. Global assessment of the trial

7.4.2.1. Positive Assessment

The implementation of the EBL2007 vaccine trial was perceived as highly positive in the following respects: 1) the ancillary care policy developed for the management of adverse

events not related to the study vaccine mentioned earlier (31), 2) the perception of this vaccination as a preparation of the province for a future Ebola epidemic, 3) the renovation of the hospital buildings, 4) provision of water, electricity and various other equipment to be returned to the general hospital at the end of the trial (offices, cold chain, generators, satellite antenna for internet, etc.) and, 5) capacity building sessions for HCPs and frontline workers prior to each scheduled visit in the trial (15).

"This study has changed our lives because when the disaster arrived in the Boende Health District, precisely in the health area of Lokolia, we saw dead bodies with our own eyes, we lost our brothers, our mothers, our fathers, our children, and when the study arrived, we were satisfied with the arrival of this study because we believed that after the study, it is the solution that will come, maybe soon, we will be more attacked by the disease." (FGD, Nurse head of health center, non-trial participant, Man).

"Yes, because first here we had a problem of water supply; with water that we have at the EBOVAC, we often see people asking, 'may I draw water?' 'Yes, you can'... Furthermore, we have power supplied 24h/24, that is very good. Our premises are arranged, it was not like that. They are really very good researchers." (FGD, Nurse, trial-participant, Man).

"[...] Well, since I left the university, I have not yet manipulated the automaton. Through this study, there was the chance to handle this device." (Interview, Nurse, staff in the trial, Woman).

Some respondents indirectly mentioned the impact of the vaccine trial process on their adoption of positive behaviors in terms of EVD prevention.

"For instance, we were used to eating bush meat that we had picked up in the forest, but through the study we understood what to eat and what not to eat [...] Since then, I have been giving the EBOVAC 4 out of 5" (FGD, Nurse trial-participant, Woman).

"This study has made me aware of how I can protect myself. The vaccine may also protect me, but I now know how to protect myself from Ebola." (FGD; First aid worker, trial-participant, Woman).

7.4.2.2. Negative assessment

Most interviewees and FGD-participants expressed their dissatisfaction with the amount of compensation provided to trial participants in terms of remuneration for their time and reimbursement of travel expenses. Several individuals referred to higher payments made in other Ebola response projects in the country.

"I worked in the control of the Ebola epidemic in the past and I was able to buy a land. Now I am in this study EBOVAC, I only got money for transportation and that's all" (FGD, nurse trial-participant, Woman).

"We noticed that our payment was insufficient. We, as staff. But as it was decided by the chiefs and there was no way to discuss, we started the work because we have to work first, and the payment comes afterwards." (Interview, trial staff, Man).

"We are involved in a study at risk where the drug could cure or potentially cause harm. We who are still alive completed the 3-year study. Did we receive compensation for the risk we took by participating in this vaccine trial? [...]." (FGD, Community health worker, trial-participant, Man).

Some few others specified that they were partially satisfied with the trial because they were expecting to receive gifts at the end as a sign of reward for their volunteering efforts or some increase in travel expenses refund.

"We are satisfied, but not completely, because we were expecting to receive gifts. We have been involved for 2 years, so we were expecting some gifts." (FGD, Nurse, trial-participant, Woman).

Let it be noted that due to the scarcity of qualified personnel with experience in conducting clinical trials in a remote area like Boende Health District, the recruitment process for local trial staff performed by the principal investigator in collaboration with the chief of the provincial health division of Tshuapa, primarily relied on local HCPs who had previously worked in the monkeypox vaccine clinical trial (32) conducted in the same area. However, some interviewees pointed out that the recruitment process for trial staff was flawed, as the true experts in the field of Ebola response were not hired. They perceived the recruitment as a mere illusion, with the researchers being influenced by local authorities in carrying out the selection process.

"I didn't like the organization because there are specialists in Boende regarding Ebola. I am there, but we were left out. On the contrary, you took the people who did not know Ebola! Normally you should have come to us, to look for – at the provincial Division of Health to look for who are experts, who have already lived, who have already handled Ebola, but you did not do that. You recruited according to you, but the experts, you abandoned us." (FGD, Medical doctor, trial-participant, Man).

7.4.3. Perspective on future Ebola virus/Ebola vaccine research

7.4.3.1. Study Site

Interviewees advocated that the trial site should become the site of future clinical trials and that establishing a well-equipped laboratory to analyze all study samples on-site could overcome beliefs/speculations that arose from shipping collected blood overseas. However, a primary concern expressed was that local authorities may lack the capacity to sustain the project equipment acquired, especially once they were no longer used in another research project.

"We would like to have a study site here and a laboratory to avoid that each time there is a study, we always have to go to a foreign laboratory, given that we are in Boende where there is an epidemic area." (FGD, Community health worker, trial-participant, Man).

"I believe that this site should not remain unused, otherwise it will be ruined. It will be destroyed. Did you find it like this? There are NGOs that come here and do not set up anything special, they make their tent and then they leave. I tell you; I am now 60 years old, and I have never seen anything like this. I worked with MSF, at the time we were paid 150$, but it was not like that; the whole community is astonished by what happened at EBOVAC, they want this work to continue, especially when the population passes by here, they are astonished by the place, wondering if it was even a hotel" (FGD, Health Facility Cleaner, trial-participant, Woman).

7.4.3.2. Perspective on the Ebola vaccine research

A significant number of interviewees suggested that the community should be involved in the study protocol design to consider the relevance of the populations to be involved in the trial. For example, they would like to see more projects determining the Ebola reservoir in the forest. Including all professional categories of the population would be a better plan for future EVD preparedness and enhance the Ebola vaccine confidence.

"When a disease arises, it does not affect the civil servants or those who work in the health sector, but the disease affects the whole community. I recommend that when you arrive, you take this initiative so that it is not limited to this place, but that it is extended to other places, and secondly, we will receive it in the same way that you have promised us". (Interview, trial-participant who withdrew the consent, Man).

7.5. Discussion

This study assessed the experiences of HCPs and frontline workers enrolled and not enrolled in the trial as well as the experiences of the EBL2007 vaccine trial staff and local health authority of province of Tshuapa.

Our findings unveiled positive experiences associated with the partaking in the trial. Commitment to improving the trial site and equipping the volunteers with necessary skills and knowledge through frequent workshops. For example, on EVD, particular emphasis was placed on the universal standards of hygiene and sanitation during workshops, as well as good practices for infection control and prevention. Information related to risky behaviors was also reiterated, such as the risk associated with bushmeat consumption or raw meat prepared from unknown animals and the handling of blood, bodily fluids. This might have contributed to a positive trial experience. Additionally, a comprehensive policy of ancillary care support was implemented during the trial, ensuring that participants received the necessary healthcare and assistance throughout the trial (31). This provision of medical support further enhanced the overall experience of interviewees and FGD-participants.

Furthermore, our findings report a broad acceptability of the study vaccine, the study site, and a tremendous willingness to support the development of Ebola immunization/research among the larger community as part of the DRC preparedness plan for likely future EVD epidemics in Ebola endemic areas. Other research exploring the experiences and perception among HCPs and frontline workers participating in Ebola vaccine trials reported similar findings (30, 33). This widespread acceptance of an experimental Ebola vaccine and positive experience of the trial may have a positive influence on the general population in the event a vaccination program is established as the large population generally rely on local HCPs to seek information regarding any new health innovation (30, 34, 35).

The objectives of the EBL2007 vaccine trial still appeared to be understood by most respondents 2 years after inclusion. This was made possible by the investigators' repetition of the study objective prior to each scheduled visit during organized workshops. However, 2 different perspectives emerged regarding how the interviewees and FGD participants perceived the achievement of the assigned objectives in the trial. From the trial staff' perspective, a sense of relief and pride was felt as the initial trial assumptions were met with high retention rate. From the trial-participants' perspective accepting the risk of receiving an experimental vaccine should be absolutely rewarded (even symbolically) by the research team. According to certain participants, a manifestation of trial participants' appreciation should be demonstrated by the research team through the provision of items such as bicycles,

t-shirts, or other tangible goods. Other interviewees/FGD-participants voiced that the disclosure of all the results from the blood samples collected during the trial would suffice, along with results of the experimental vaccine. To comply with this request, the research team plans to organize a conference in Boende to disseminate the main findings of the study once they are available and all the trial-participants will be invited. However, results were not yet available at the time of these interviews and FGDs.

Furthermore, a considerable number of participants perceived the time offered and travel cost refund provided during the trial visits as insufficient. Several authors have described the pursuit of an immediate financial gain beyond the benefit of improving one's health as inducements to volunteer in a clinical trial in developing countries, as well as throughout the world, regardless of social or educational status (36-38). However, some studies indicated that excessive compensation is also ethically questionable and can be considered as excessive inducement to participate in a trial (39-41). Yet, studies examining the experiences of HCPs volunteering in Ebola vaccine trials elsewhere reported different results. In fact, the primary motivation to receive an experimental vaccine ahead of the general population has been identified as a strong desire to contribute to the search for an Ebola vaccine (30, 42).

Most trial participants had concerns regarding blood collection. Blood was perceived to be either bought or stolen by the researchers through blood collection. The shipment of collected blood samples from the trial location to other countries reinforced this belief, leading to rumors among study participants' social circles, accusing them of exchanging their blood for the money provided for their travel costs and time offered. The refusal to continue in a clinical trial due to religious beliefs prohibiting the collection of human blood has also been reported in previous studies (43, 44). Likewise, many people discontinued in studies conducted elsewhere in Africa because they were afraid of getting their blood drawn (45, 46). Reluctance towards blood collection in clinical trials may be due to beliefs that blood collected or donated from someone who is not sick, it is often used for mystical rituals (47). Some African cultures often have strong spiritual beliefs and traditional healing practices. Some individuals may believe that blood holds a substantial spiritual or life force, therefore withdrawing blood could be seen as potentially harmful or weakening. Hence, the use of blood samples in trials conducted in Africa should always be given special attention, and investigators' efforts should focus on providing consistent and clear risk communication on study procedures during the informed consent process.

Trial staff did not receive the vaccine under investigation to prevent any interference with the evaluation of reactogenicity outcomes among vaccinated participants (interpretation bias). However, this was perceived by some interviewees and FGD participants as there might be adverse long-term outcomes from the study vaccine, like death or reduced life expectancy.

This study highlights the complexity of research in resource-limited settings where researchers and trial volunteers are seemingly living in 2 different worlds. This may suggest that the information provided by the researchers to the trial volunteers prior to signing a consent form would not significantly contribute to adequately enlightening them about all the procedures and planning following the defined research protocol on the researchers' side (48). Hence, the active commitment of the community during the preparatory phase of research, going beyond mere participation, has increasingly become a crucial and beneficial factor, specifically in clinical trials (28-31). An early partnership between researchers and the community could provide an opportunity for researchers and the community to work together, sharing knowledge and responsibility from the beginning until the end of the project, in order to create, revise, and generate research knowledge (49). In the context of the EBL2007 vaccine

trial, early involvement of the conceptual phase of community (the protocol), would have created opportunities to consider real-life scenarios. In doing so, problems related to the volunteers' expectations regarding the reimbursement of time offered and transport, the duration, and the number of reasonable blood samples could have been discussed before the trial started recruitment.

7.6. Limitations and strengths

Firstly, our study included some HCPs and frontline workers who had experienced an Ebola outbreak in the past. The perception of disease risk among interviewees/FGD-participants who had faced the disease could have affected our findings and thus these findings might not be applicable to those who have not faced a previous Ebola outbreak. Secondly, the involvement of one of the qualitative research team in the EBL2007 vaccine trial as sub-investigator during conversations may have affected our findings. Finally, conducting interviews in locations close to the study site could influence the views of some participants and potentially impact our results. Some of the selected conversation locations were situated within the Boende HGR premises, which served as the EBL2007 vaccine trial site. This choice of location might have created a sense of unease among participants regarding the expression of opinions or views that could potentially upset or offend the researchers. The fear of potential reprisals from their hierarchical superiors at the hospital could have contributed to this apprehension.

However, including different job professions and genders in the FGDs and individual interviews allowed us to gather a detailed understanding of the participants' experiences in the clinical trial and their overall acceptance of the vaccine being studied as well as those of non-participants, trial staff and members of local health authorities. This approach helped us capture a more comprehensive picture of the trial experience among the individuals we investigated.

7.7. Conclusion

Interviewees and FGD-participants voiced positive experiences gained from volunteering in the trial. These included Ebola prevention training, Ebola vaccination, ancillary care provided by researchers, and hospital renovations at the trial site. Furthermore, a widespread acceptance of the study vaccine and the trial site, as well as a strong willingness to support the development of Ebola vaccination and research in the wider community as part of an EVD preparedness plan was reported.

Areas of uncertainty and ambiguity pertaining to the understanding of compensation costs, as well as concerns regarding blood collection and its exportation to other countries, were raised. By involving the community of the study area in which the research will take place already in the study design, researchers can introduce acceptable trial procedures before the start of recruitment or anticipate any concerns. Next to that, it can enable a better community comprehension of the reasons why a certain population is chosen to participate in a trial, and the challenges they may face.

These elements combined can add to fair expectations of both trial participants and community members, which in turn can contribute to their trust in the research.

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7.10. Potential conflicts of 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 8 Longitudinal assessment of an Ebola vaccine trial understanding among healthcare providers in the Democratic Republic of the Congo

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8.1. Abstract

Background: The long-term retention of information disclosed during the informed consent in clinical trials lasting over a year cannot be guaranteed for all volunteers. This study aimed to assess the level of participants' retention and understanding of the trial information after two years of participation in a vaccine trial.

Methods: In total, 699 health care providers (HCPs) and frontline workers were enrolled in the EBL2007 vaccine trial conducted between February 2019 and September 2022 in the Health District of Boende, Democratic Republic of the Congo (DRC). Individual scores obtained from a questionnaire (test of understanding, TOU), specifically designed to assess the understanding of the consent at baseline, were collected before the clinical trial started and at one-year and two-year intervals.

Results: TOU scores were high in the beginning of the trial (median TOU=10/10), but significantly decreased in both the first and second years following (median TOU = 8/10 in year 1 and median TOU = 9/10 in year 2, p-value <0.0001). The decrease in scores was significantly higher among individuals with occupations requiring shorter education such as midwives (median TOU=7/10 in year 1 and 8/10 in year 2, p-value=0.025). Furthermore, older participants exhibited poorer retention of information compared to younger individuals (median TOU=8/10 vs 9/10, p-value=0.007).

Conclusion: We observed a significant decline in the informational knowledge of informed consent, specifically in terms of basic knowledge on the study vaccine and trial procedures. As participant safety and understanding is a paramount ethical concern for researchers, it is crucial for participants to fully comprehend the study's objectives and potential risks. Therefore, our findings suggest the need for clinical researchers to re-explain participants to optimize the protection of their rights and wellbeing during the research.

Keywords: Clinical trial participants, comprehension, assessment, test of understanding, consent form, informed consent

8.2. Background

Prior to being recruited in a clinical trial, potential volunteers are informed of the trial aims, methods, reasonably anticipated benefits, potential risks or discomfort and general study requirements. By providing key study aspects, the informed consent allows for potential participants to decide which risks, benefits, and procedures are acceptable to them in the study, making it possible to adequately decide to continue with the trial (50). A series of regulatory and ethical guidelines (e.g., The Council for International Organizations of Medical Sciences guidelines) highlight the need for potential trial participants to understand the information provided during the informed consent (50-53). Both informed consent and understanding are core ethical imperatives for entering a clinical trial. Unfortunately, it has been reported that some volunteers in clinical trials limit their consent to a document designed to protect the investigators in the event of an intervention-related complication, completely ignoring their autonomy and their right to be protected from harm (54, 55).

Evidence in the literature repeatedly indicates that research participants and patients undergoing medical procedures do not always correctly understand the research protocol involved (56-60). Besides, most of the research participants may be illiterate and unfamiliar with medical research, especially for trials conducted in low-income countries (61). Furthermore, in long-term studies involving extended participant follow-up periods (62-64), decreased ability to retain information studied over time is an additional barrier to a complete understanding of the risks and benefits of the research (65, 66). This issue is especially pertinent in vaccine trials, as participants need to maintain their understanding and consent form over extended periods, spanning months or years(60, 64).

Despite the fact that international research ethics guidelines emphasize the continuing nature of informed consent, a limited amount of investigators have published results related to assessments of participants' level of understanding of the clinical trial prior to enrolling and during the clinical trial (50, 59, 67-69). In addition, there are few practical guidelines on how to optimize the safety of volunteers to guarantee understanding of the consent form, which should be seen as a continuous dynamic process rather than an isolated event during the clinical study (70). Therefore, it is essential to incorporate periodic reassessments of consent understanding during the follow-up phase (60). This approach may help to enhance the optimization of participants' comprehension and retention of informed consent content-related information in vaccine trials. The challenges of ensuring proper understanding of study information have led some researchers to recommend that participants' understanding be assessed after the consent discussion (71-74).

In Boende, the Democratic Republic of the Congo (DRC), we conducted an Ebola vaccine trial (EBL2007) which included registered healthcare providers (HCPs) and frontline workers. This randomized, open-label, phase 2 study aimed to assess the safety and immunogenicity of a heterologous prophylactic vaccine regimen followed by a booster dose one or two years after the initial dose (14). One of the eligibility criteria for participation in the trial was the ability to successfully answer at least 9/10 questions of the Test of Understanding (TOU, Supplement 1). This TOU consisted of a true/false questionnaire to assess the understanding of trial consent among participants at baseline and when the trial was ongoing one and two years later. This sub-study collected the answers and scores of participants on the TOU and assessed

whether participants understanding of the consent/EBL2007 vaccine protocol waned over time, in a trial that was two years and half in duration.

8.3. Methodology

8.3.1. EBL2007 vaccine trial and TOU assessment

EBL2007 vaccine trial screening and enrolment procedures started in December 2019 and were completed in February 2020. Forthcoming trial participants were invited to attend an introductory workshop where the study protocol and activities were explained. They were also provided with a copy of the consent form to review at their leisure. If they expressed interest in participating, they were requested to return on the following day for screening and formal consent (Day 1).

Alongside the screening process on Day 1, a pretested and structured TOU was foreseen for potential participants following the informed consent discussion/dialogue and prior to signing the consent form.

The TOU helped the investigators to determine to which extent potential participants had basic knowledge of the study vaccines, trial procedures, purpose of the trial, acceptable risks, and volunteerism in the trial. The TOU questionnaire was translated from English to French, and afterwards, it was translated from French into Lingala by an experienced translator. To ensure translation accuracy, a back-translation process was applied at each stage. This involved translating the text from Lingala to French, and subsequently, from French back to English, each step performed by a different translator than the original.

Participants eligible for enrolment in the trial had to be able to correctly answer at least nine of the ten test questions (≥9/10) across three attempts in the preferred language. If the participant failed the first attempt, he/she was retested. Two repetitions were allowed, and the study nurses provided additional information regarding the protocol before and between each attempt. If participants failed the third attempt, they were not allowed to join the EBL2007 vaccine trial.

To measure their understanding over time, starting from late November/early December 2020, the EBL2007 vaccine trial protocol was amended so that the TOU could be repeated (one attempt) among enrolled participants approximately one- and two-years following inclusion, without impacting their continuation in the trial. When a participant failed, he/she was reminded of the key information related to the informed consent such as knowledges on the study vaccine, voluntary participation, benefits, risks and trial procedures. Participants scoring below 9/10 in first and second-year assessments had only one TOU attempt.

8.3.2. Data collection

The data for this study consisted of EBL2007 vaccine trial participants' demographics and TOU scores obtained at baseline and then approximately 1 and 2 years later]. Scores were extracted from paper TOU at the end of enrollment (March-April 2020) and at the end of the Year 1 follow up visit (March-April 2021) and the Year 2 (March-April 2022) follow-up visits. Two study staff members entered (double data entry) these data into a purpose-built Redcap database using tablets. The original paper questionnaires contained non-identifying information of the participants in the EBL2007 clinical trial. The extracted data included the following variables: participant ID; day of the visit; month of the visit; year of the questionnaire administration; signature of the person who administered the questionnaire; answers given by the participant to each of the 10 true/false (supplement 1).

8.3.3. Quality assurance

An independent person performed quality assurance by checking the consistency of the data entered in the two Redcap databases. Any inconsistencies found were corrected by comparing them to the original paper TOU. Additional data, including demographic variables of the study participants, were obtained from the EBL2007 study database, and linked to the survey data.

8.3.4. Data analyses

To check the change in TOU (as proxy for information retention) over time and to see if it differed between participants, we performed a beta regression analysis. This technique is recommended when the dependent variable (TOU) represents proportional data derived from counts of "successes" (correctly answered questions) and "failures" (wrongly answered questions)(75). Different models were developed with the TOU score as dependent variable and study year, age, sex, and occupation as explanatory variables. To test if the TOU decreased over time in particular participant categories, we considered a combination of the study year with the other explanatory variables (year*age and year*occupation). Because the sample size for some occupations was too low (<10 participants), we grouped caregivers, laboratory technicians, pharmacy assistant, facility maintenance worker, under the category 'others'. We also divided the ten questions of the TOU (supplement 2) according to five different categories: 1) 'Basic knowledge on the study vaccine' grouping questions 1 and, 3 3) 'Study procedures' for question 2, 3) 'Purpose of the trail' for question 4, 4) question 5 and 7 as 'Voluntarily participation' and 5) questions 6,8,9, and 10 were grouped as 'Safety risks (Supplement 2). To test for difference between years for each question category, we performed a generalized linear model with binomial distribution. Different models were developed for each question with "questions correctly answered" as binary response variable and time (year 0-2) as response variable. Similarly, we investigated if the "occupation" and age could also significantly affect the correctness to the answer. Analyses were performed using -the R-packages" betareg" and "emmeans", "lmer" and boxplots were created with "ggplot2".

8.3.5. Ethical Review

The National Ethics Committee of the DRC Ministry of Health (approval reference n ° 211/CNES/BN/PMMF/2020) approved the current sub-study nested in the amended EBL2007 study protocol. The EBL2007 vaccine trial was registered at clinicaltrials.gov (NCT04186000).

8.4. Results

8.4.1. Baseline Characteristics of Participants of EBL2007 vaccine trial

A total of 720 HCPs and frontline workers participants were screened for inclusion in the EBL2007 vaccine trial, of which 699 (97.08%) were eligible at baseline (Day 1). Out of the 21 individuals who did not successfully pass the screening process, four of them had not attained the stipulated score (≥9/10) on the comprehension test after three attempts, and the other trial inclusion criteria were not met by the remaining 17. Approximately one year following the baseline, 671 participants returned and underwent re-administration of the TOU, whereas after two years, 651 participants returned. The demographic characteristics of the study population are summarized in Appendix 1. The scores of the TOU ≥9/10 at baseline were available for 698 (99.9%) participants. The study population was predominantly male (76.5%). The EBL2007 vaccine trial had a retention rate of 93.1% from Day 1 to Day 730.

8.4.2. TOU scores at baseline, year 1 and year 2

TOU scores dropped from a minimum score of 9/10 and a median of 10/10 to a median of 8/10 one year after inclusion (p-value <0.0001) and a median of 9/10 in year 2 (p-value<0.0001) (Figure 1).

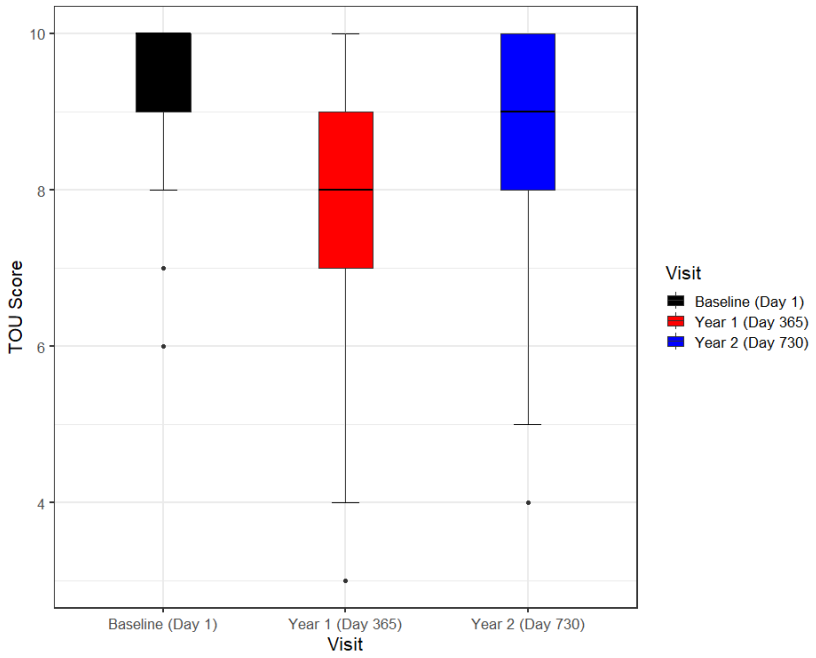

Figure 1: Ability of healthcare providers and frontline workers to provide correct answers to the TOU over time.

The decrease in TOU score over time differed between occupations (df=12, p-value<0.0001). Midwifes scored lower on the test at subsequent years compared to the other occupations. The drop was significant at year 1 (with a median TOU score =7/10, p-value=0.025), and not significant at year 2 (with a median TOU score=8/10; p-value=0.062). Doctors showed the lowest decrease in TOU score over time (TOU score = 8.5/10 at year 2 and 10/10 at baseline, p-value=0.34 (Figure 2).

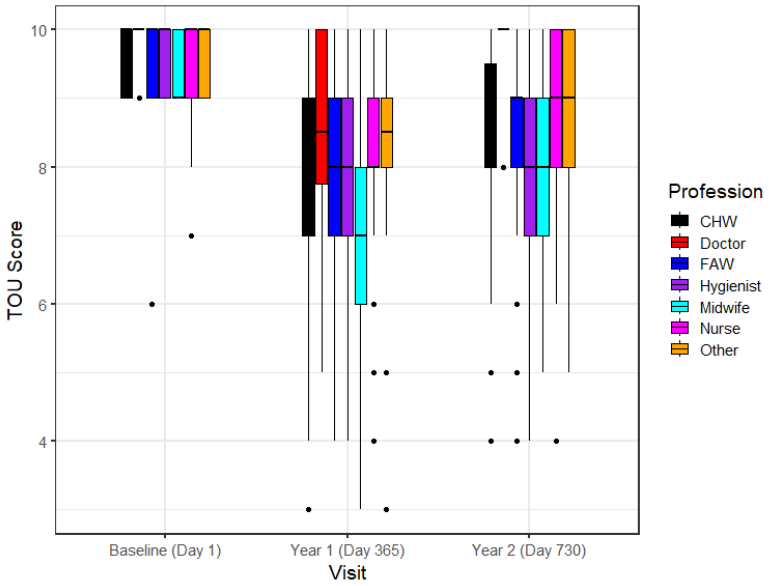

Figure 2: Ability of healthcare providers and frontline workers to provide correct answers to the TOU over time and profession. At baseline and in year two, the minimum and maximum scores obtained by all the Doctors are equal.

The analysis across different age groups revealed similar performance in all categories, with a notable exception observed in year two for the oldest age category (61-75 years old). In this group, there was a decrease in scores compared to the younger categories, particularly the 18-30 years old group (median TOU=8/10 for 61-75 years old vs 9/10 for 18-30 years old, df=6, p-value =0.007). This indicates that while the performance was generally consistent across most age groups, the 61-75 year old group demonstrated a distinct deviation at year 2 (Figure 3).

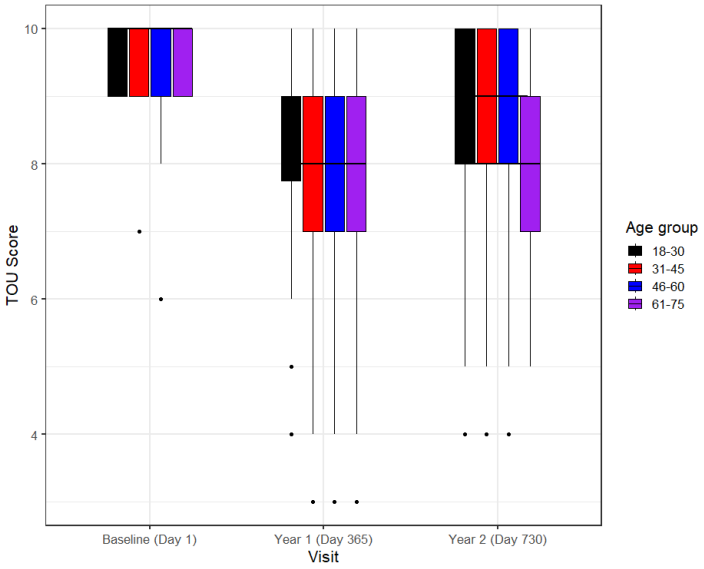

Figure 3 Ability of healthcare providers and frontline workers to provide correct answers to the TOU over time and per age category.

Table 1 and figure 4 describe the observed differences among participants according to the TOU question categories. The strongest differences were observed for “Basic knowledge on the study vaccine” and “study procedure” questions (pvalue<0.0001) for which a clear decrease in proportion of correctly answered questions was observed one year after the start of the study (Table 1). Although a decrease in proportion of correctly answered questions was also observed for the other questions groups such as purpose of the trial (pvalue=0.05), safety risks (pvalue=0.02) and voluntarily participation (pvalue=0.35), they were answered more correctly overall (Figure 4).

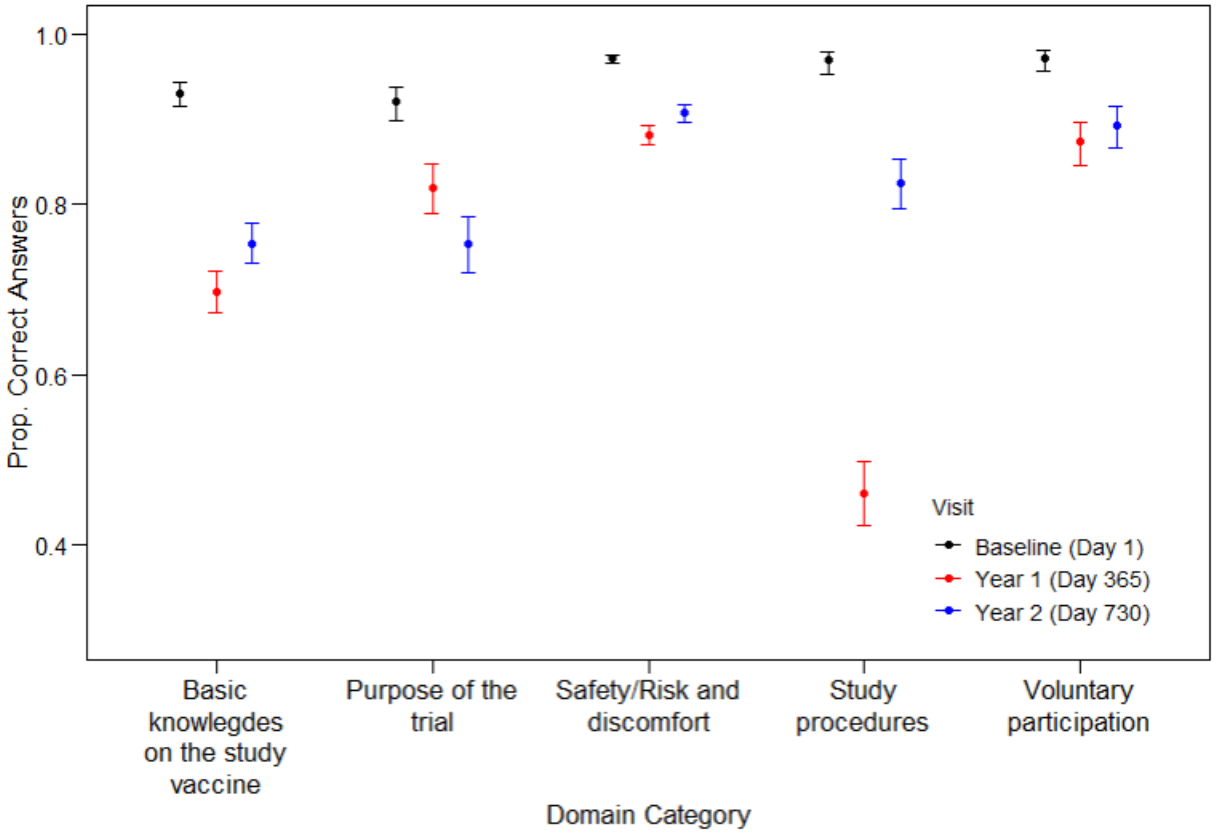

Figure 4 Effect of time on the Proportion of questions that were answered correctly during the EBL2007 vaccine trial separated for different categories of questions (Basic knowledge on the study vaccine, study procedures, Purpose of the trail, Safety risks and Voluntarily participation).

Different colors represent different years. Error bars represent 95% binomial confidence intervals

Furthermore, we observed that the questions related to" Basic knowledge on the study vaccine" were answered more incorrectly by certain occupations (p-value<0.0001). Indeed, 50% of participants among occupations like community health care workers, doctors, midwifes and nurses answered these questions incorrectly. Although doctors and community health care workers had more correct answers at year 1, nurses and midwifes made more mistakes in year 2 (Figure 5). In contrast, the questions related to "Study Procedures" were answered equally incorrect by all occupations in year 1 and equally better in year 2 (p-value = 0.3312) (Figure 5). The other question categories were not answered significantly different by the different occupations over the years (Table 1).

Table 1 Effect of time (years) and occupations on % of correctly answered questions since the trial started for different categories of questions

	Questions	df	Chi²	p-value
Effect of Time (years)	Q1,3: Basic knowledges on the study vaccine	2	27.481	**<0.0001**
	Q2: Study procedures	2	77.922	**<0.0001**
	Q4: Purpose of the trial	2	6.136	0.05
	Q5,6,8,9,10: Safety risks	2	8.1034	0.02
	Q7: Voluntarily participation	2	2.1003	0.35
Effect of occupation	Q1,3: Basic knowledge on the study vaccine	6	29.578	**<0.0001**
	Q2: Study procedures	6	6.8894	0.33
	Q4: Purpose of the trial	6	4.7456	0.58
	Q5,6,8,9,10: Safety risks	6	5.2794	0.51
	Q7: Voluntarily participation	6	12.723	0.05

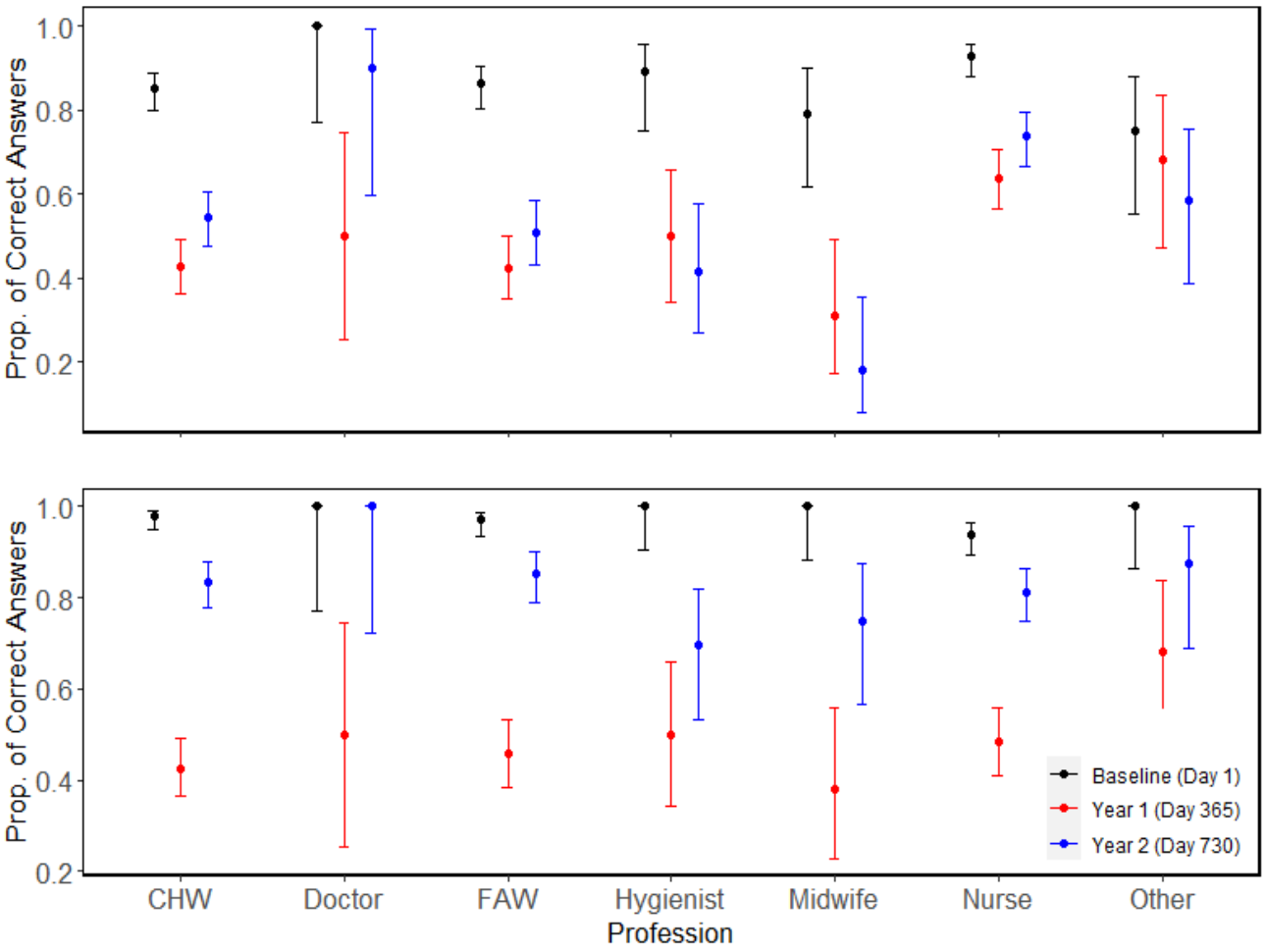

Figure 5 Effect of occupation on the proportion of questions that were answered correctly during the vaccine trial separated for different years. *Above* correctly answered questions related to "Basic knowledge on the study vaccine". *Below* correctly answered questions related to "study procedures". Different colors represent different years. Error bars represent 95% binomial confidence intervals. CHW (Community health care workers) and FAW (First aid worker).

8.5. Discussion

This study assessed comprehension of a vaccine trial among 699 HCP and frontline worker participants over two years.

A substantial reduction in the overall TOU score was observed during planned visits in the first and second years after inclusion, suggesting that consent should be repeated in longitudinal studies that span over time N(64, 76). The TOU score at screening might merely be a literal reminder prior to signing the consent form, becoming vague afterward (60). Recall of informed consent declined, especially regarding basic vaccine knowledge and trial procedures. The inability to remember the topic in regards with basic knowledges about the investigational product in a trial, has been reported in previous studies (60, 77-80). These studies indicate a complete lack of retention of basic details about the investigational product or study vaccine among participants enrolled in a long-term clinical trial ranging from one to five years. Similarly, challenges in recalling study procedures among trial participants were reported in previous studies conducted in both high and low-income countries (60, 64, 77). In these studies, some respondents were unable to correctly recall or explain certain procedures/concepts used during the consent process, such as randomization, placebo, blinding (79-82).

The decline in retention of the consent form content was less pronounced two year after the commencement of the trial. Noteworthy that individual sessions to clarify the content of the informed consent followed each trial participant, mostly in the event of weak TOU performance. Furthermore, the recapitulation of the same TOU questionnaire in the trial (Day 1, Year 1, and Year 2) likely contributed to a slight improvement in the overall score in Year 2 compared to Year 1. Similar findings were reported by Chaisson et al., who conducted the same comprehension questionnaire at enrollment and during follow-up (59). The results indicated that participants exhibited an improved understanding of the key study information (83).

Several reasons may have helped participants achieve the baseline TOU score, including the use of printed trial information sheets, concise consent and its translation into the local language, workshops explaining the protocol with multimedia and video during the screening process.

The decline in the TOU score over time differs across occupations. Similar findings were reported in other studies conducted in Africa showing that more years of education was associated with a deeper level of understanding in medical research (71, 84, 85). Some professional categories, such as doctors and midwives, were underrepresented in the trial. This is likely to be related to the scarcity of specific occupations among HCPs in remote health districts of the DRC , such as Boende, where most of HCPs likely head for the cities, which offer better infrastructure and financial incentives(86).

Likewise, the understanding level of the informed consent decreased in older participants. A similar situation was apparent in the age category in studies conducted elsewhere where the decrease in understanding was more pronounced over time for older than for younger people (80, 85, 87). Compared to younger people, older people may feel less comfortable and confident asking questions or expressing concerns during the informed consent process. The motivation to participate may be different from that of younger people as well. For example, older people may be more motivated by the potential benefits of research participation for their health needs than by the aspects of informed consent.

Importantly, it was not possible to check the effect of sex and the TOU score over the years, as it was confounded with occupation (supplement 3). Moreover, our analysis revealed that

certain professions within our participant pool were sex-specific, which limited the scope for investigating gender differences in these categories. However, in mixed-sex professions, statistical tests such as the Welch Two Sample t-test showed no significant gender-based differences in TOU scores, except in specific cases like First-Aid Workers (FAW: t = -3.202, df = 180.92, p-value = 0.001613) where a significant sex difference was observed, sex did not generally have a statistically significant impact on TOU scores across most professions in our study. The distribution of males and females in the FAW is 134 males and 43 females. The negative t-value of -3.202 indicates that females have a lower mean score compared to males.
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The use of true/false questions was a limitation of this survey. The used TOU may have led to an overestimation of the participant comprehension at baseline or in how participants incorrectly responded at one and two years after the trial initiation. The use open-ended or multiple-choice questions might better reflect the actual level of understanding of the participants. Furthermore, as the order of questions in the TOU questionnaire used in Year 2 did not change from the baseline, the slight improvement observed in Year 2 compared to Year 1 could be the result of recalling correct answers as clarified in Year 1, rather than an indication of improved understanding of the trial due to further explanations about the study provided in year 1.

Another limitation to our findings is that our population group of HCP and frontline workers, with a typically higher educational level than the general population, may have maintained a better level of the study comprehension than with other population groups.

Nevertheless, the greatest strength of this survey resides within the extent to which it has brought together data on the understanding of consent in a longitudinal manner. The results generated are further evidence of the need to consider consent as an ongoing and not an isolated process in long-term studies like vaccine trials.

To enhance participants' understanding, engagement, and autonomy in long-term clinical trials like vaccine trials, we propose following recommendations: 1. Regular and periodic rehearsal of the informed consent throughout the duration of the vaccine trial; 2. Periodic recapitulation of a TOU with open-ended questions, allowing participants to explain in their own words what they have retained from their consent to the trial, or multiple choice questions; 3. The use of tailored wording for the TOU that considers participants' age, level of education, and health literacy proficiency; 4. A periodic reconsent of participants failing the TOU; and 5. Paying specific attention to more vulnerable participants (low education and older age).

8.6. Conclusion

We conclude that participants of clinical trial can forget crucial information on the study over time. Therefore, we recommend assessing the understanding of consent as a prerequisite to each study visit, as this may safeguard the autonomy, respect and beneficence of participants in volunteering studies.

8.7. Acknowledgments

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8.9. Potential conflicts of 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. All authors attest they meet the ICMJE criteria for authorship.

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8.11. Appendix 1 . Baseline characteristics of participants in the EBL2007 vaccine trial

		Mean (SD)	Min	Max
Age (year)	N=698	45.0 (12.0)	19	75
18 – 30, n (%)	102(14.6%)			
31 - 45, n (%)	231 (33.2%)			
46 - 60, n (%)	297(42.5%)			
61-75, n (%)	68(9.7%)			
Sex, n (%)				
Female	164(23.5%)			
Male	534(76.5%)			
Occupation, n (%)				
Community Health Worker	236(33.8%)			
Nurse	181(25.9%)			
First Aid Worker	177(25.4%)			
Hygienist	37(5.3%)			
Midwife	30(4.3%)			
Medical Doctor	13(1.9%)			
Health Facility Cleaner	10(1.4%)			
Care Giver	7(1%)			
Other	3(0.4%)			
Laboratory Technician	2(0.3%)			
Pharmacist Assistant	2(0.3%)			

8.12. Supplement 1

Table 1 Test of Understanding (English copy)

Please read each question and answer whether the statement is True or False.

True ☐	False ☐	1. The vaccines you will receive in this study will definitely protect against Ebola.
True ☐	False ☐	2. You will need to give 5 blood samples during this study
True ☐	False ☐	3. The vaccines in this study can give Ebola Virus disease.
True ☐	False ☐	4. One purpose of this study is to determine if these vaccines are safe to administer to humans.
True ☐	False ☐	5. Participants in this study will need to avoid engaging in activities that may expose them to Ebola virus.
True ☐	False ☐	6. You may take other experimental (test) products while you are taking part in this study.
True ☐	False ☐	7. You may withdraw from the study at any time if you choose.
True ☐	False ☐	8. Women participating in this study are permitted to become pregnant during the study.
True ☐	False ☐	9. A participant in this study may experience side effects after vaccination.
True ☐	False ☐	10. Some participants in this study may develop a positive Ebola test result, despite the fact that they do not have Ebola disease.

Table 2 Test of Understanding (Lingala copy)

Mituna mpo na koyeba soki okangi ntina ya boyekoli oyo
Tángá motuna mokomoko mpe yanolá: Solo to Lokuta

Solo ☐	Lokuta ☐	1. Mitindo ya mangwele oyo okozwa na boyekoli oyo ekobatela yo mpenza mpo ozwa Ebola te.
Solo ☐	Lokuta ☐	2. Osengeli kopesa mwa ndambo ya makila na yo mbala 5 na boumeli ya boyekoli oyo.
Solo ☐	Lokuta ☐	3. Mitindo ya mangwele ya boyekoli oyo ekoki kopesa maladi oyo euti na virisi ya Ebola.
Solo ☐	Lokuta ☐	4. Mokano moko ya boyekoli oyo ezali ya koyeba soki mitindo ya mangwele yango ezali likama mpo na bato.
Solo ☐	Lokuta ☐	5. Baoyo bandimi kosangana na boyekoli oyo basengeli koboya misala nyonso oyo ekoki kotya bango na likama ya kozwa virisi ya Ebola.
Solo ☐	Lokuta ☐	6. Bakoki kopesa yo bankisi mosusu, oyo ezali komekama, ntango ozali kosangana na boyekoli oyo.
Solo ☐	Lokuta ☐	7. Okoki komilongola na boyekoli oyo soki olingi mpe ntango olingi.
Solo ☐	Lokuta ☐	8. Basi oyo bandimi kosangana na boyekoli oyo bazwi ndingisa ya kozwa zemi na boumeli ya boyekoli yango.
Solo ☐	Lokuta ☐	9. Moto oyo andimi kosangana na boyekoli oyo akoki komiyoka mabe nsima ya kozwa mangwele.
Solo ☐	Lokuta ☐	10. Mpo na bato mosusu oyo bandimi kosangana na boyekoli oyo, egzame ya Ebola ekoki kolakisa ete bazali na virisi yango, nzokande bazali kobɛla maladi ya Ebola te.

Table 3 Test of Understanding (French copy)

Test de compréhension de l'étude

Veuillez lire chaque question et indiquer si la déclaration est Vraie ou Fausse

Vrai ☐	Faux ☐	1. Les vaccins que vous recevrez dans cette étude protégeront certainement contre Ebola.
Vrai ☐	Faux ☐	2. Vous devrez donner 5 échantillons de sang pendant cette étude.
Vrai ☐	Faux ☐	3. Les vaccins de l'étude peuvent donner la Maladie à Virus Ebola.
Vrai ☐	Faux ☐	4. Un des objectifs de cette étude est de savoir si ces vaccins sont danger pour les humains.
Vrai ☐	Faux ☐	5. Les participants à cette étude devront éviter de se livrer à des activités pouvant les exposer au virus Ebola.
Vrai ☐	Faux ☐	6. Vous pouvez prendre d'autres produits expérimentaux (tests) pendant que vous participez à cette étude.
Vrai ☐	Faux ☐	7. . Vous pouvez vous retirer de l'étude à tout moment si vous choisissez
Vrai ☐	Faux ☐	8. Les femmes participant à cette étude sont autorisées à tomber enceintes au cours de l'étude
Vrai ☐	Faux ☐	9. Un participant à cette étude peut ressentir des effets indésirables après la vaccination.
Vrai ☐	Faux ☐	10. Certains participants à cette étude peuvent développer un résultat positif au test Ebola, même s'ils ne sont pas atteints de la Maladie à Virus Ebola.

8.13. Supplement 2

Table 4 Question of the TOU and Informed consent components categories

Questions of TOU	Informed consent components categories
1. The vaccines you will receive in this study will protect against Ebola	Basic knowledges on the study vaccine
2. You will need to give 5 blood samples during this study	Procedures of the trial
3. The vaccines in this study can give Ebola Virus disease.	Basic knowledges on the study vaccine
4. One purpose of this study is to determine if these vaccines are safe to administer to humans.	Purpose of the trial
5. Participants in this study will need to avoid engaging in activities that may expose them to Ebola virus.	Safety/Risk and discomfort
6. You may take other experimental (test) products while you are taking part in this study	Safety/Risk and discomfort
7. You may withdraw from the study at any time if you choose	Voluntary participation
8. Women participating in this study are permitted to become pregnant during the study	Safety/Risk and discomfort
9. A participant in this study may experience side effects after vaccination	Safety/Risk and discomfort
10. Some participants in this study may develop a positive Ebola test result, even though they do not have Ebola disease.	Safety/Risk and discomfort

8.14.Supplement 3

Table 5 Ability of healthcare providers and frontline workers to provide correct answers to TOU questions over time, relative to their age, occupation and sex

	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	2.39485	0.06580	36.396	**<0.0001**
Year 1	-1.13894	0.04823	-23.616	**<0.0001**
Year 2	-0.71115	0.04939	-14.399	**<0.0001**
Age	-0.005267	0.001664	-3166	**0.01**
Sex	0.07021	0.05036	1.394	0.16
Doctor	0.66963	0.16089	4.162	**<0.0001**
Hygienist	-0.11819	0.08817	-1.340	0.18
Midwife	-0.35084	0.10256	-3.421	**0.0006**
Nurse	0.28013	0.05117	5.474	**<0.0001**
Other	0.22257	0.11010	2.022	**0.04**
First aid workers	-0.02037	0.05041	-0.404	0.68

Chapter 9. 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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9.1.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.

9.2. 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 countries (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.

9.3. 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), informed consent forms (ICFs), etc.) between	A storage method/system was developed using the study visit and subject identification number, so that information remained coded. A travel plan was developed together with the sponsor in which it was determined which	Develop an archiving system for study documents before the start-up of the study. 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.

Boende (site location) and Kinshasa (headquarters PI).	documents were needed at the site before each active period started.	
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.

	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.

Impossible to fully rely on the hospital pharmacy (or other external pharmacies)	An adapted version of the World Health Organization (WHO) *Interagency Emergency kit 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.	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.

	Additionally, participants were invited for a workshops 24 hours before each study visit. During this workshop, the PI took the 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	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.

equipment, operating funds, etc.		
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. 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 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.	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. 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.	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 coming across unexpected situations. Foresee a buffer in study budgets for unforeseen additional expenses (e.g., Covid testing, longer research stays due to quarantine).

• Additional cost of testing to travel to site (UNIKIN)/DRC and site (sponsor).		
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 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;	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.

no sample results could be shared until approval was obtained.		
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.
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.

9.3.1. 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).

9.3.2. 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 (88). 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.

9.3.3. 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 24 hours) 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; Figure 1).

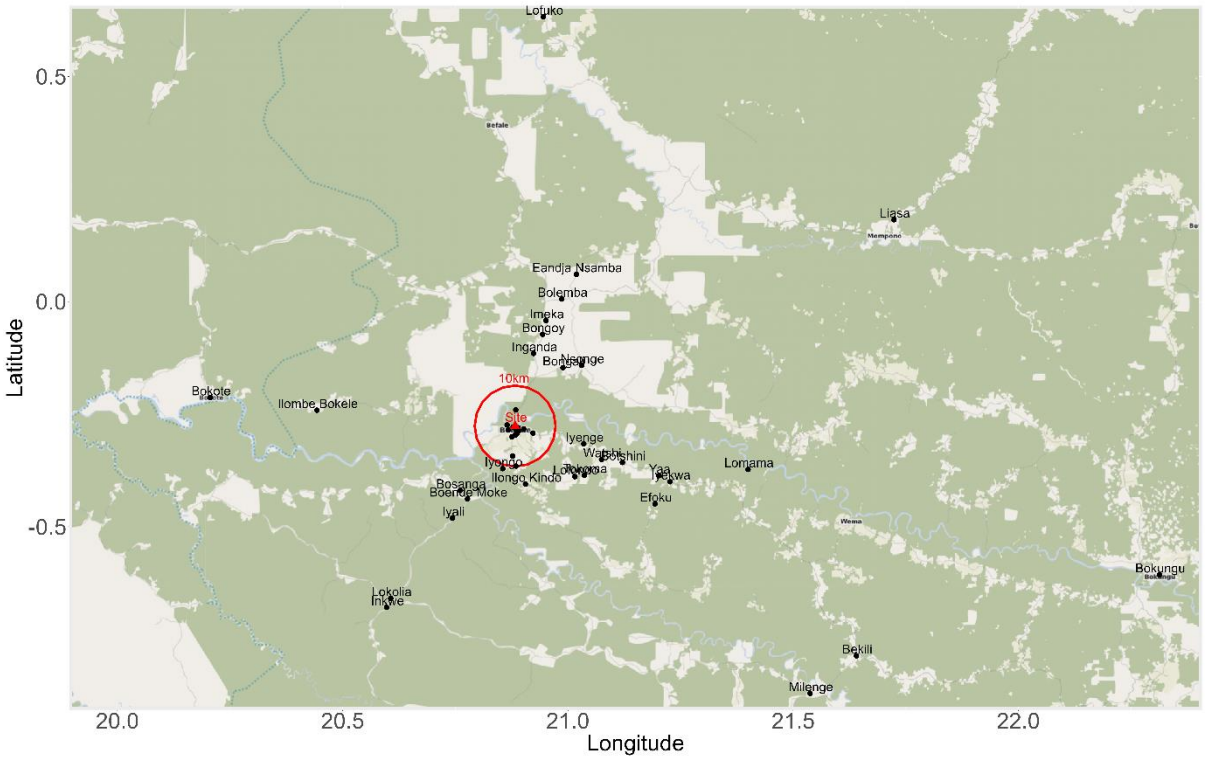

Figure 1 Mobile phone network range around Boende

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).

9.3.4. 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 (Figure 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.

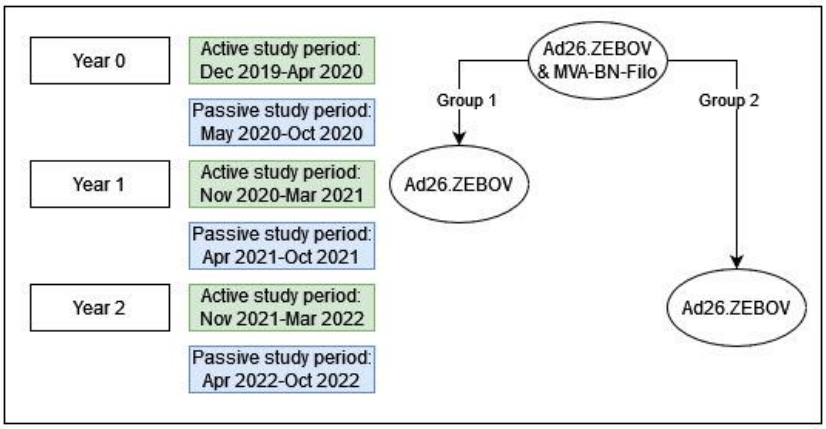

Figure 2 Simplistic overview of the EBL2007 Ebola vaccine trial

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 (Figure 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 (Figure 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.

9.3.5. Participant's recruitment and follow-up visits

During the initial enrollment visits, participants spent an average of 2 hours at the trial site. This time was eventually reduced to less than 45 minutes 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 (Figure 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 hours (approximately >25km) 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 (Figure 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.

9.3.6. 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 hours 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 hours 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.

9.3.7. 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 (Figure 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 (Figure 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, Figure 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.

9.3.8. (Inter)national collaborations

With many international teams involved directly or indirectly in the EBL2007 trial activities (Figure 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 reassessing, 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.

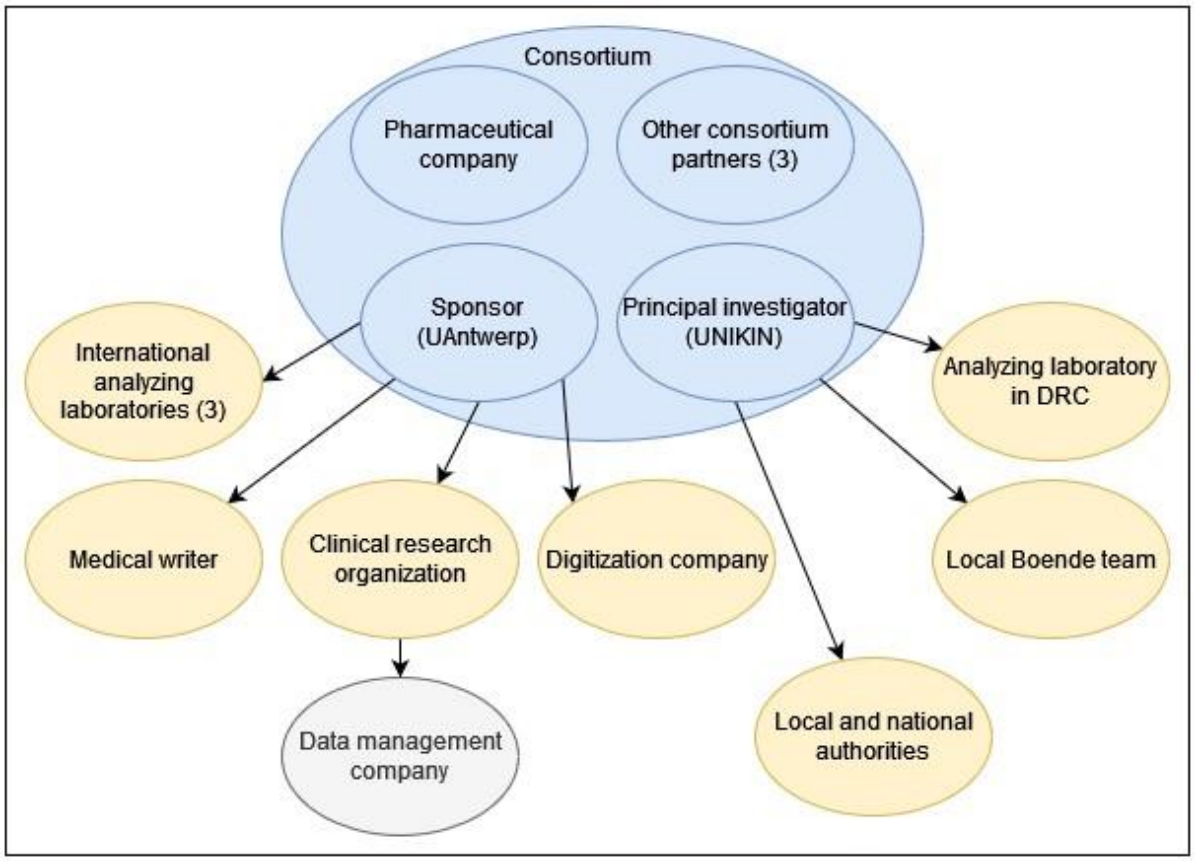

Figure 3. International collaboration diagram of the EBL2007 trial based on contractual links

9.3.9. 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.

9.4.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.

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9.6. Conflicts of interest

Authors have no conflicts to declare.

9.7. Acknowledgements

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All authors attest they meet the ICMJE criteria for authorship.

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Chapter 10. General Discussion

10.1. Accurate Identification and Maintenance of sources documents in the remote health setting of Boende, DRC

Even though a preventive vaccination strategy against EVD in endemic regions like Boende Health District would primarily target at-risk populations, particularly HCPs and frontline workers, the lack of reliable demographic data at population level needs to be addressed. Identifying HCPs and frontline workers participating in the EBL2007 vaccine trial was one of the major challenges. The trial's goal was to collect related safety and immunogenicity data for a vaccine given at 56 days interval, with booster shots administered at one or two years apart. To identify HCPs and frontliners participants in the EBL2007 vaccine trial, we utilized iris scanning technology as a biometric identification tool.

Although iris biometric has proven to be effective (1-3), its widespread adoption in vaccine trial has been limited. Yet, it is generally observed that humans possess an innate capability to identify individuals by looking at their eyes (4). In this doctoral thesis, we reported findings of the feasibility, cultural acceptability, and long-term perception studies of this tool (chapter 2).

Before and during the follow-up visits of the trial, we discovered that biometric identification using iris scanning achieved a 93.1% accuracy rate in correctly identifying participants, with an impressive 99% acceptance rate. Similar acceptance rates for iris scanning biometrics have been reported in diverse populations in low- and middle-income environments, including patients at HIV treatment clinics in Kenya within routine health information systems (99%)(8), and among participants in clinical research in Uganda, Tanzania, and Kenya (98%). Likewise, comparable accuracy was observed in the pediatric population enrolled in a genetic study in Brazil, where a 94% success rate was noted in avoiding identification errors even among identical twins (9).

Utilizing iris scanning in the EBL2007 vaccine trial significantly eased the investigators' workload by ensuring that the same individuals vaccinated initially were followed throughout the trial period (2.5 years). Additionally, the iris scan tool helped avoid potential errors in documenting demographic data by providing a unique identifier, supplemented by an identity photo and iris scan stamps for each participant, thus significantly reducing the likelihood of intentional or unintentional fraud from participants or trial staff. Thus, iris scan identification in vaccine research may ensure that participants use their real identities during enrollment and follow-up visits. Furthermore, this method eliminates the use of someone else's identity documents or false personal data, as identity verification is tied to unique biometric characteristics. Additionally, iris recognition is suitable for healthcare environments because it eliminates the need for physical contact. This non-contact approach ensures hygiene,

safeguarding users against germ transmission and illnesses that might arise from contact-based biometric methods like the traditional and widespread fingerprint.

However, nearing the EBL2007 trial, some participants expressed concerns about the long-term safety (nearly two and a half years) of iris scan use on their vision. Despite initial thorough explanations from the trial staff about the iris scanner's safety, highlighting the minimal infrared radiation levels insufficient to damage vision, the interviewees appeared likely to have forgotten the clear explanations regarding the safety of the iris scan tool. These concerns raised near the trial's end may likely stem from participants' difficulty in accurately recalling details over two years later as revealed in another study conducted (Chapter 7) to assess their ability to recall information explained at the beginning of the vaccine trial. This aligns with several studies indicating that participants often struggle to remember consent form information and clinical trial procedures (5-7). Additionally, the onset of vision difficulties commonly starting at age 40, with an average participants age of 45 in the EBL2007 vaccine trial, could have contributed to doubts about long-term safety of the iris scanning technology favouring the perception of causality rather than coincidence.

Implementation of iris scan in clinical trial may simplify the process for both participants and investigators by eliminating the need to recall or secure the participant's unique identifier, as this is necessary in traditional authentication methods (4). This implies that, a participant can attend his/her follow-up visit without needing to bring an ID card, vaccination card, or trial participation card. He/she can be accurately identified through an iris scan, which also helps retrieve his/her unique identifier in the trial.

Concerns are increasingly being raised regarding iris identification, with apprehensions that the infrared rays from iris scanners might impact vision (4, 8). While most infrared light-emitting diode (LED) sources are unlikely to cause direct ocular damage due to their incoherent light emission, the potential use of LED arrays that could pose a threat is a growing area of concern (9). In the realm of iris identification, infrared light is favored over visible light for its superior resolution. This is attributed to its lower absorption by melanin, the principal pigment of the iris, resulting in higher-contrast images of iris structures. Human eyes lack protective reactions against infrared rays. Since infrared light is imperceptible, we cannot determine when we are exposed to it, and unlike with bright light, the eyes do not respond by constricting the pupil to this type of radiation (10). However, it is well known that infrared radiation, though it increases the overall temperature of the aqueous eye, primarily affects the cornea and the aqueous humor, is highly inefficient in causing eye damage (11).

Furthermore, the process of capturing an image for iris recognition is non-invasive and user-friendly. The subject can stand up to 25 centimetres away from the scanner, and wearing glasses or contact lenses does not affect the accuracy. The process typically may generally take between 2 to 4 seconds, mostly for the subject to align their eyes (4). On average, iris verification takes about 10 seconds (4, 12, 13). The use of infrared light from LEDs is safe for the eyes, as the light is incoherent. While a single LED poses minimal risk, multiple LEDs might

be harmful if not properly designed and used (4, 14). However, iris scanning may not always require such illumination.

Compared to retinal scanning, concerns about eye safety are generally less significant with iris scanning (14). The image capture procedure for iris recognition in the EBL2007 vaccine trial was notably brief (less than 1 minute) for the majority of participants (97%), taking less than one minute (chapter 3)

To alleviate similar concerns in future clinical research, it might be advisable to consider participant identification for those with vision problems, allowing them to wear their glasses, even if tinted, during the identification process, if the manufacturer's recognition algorithm can accommodate this. Additionally, in fragile environments, other promotional and preventive activities aimed at improving eye health may be necessary. Such initiatives could further enhance trust within the community of participants in vaccine trials, especially in remote, low-resource countries.

To enhance Sub-Saharan Africa's involvement in clinical trials, it is crucial to address issues of actual or perceived corruption and its impact on data quality, patient safety, and investor engagement in the region (15). Concerns regarding corruption can be addressed by promoting the development of innovative biometric identification tools in clinical trials conducted in these settings.

Based on our findings regarding iris scanning, we can assert that the iris scan contributed significantly to the credibility of the data quality in the EBL2007 vaccine trial in a remote and fragile setting like Boende. This is evidenced by confirming, for example, that blood samples were consistently taken from the same participants who actually received the vaccine at all scheduled visits (Day 1, Day 56, Day 78, Year 1, and Year 2). In the reported fraud cases (Chapter 3 and 4), t the majority involved participants who were not originally enrolled, substituting themselves during planned visits to avoid missing transportation allowances and compensation for time spent during study site visits. The Council for International Organizations of Medical Sciences (CIOMS) emphasizes the growing importance of introducing new and digitally adapted technologies in low-resource country contexts (16). Conducting trials in low-income countries can be advantageous, as it introduces healthcare innovations. For instance, the use of iris scan in the EBL2007 vaccine trial as an identification tool was an innovation introduced by the EBOVAC 3 Project, under which this doctoral thesis is written. This innovation demonstrates how the implementation of clinical trials in a weak healthcare system can play a significant role in contributing to healthcare system improvement (17). Furthermore, this tool proved to be invaluable for precise participant identification, enhancing the integrity and reliability of the study. This utility extended beyond the initial trial, as the established iris scan database was instrumental in the subsequent COVID-19 vaccine trial, ensuring accurate screening and avoiding the inclusion of individuals previously vaccinated in the EBL2007 trial, in line with the study's exclusion criteria.

10.2. Adhering to Good Clinical Practice in Challenging setting

The DRC is among the developing countries that are generally underrepresented in research due to a lack of commercial viability and qualified researchers (18, 19). Despite this challenge in research representation, it was crucial to take into account scientific factors, the strict regulations of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), and Good Clinical Practice (GCP) guidelines during the implementation of the EBL2007 vaccine (17). The conduct of this trial undoubtedly involved proposing in advance how to address potential logistical challenges, particularly in terms of consistently adhering to GCP to generate viable evidence.

After developing the trial protocol and submitting it for ethical committee approval at a very early stage in accordance with ICH-GCP guidelines (Chapter 6) (20), the key approaches outlined in Chapters 5 and 9 were employed to conduct the trial in accordance with standard GCP norms : 1) Continuous education and retraining were almost consistently maintained for both the trial participants and the staff involved throughout the trial over the 2.5 years. The level of knowledge about the trial (for example: basic knowledge of the vaccine and trial procedures) was reiterated at year 1 and two years to ensure informed participation of participants. This refreshing of knowledge was preceded by an evaluation that showed a significant decrease in the ability to recall information received through the consent at inclusion, year one, and year two, compared to inclusion. The concepts GCP, the protocol, and the SOPs among trial staff were regularly revisited during the same period to ensure thorough understanding and active engagement of the staff, thus contributing to the success and integrity of the trial; 2) Data were copied daily from the local server to the central server using a satellite uplink. Both servers were fully compliant with the 21 CFR Part 11 standard; 3) In relation to the remote location of the study site, whose main sure and efficient means of access was by plane, simulations (dry-run) of vaccine shipments from Belgium to the site (Boende, DRC) via Kinshasa (the capital city of DRC) and sample shipments from Kinshasa to the site were conducted during the preparatory phase to mitigate risks associated with suboptimal cold chain performance (management of blood samples and investigational products). These simulations were crucial for making necessary adjustments to ensure the proper functioning of the cold chain during storage and transport/shipping of samples (temperature recording, documentation). Indeed, these simulations, for example, helped to limit the loss in quality of the samples and vaccines; 4) All source documents without patient identifiers were digitized and retained by both the PI and the Sponsor for the same duration as the main trial file, which follows European legislation and amounts to 25 years, given the ambiguity in the legislation in the DRC.

These approaches were effective thanks to the establishment of good collaboration with local health authorities, creating a bridge for community engagement, and utilizing existing health infrastructures and resources for the trial. This collaboration proved to be very beneficial, contributing to capacity building, from infrastructure to education and training for other research that could be conducted in the region.

In addition to these approaches, addressing challenges related to compensation for research-related harm, healthcare for participants, and remuneration for participation were also major hurdles to overcome in the conduct of the EBL2007 vaccine trial. Given the potential for vaccines to cause adverse effects (AE), a policy was designed to manage all AEs or serious adverse events (SAE) in the same manner, regardless of their association with the experimental vaccine (21). This policy was implemented in the field through an algorithm consisting of a series of consecutive questions with binary response options, leading to structured, non-arbitrary, and consistent support and management for each AE and SAE unrelated to the study vaccine. It was advised to report every (S)AE to the study team, whether it was considered an AE or not, and for which a potential outcome of support would be determined. The algorithm provided guidance to cover (medically and financially) all medical needs beyond vaccine-related damages (covered by the clinical trial's insurance) and included events that were treated off-site >(22). This policy was the result of dialogue and collaboration between the sponsor (UA) and the principal investigator (UNIKIN), a review of the literature, and inputs from experts in research ethics and social sciences.

In striving to enhance compliance with GCP standards, the digitization of source documents was essential for each anonymized participant in the EBL2007 vaccine trial. If each participant's record could be constructed electronically (electronic data capture), and it could be synchronized with the biometric iris scan identification data through a single centralized server at the site level, this could further optimize the management of source documents and reduce the workload for both local investigators and the sponsor's monitors. This single server would further reduce the risk of duplicates, wastage of paper resources, and cartridges. Moreover, reviewing a participant's study chronology during the evaluation of the treatment timeline and trial completeness during analysis would be more rational. It had been shown that a system with unique digital identification offers significant gains in efficiency and effectiveness compared to precarious, fragmented, paper-based health systems (23).

The remote approach to monitoring clinical trials was proposed to enable the continuation of trials while adhering to GCP standards during the COVID-19 pandemic (24). The hybrid model of clinical trial monitoring, combining remote and on-site follow-up, was shown to improve efficiency, to reduce costs, and to ensure compliance with GCP standards (25). Thus, digitized documentation or the electronic capture of electronic case report forms and trial records (investigator site file and main trial file) from the start of the trial would have facilitated remote monitoring of trials during the COVID-19 lockdown period.

However, the use of essential research tools such as computers, printers, scanners, and particularly access to the internet, was not commonplace among the local staff, presenting challenges. Moreover, climatic conditions, especially the hyper-humidity unfavorable for long-term preservation of paper documents, motivated the research team (PI and Sponsor) to opt for manual record-keeping, and subsequent scanning of the source documents.

One of the primary goals of the sustainable development program is to ensure optimal health for everyone by providing universal access to essential medicines and vaccines (26).

Conducting quality research to identify and address the unmet health needs of people living in resource-limited environments is essential (16). Particularly in remote and vulnerable areas of the DRC, such as Boende, adhering to GCP standards in the EBL2007 trial is an encouraging sign for making strategic decisions in favour of expanding clinical vaccine research, especially against vaccine-preventable diseases like EVD. This success highlights the importance of local and international collaboration to develop solutions tailored to the specific health challenges in the DRC, a crucial approach to addressing the complexities of endemic diseases. Indeed, CIOMS recommends the optimization of clinical research by leveraging the experiences of various stakeholders (16, 27). Researchers from LMICs and sponsors/pharmaceutical companies should collaborate to create and maintain permanent clinical research networks, with core functions that could serve both academic clinical trials and those conducted by the industry, as was the case between UNIKIN and UA in the conduct of the EBL2007 trial. Experienced mentors are needed to continue building an African culture of research in Africa (27, 28). This type of collaboration in the EBL2007 trial is also supported by the WHO's research and development (R&D) action plan. This plan is committed to accelerating research on emerging diseases, reducing the time to develop safe and effective medical countermeasures, as part of a proactive strategy to improve preparedness and response to future epidemics and pandemics (29). This targeted approach aims not only to expedite the development of vaccines and treatments but also to fill gaps in our understanding of similar viruses, thereby emphasizing the importance of conducting vaccine trials in LMICs. Clinical research conducted in LMICs, particularly in Sub-Saharan Africa, enhances research capabilities and healthcare provision (30), contributing to the strengthening of healthcare systems, and providing an evidence base for future responses to health crises (31, 32).

10.3. Baseline Seroprevalence and Challenges Related to Cutoff

Previous studies have shown the long-lasting persistence of IgG antibodies in EVD survivors [40,57,58]. ELISA was reported as a suitable assay in epidemiological studies (33-36). The development validation of the FANG ELISA for human serum have been documented through studies evaluating vaccine and therapeutic efficacy against Ebola virus disease (37). Likewise, the Luminex assay showed a high level of accuracy for detecting past EBOV infections and was useful for epidemiological surveys (38). In the EBL2007 vaccine trial, minimal seroreactivity (0.8%) to GP-EBOV-m and GP-EBOV-k surface antigens was found in healthcare professionals and frontline workers who already had IgG antibodies specific to EBOV before vaccination, using FANG ELISA and Luminex assays (chapter 6). Importantly, our analysis revealed no correlation between seropositivity and previous exposure to EBOV-m suggesting the possibility of false positives in seropositive results.

However, the establishment of baseline seroprevalence for EVD was complex due to the lack of standardized EVD assays. Despite numerous studies investigating the seroprevalence of ebolavirus IgG since the initial outbreak in Yambuku, reaching a consensus on the findings has been challenging (39). The indirect fluorescent antibody test (IFAT) was introduced in 1977 to differentiate the newly discovered Ebola virus from the closely related Marburg virus, based

on the specificity of viral antigen in the convalescent serum antibodies of individuals who had recovered from these pathogens (33, 40). However, IFAT was deemed to have suboptimal sensitivity and specificity in populations with no apparent probability of infection with the African filoviruses (34, 41). Furthermore, its requirement for Biosafety Level 4 (BSL-4) containment rendered it unsuitable for large-scale diagnostic efforts (33). The introduction of enzyme-linked immunosorbent assay (ELISA) tests for the detection of Ebola virus-specific IgM and IgG antibodies marked a significant advancement, offering a faster and more efficient approach to serological testing (33). A study examining the cross-reactivity of IgM and IgG antibodies in convalescent phase sera from different Ebola virus species indicated minimal cross-reactivity of IgM antibodies among different Ebola virus species (42). In contrast, IgG antibodies showed broader reactivity with antigens from multiple Ebola virus species (33).

Recombinant antigens are extensively utilized in various ELISA assays, including both the Luminex and ELISA FANG assays, which employed EBOV (Ebola virus) recombinant antigens (38). The advantage of using recombinant antigens in ELISA tests lies in their ability to be produced in large quantities with high purity, thereby facilitating the detection of antibodies with notable specificity and sensitivity (43). However, a primary disadvantage of employing EBOV recombinant antigens in seroprevalence studies is the risk of cross-reactivity, leading to false positive results (44). Thus, establishing a baseline seroprevalence for the EBL2007 vaccine trial in the remote area region of Boende, where health system infrastructure is minimal, posed significant challenges. Neutralization assays, considered the gold standard for serological testing, are labor-intensive and time-consuming as they require the use of infectious cells (45). Additionally, conducting these tests for viruses like EBOV necessitates a Biosafety Level 4 (BSL-4) laboratory, which is rare and expensive to operate. This further complicates the process in resource-limited environments (46). Seropositivity rates in this study are lower compared to previous studies where different kits were used (Table 1, chapter 6).

Conducting a preliminary study on reference samples to compare the sensitivity and specificity of Luminex and FANG assays with the tests/kits used in prior serological surveys in the Boende Health District region would be a valuable addition. However, a previous study by Ayouba A et al.(38) compared the results of the LUMINEX test with the commercial ELISA assay employed in previous EBOV serological surveys in the DRC (Alpha Diagnostic, San Antonio, TX) using survivor samples from the EBOV outbreak in Guinea (2014-2016) and negative samples from patients in France. This study found that the Luminex test had higher specificity (95.4%: 95% CI 89.6-98.0) and similar sensitivity (96.8%: 95% CI 91.3-98.9) compared to the commercial ELISA assays when considering seroactivity to a single EBOV GP antigen (the specificity of the commercial ELISA kits was 92.6% (95% CI: 86.1-96.2) and the sensitivity was 96.8% (95% CI: 91.3-98.9)). Another study by Wei Wu et al. (47) demonstrated that ELISA tests exhibited lower specificity compared to the Luminex assay for serological detection of antibodies specific to viruses causing hemorrhagic fevers. The specificity of the Luminex assay was found to range from 66 to 100%, with a sensitivity of 90 to 98%. Another study by Logue et al.(37), compared the FANG ELISA assay to the commercial ELISA assay (Alpha Diagnostic International, ADI). The findings from this study revealed that FANG ELISA assay was

substantially more precise, with less regional background noise than ADI ELISA, which has been widely used in previous EBOV tests.

This doctoral project, conducted under the EBOVAC 3 consortium nearing the end of its budget allocation, faced practical limitations in conducting further analyses with alternative assays/kits to compare our results with those of other researchers in the field. Undertaking such additional investigations would have introduced unexpected logistical and financial challenges not accounted for in the project's initial planning.

In our study, a high cutoff estimation using change-point analysis (48) was utilized for Luminex and FANG ELISA tests, resulting in a lower GP-EBOV-m seroprevalence value (0.8%, Chapter 6) compared to previous studies. Based on this cutoff, the GP-EBOV seroprevalence using FANG and Luminex assays was similar (0.8%,) to that found using the literature threshold (determined based on control samples). Hence, we believe our results may reflect the real exposure levels of HCPs and frontline participants in the EBL2007 vaccine trial. This group likely adhered stringently to infection control and prevention measures during past outbreaks, resulting in minimal exposure, or they may have experienced a reduction in antibodies over time, highlighting the need for vaccination.

10.4. Perceptions of the trial among HCPs and frontline workers participants

Positive perceptions of the vaccine trial were influenced not only by the role of the study vaccine in preparing for future possible EVD in the Health District of Boende but also by improvements brought in the local health infrastructure. These improvements, alongside the provision of essential resources and the capacity-building activities for HCPs and frontline worker participants, contributed significantly to the favourable view of the trial (chapter 7). Furthermore, our findings indicate a broad acceptance of the experimental Ebola virus vaccine and trial location (study site, General Hospital of Boende) as well as the choice of the target HCPs population. This acceptance highlights a significant commitment within the community to support Ebola virus vaccination efforts and associated research.

There was an argument suggesting that recruitment for experimental EVD vaccine research during the 2014-2016 West African epidemic should not focus solely on HCPs population (49). Such approach targeting solely HCPs could potentially exacerbate feelings of marginalization in communities not involved in trials (51). However, the pivotal role of HCPs in building patient trust in vaccines is well recognized (50). Studies in pediatric groups for example (51, 52) have underscored the importance of strong vaccination recommendations from HCPs. In the context of COVID-19 vaccines, amidst prevailing uncertainties, HCPs were key in providing accurate vaccine information in several areas globally (50, 53). Vaccinating HCPs is seen as essential, as it may not only boost confidence in the vaccine but also plays a critical role in reducing transmission during epidemics (50). Therefore, the EBL2007 vaccine trial's target population was broadly defined to include not just nurses but also laboratory staff, health facility cleaners, first aiders, morgue workers, and community health workers. Essentially, if

the vaccine is effective, targeting HCPs will serve a dual purpose: enhancing trust in the vaccine and helping control the spread of the disease. Moreover, despite the increasing use of internet searches for health information, HCPs are increasingly reported to the most trusted source for vaccination information (54-56).

Some interviewees expressed dissatisfaction with the compensation of the time spent for participating in the trial, the risk taken by participating in it, high frequency of their blood drawn, and the travel expenses. Some were only partially satisfied, expecting additional rewards or higher travel expense refunds. A few persons expressed concern about the recruitment process of trial staff, stating that true experts in the Ebola response were not hired and suspecting that local authorities influenced the selection process. Early partnership between researchers and potential participants in the EBL2007 vaccine trial could enhance collaboration, and the EBL2007 vaccine protocol understanding. This early partnership might aid in aligning and clarifying issues such as reimbursement for transportation for transportation costs, concerns about frequent blood draws, the need to send samples abroad for analysis, and the timeline for returning trial results. While addressing participants' concerns about inadequate compensation for travel costs and time in the vaccine trial, it is essential to consider that, we carefully balanced the need to fairly compensate participants for their time and expenses avoiding too much incentives that could unduly influence their decision to participate in the trial.

A study showed that early community involvement in trial preparation helped address participant opinions and concerns and identified effective methods to resolve emerging issues (57). Similarly, it is well known that community engagement is crucial as it allows researchers to gain insights into the research community's needs and priorities (58). Thus, our findings underscores the relevance of early community involvement in the conceptual phase of a vaccine trial in remote areas of LMICs countries.

This early engagement may be crucial for a broader understanding of real-life scenarios more accurately by the researchers. Furthermore, the global imperative for public involvement in healthcare was established by the WHO's Alma-Ata Declaration in 1978 (59). This declaration emphasized the fundamental 'right and duty' of every individual to actively engage in shaping and implementing their own healthcare services. Patient and public involvement (PPI, also known as “public involvement”) for example, has been defined as “research being carried out ‘with’ or ‘by’ members of the public (including patients and carers) rather than ‘to’, ‘about’ or ‘for’ them” in the United Kingdom (UK) (60, 61). This entails that patients and the public are active partners in research, rather than simply being used as participants in research. PPI is increasingly recommended and even required by some research funders like the National Institutes of Health (NIH) and the Wellcome Trust (62), and it is mandatory in contexts such as guidelines issued by the United Kingdom's National Institute for Health and Care Excellence (NICE)(63). Implementing such approach in clinical trials conducted in LMICs can help in tailoring communication strategies to local contexts, addressing cultural sensitivities, and ensuring that participant concerns are adequately considered and addressed (64). This type of approach, which emphasizes a more equal partnership and collaboration, enables clinical

trial participants from remote areas of LMICs to effectively engage in setting up and implementing research. Furthermore, it may ensure that participants do not perceive the research as exploitative, as they take ownership of the research process (64, 65). Hence, we believe that PPI might be a better strategy to address some challenges with respect to the raised concerns of EBL2007 vaccine trial's participants.

The delay in receiving feedback from the EBL2007 vaccine trial became a significant issue for participants. They were unable to see the study vaccine results at the study's end due to delays caused by the COVID-19 pandemic and lockdowns. This resulted in the immunogenicity findings, processed by Q-square Solutions in the USA, being available almost two years after the trial started. Participants in longitudinal studies like this often expect timely feedback, especially after dedicating considerable time to the study, making this delay particularly impactful. The challenge in providing feedback is often exacerbated when comprehensive laboratory facilities are not readily available.

10.5. Two-year maintain of informed participation in the EBL2007 vaccine trial

Prolonged engagement in vaccine trials may introduce ethical challenges, particularly regarding informed consent (66). Initially, participants' consent may evolve as the study progresses, highlighting the need for an adaptable approach to informed consent throughout its duration. Furthermore, respecting autonomy is a key ethical principle underpinning the requirement for informed consent (67). This involves making a voluntary decision without coercion or manipulation by others, and having access to adequate and understandable information (66, 68). This consideration was particularly critical in conducting a study whose duration (2.5 years) and complexity required continuous understanding from participants to ensure their protection. We have recorded a significant decrease in the TOU score at years one and two post-inclusion, prior to the trial re-explanation, suggesting that clinical trial participants can forget crucial study information over time (Chapter 8). EBL2007 vaccine trial's use of a TOU contributed to address concerns about exploiting vulnerable populations in trials in Low- and Middle-Income Countries (LMICs), a region where fraud and ethical challenges may impede recruitment (15) (64). Implementing TOU in a poor resources setting may foster informed participation, allowing participants the freedom to withdraw their consent at any time. In the supplementary material of Chapter 8 (Supplement 3, Table 4), the ability of healthcare providers and frontline workers to provide correct answers to TOU (Test of Understanding) questions over time was analyzed relative to their age, occupation, and sex. A generalized linear model was used to assess the effects of covariates such as age, sex, and occupation on the outcome variable (score of TOU over a year per participant). The table indicated significant effects of age (over 60 years) and certain occupations like doctors, midwives, and nurses on the TOU scores among participants over the year. However, while the used model represents a fixed effect analysis, which does not account for potential random effects that may arise from the hierarchical structure of the data, such as repeated measurements of the TOU from the same participants, the omission of the results from the

random effects model in the supplementary material was an oversight. The main findings of this chapter, based on the omitted random effects model (Additional material), showed that only age (over 60 years), doctors, and midwives have a significant effect on the score over the year. The effect of the nurses' occupation, although significant in the fixed effect model, was not found to be significant in the random effects model.

10.6. Strengths and limitations

One of the key strengths of this thesis is its in-depth examination of the challenges faced during the implementation of Ebola vaccine trials in the remote and endemic areas of the DRC, the country most affected by EVD. This exploration addresses a crucial aspect of public health and significantly contributes to our understanding of managing vaccine trials in hard-to-reach regions. The thesis highlights the real-world challenges in remote and underdeveloped areas, including managing logistics in difficult locations, respecting and adapting to local cultures, and addressing unique ethical concerns. These insights are invaluable for adapting vaccine trials to various environments, particularly those with limited resources. However, this thesis has limitations, primarily its focus on the specific area of the Boende Health District in the DR Congo. The findings from this unique setting, characterized by limited resources and challenging logistics, might not be universally applicable. It's uncertain if the same results would be observed in different regions with varying conditions. Additionally, the study navigated challenges related to cultural and language homogeneity, as the dominant ethnic group in the area shares similar culture and language. This similarity could have influenced our interpretation of participant responses and the execution of the vaccine trial. Furthermore, the Boende health district's HCPs and frontliners have a firsthand experience of the 2014 EVD outbreak (7th EVD outbreak), which began in a hospital without an available vaccine. This experience likely increased their awareness of Ebola risks, potentially affecting certain study aspects, such as vaccine acceptance and iris scan participation. This heightened compliance rate in this specific group should be considered when interpreting the findings, as it may affect the generalizability of the results to other communities or setting.

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Chapter 11. Conclusions and Recommendations

11.1. Conclusions

To accommodate the diversity of experiences and exposures of different populations, clinical research should adequately include different racial and ethnic groups, as well as socio-economically disadvantaged populations like those in remote regions of LMIC countries. This inclusion is vital for meeting the objectives of activities related to addressing and mitigating the impact of pandemics, as outlined in the R&D blueprint blueprint (89). To safeguard individuals potentially facing an EVD outbreak, it is desirable to have a vaccine that can offer long-term protection, given that virus outbreaks are sporadic and unpredictable. This necessitates additional vaccine trials with various vaccine candidates in these settings. Conducting more pre-registration studies for Ebola vaccines in a country like the DRC is crucial. The EBL2007 vaccine trial conducted in Boende's remote health districts illustrates the practicability of pre-licensure trials in isolated LMIC settings. Effectively planning and executing such a trial in resource-constrained environments demands adaptability, inventiveness, and deep local involvement. Researchers should be prepared for potential bureaucratic hold-ups with ethical and regulatory feedback. It might be wise to submit trial protocols early and to anticipate unforeseen queries from regulatory agencies. Vaccination of study personnel is paramount for readiness in face of possible epidemics. Fostering positive relationships with local authorities and establishing definitive algorithms for financial and medical decision-making are key to circumventing budgetary complications. For managing source documents, having a robust archival system, along with plans for document transit and storage, is essential, particularly when electronic data capture poses challenges. Employing biometric identification tools can help deter fraud in participant recruitment and follow-up. Comprehensive training for local staff, the conduct of trials, and having backup plans for logistical hurdles should be imparted to both administrative and financial staff from the outset. Early and continuous engagement with the community and participants is vital. This should include addressing concerns about compensation, healthcare provision, and the transparency of the recruitment process.. Investment in Local Research Infrastructure: Strengthening local research capabilities and infrastructure in low-resource settings is crucial for enhancing the quality and credibility of clinical trials.

11.2. Recommendations

In light of the conclusions and findings presented in this thesis, the subsequent recommendations are proposed:

• Future clinical trials in remote settings, especially in low-resource settings, should consider integrating biometric technologies like iris scanning for participant identification to enhance data accuracy and minimize fraud.

• Ongoing education and transparent communication are crucial to address participants' concerns, particularly regarding the safety of biometric technologies. This includes reinforcing safety information periodically to ensure long-term comprehension and trust. Consideration should be given to participants with special needs, such as those with vision problems, when using iris scanning, ensuring that identification methods are inclusive and adaptable.

• Standardized approaches for establishing baseline seroprevalence should be developed, particularly in areas with limited health infrastructure, to improve the accuracy of clinical trials. Adherence to GCP Standards: Rigorous adherence to GCP guidelines is essential, alongside innovative approaches like digitized documentation and remote monitoring to overcome logistical challenges in challenging settings.

• Early and continuous engagement with the community and participants is vital. This should include addressing concerns about compensation, healthcare provision, and the transparency of the recruitment process. Investment in Local Research Infrastructure: Strengthening local research capabilities and infrastructure in low-resource settings is crucial for enhancing the quality and credibility of clinical trials. Promoting Ethical Conduct and Informed Participation: Continuous effort is needed to ensure informed participation throughout the trial duration, respecting participant autonomy and addressing ethical challenges. Leveraging Digital Technologies: The utilization of digital technologies for data management and participant tracking should be maximized to enhance efficiency and trial integrity.

• Building and maintaining international research collaborations can help address endemic diseases effectively and contribute to healthcare system improvements in low-resource settings. These recommendations aim to enhance the efficacy, integrity, and ethical conduct of clinical trials, particularly in remote and resource-limited environments, thereby contributing to global health advancements and the well-being of diverse populations.

Additional material

Table 1. Ability of healthcare providers and frontline workers to provide correct answers to TOU questions over time, relative to their age, occupation and sex

Coefficients	Estimate	Std error	Z-value	p
Year 1	-1.41877	0.06118	-23.189	**<2e-16 *** **
Year 2	-0.88124	0.06034	-14.605	**<2e-16 *** **
Sex	0.12502	0.0704	1.776	
Doctor	0.4637	0.34094	1.36	
Hygienist	-0.55397	0.29228	-1.895	
Midwife	-0.77084	0.30047	-2.565	**0.0103** *
Nurse	-0.07486	0.27518	-0.272	
Other	0.2156	0.48237	0.447	
First-Aid Workers	-0.46929	0.27523	-1.705	
Age 30-45	-0.12811	0.08497	-1.508	
Age 45-60	-0.12251	0.08258	-1.484	
Age>60	-0.29144	0.11124	-2.62	**0.0088** **

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Oct 2022-January 2023, Supervisor: Dr Nelly Mukonda (nmukonda@path.or), Cyril Nogier (cnogier@gavi.org)

- Principal Investigator in the Project Study of the effectiveness of Ebola vaccines. Study carried out in a consortium involving the CDC-Atlanta, PEV-RDC, School of Public Health of Kinshasa-DRC, the Epidemiological Surveillance Department, the INRB and the University of Kinshasa, 2021- ongoing, supervisor: Reena Doshi: hqo3 @cdc.gov.
- Teaching Assistant, Department of tropical Medicine, University of Kinshasa, 2014-to date
- Civil Servant, Expanded programme on immunization of DRC, 2020-to date

- List of associated functions and main achievements:
- Deputy chair of the work thematic sub-group vaccine delivery in the national immunization coordination committee:
  - ✓ Lead in the drafting and approving the national deployment plan of COVID-19 vaccination in the DRC (November 2020 - February 2021)
  - ✓ Contributions to updating the VIRAT tool as part of monitoring the preparations for the introduction of COVID-19 vaccines in the DRC (November 2020-February 2021)
  - ✓ Significant contributions to the organization of the consultation workshop on appropriate strategies for the vaccination of target populations against COVID-19 in the DRC by PEV-RDC and its partners (February 2021)
  - ✓ Significant contribution to the development, planning, and implementation of training tools on the introduction of COVID-19 vaccines in the DRC (February - April 2021)
  - ✓ Providing support to the Technical Advisory Group on Vaccination (GTCV) in issuing opinions on the choice of COVID-19 vaccines, vaccination strategies as well as priority groups (February 2021)
  - ✓ Significant contribution to the organization of the first intra-action review of COVID-19 vaccination in the DRC (July-August 2021) and report preparation
  - ✓ Conference panelist at the scientific day co-organized by EPI and WHO on COVID-19 vaccination to promote vaccine confidence, at the University of Kinshasa, March 27, 2021.
- Trial Site Coordinator in the EBL2007 vaccine trial in Boende (EBOVAC 3 projects, Tshuapa Province, DRC): Phase 2 open-label clinical trial aimed at evaluating the immunogenicity and safety of prophylactic vaccination in healthcare professionals by administering of a heterologous vaccine regimen (Ad26ZEBOV and MVA-BN-Filo, Johnson & Johnson) against Ebola Virus Disease in the Democratic Republic of Congo (EBL2007, EBOVAC 3 Project)
  - ✓ Contribution in the Protocol and Informed consent form preparation
  - ✓ SOPs and trial activities plans development
  - ✓ Providing protocol training to the study personel
  - ✓ Quality check of the study source documents, blood samples collection, and vaccination.
  - ✓ Supervision of the activities of all positions during the recruitment and follow-up of volunteers to the clinical trial
  - ✓ Preparing the completeness of the site investigator file
  - ✓ Investigational Product management (pharmacy and cold chain oversight)
  - ✓ Active monitoring and Safety reporting
- Project coordinator of the phase 2b clinical trial titled “A Multi-Centre, Randomized, Double Blind, Phase 2b Trial to Evaluate the Safety and Immunogenicity of Janssen Ad26COVS1 and Novavax NVX-CoV2373 COVID-19 vaccines for Homologous and Heterologous Boosting in Adolescents and Adults Aged 12 to 64 Years with and without HIV infection in 3 African Countries (Kenya, Democratic Republic of Congo, and Rwanda)”
  - ✓ Preparing the completeness of the site investigator file

- ✓ Site respondent on Elonga site progress, overseeing site activities and team as assigned in delegation log (screening, randomization, and follow-up visit, attending weekly VIBRI-ACE meeting,
- ✓ Completeness of the protocol deviation report,
- ✓ Reporting of serious adverse events, managing the daily activities of the trial according to the protocol,
- ✓ Close interaction with sponsor CRAs and scheduling patient visits for protocol required assessments.

- Principal Investigator in the Project Study of the effectiveness of Ebola vaccines.
  - ✓ Involvement in the development of a protocol for a vaccination program targeting contacts of Ebola survivors in Equateur Province, DRC (February 2021-March 2023). This program involved partnerships with CDC Atlanta, the Ebola Survivors Programme, UNIKIN, the Institut National de Recherche Biomédicale (INRB), Kinshasa School of Public Health, and the MoH of DRC.
  - ✓ Mobilizing all necessary partners, including those from the DRC Ministry of Health, the Expanded Programme on the Immunization, and the University of Kinshasa to finalize the protocol and revise the surveillance forms for future outbreaks to enable collection of improved vaccination data

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Professional development

- How to be a Good Principal Investigator, The Global Health Network and MRC clinical trials unit, Webinar, June 7th 2023
- WHO costing, budgeting, financing and delivery of COVID-19 vaccines: how to apply for GAVI CDS funding through the partners platform, OMS, virtual training, July 13, 2021
- COVID-19 Vaccine Development & Implementation Workshop 2021 - June Edition, Virtual, Netherlands, 06/29/2021-06/30/2021
- Management and facilitation of an intra-action review (IAR) of COVID-19 in a country, WHO, Virtual course, 23 July 2021
- Infodemic, managing rumors about COVID-19 vaccines, MOMENTUM -Routine Immunization Transformation and Equity (M-RITE) and BREACKTHROUGH ACTION, Kinshasa, DRC, 2021, 16-21 July 2021
- Infection Prevention and Control – Controlling Antimicrobial Resistance Transmission, the global health network, virtual workshop, 18-19 November 2020
- Quality control and assurance, Monitoring editing and site inspection, Africa Contact Organization Research Clinical trial (ACE Research Africa), WP2F+RVC, Kisumu, Kenya 2019, 22-27 November 2019
- Management and leadership, Wallonie-Bruxelles International (Brussels/Belgium), Pl. Sainctelette 2, 1080 Brussels, Belgium, 01 to 05 June 2018
- Impact of Belgian support to the Congolese administration (Audit), Belgium Development Agency (ENABEL/DRC), Cinquantenaire building, Boulevard du 30 juin, 133 – Gombe, Kinshasa Congo, Democratic Republic, from September 18 to 20, 2020

Scientific publications

1. Vinkeles Melchers NVS, Mollenkopf S, Colebunders R, Edlinger M, Coffeng LE, Irani J, **Zola T**, Siewe JN, de Vlas SJ, Winkler AS, Stolk WA. Burden of onchocerciasis-associated epilepsy:

first estimates and research priorities. Infect Dis Poverty. 2018 Sep 19;7(1):101. doi: 10.1186/s40249-018-0481-9. PMID: 30253788; PMCID: PMC6156959.

2. **Matuvanga, T.Z**., Zimbombe, F.V., Ntita, F.M., Muamba, M.M., Di-Mosi-Nkoy, R.W., Tevuzula, V.M., Mulopo, P.M., Rika, J.M., Claude, J., Katumbayi, K. and Muhindo-Mavoko, H., 2020. Amibiase intestinale: controverse sur l'implication des especes non pathogenes, revue generale. Intestinal amoebiasis: controversial implication of nonpathogenic isolates. Revue Médicale des Grands Lacs Vol, 11(02).
3. Lemey G, Larivière Y, **Zola TM**, Maketa V, Matangila J, Mitashi P, Vermeiren P, Thys S, De Bie J, Muhindo HM, Ravinetto R, Van Damme P, Van Geertruyden JP. Algorithm for the support of non-related (serious) adverse events in an Ebola vaccine trial in the Democratic Republic of the Congo. BMJ Glob Health. 2021 Jun;6(6):e005726. doi: 10.1136/bmjgh-2021-005726. PMID: 34183329; PMCID: PMC8240587.
4. **Zola Matuvanga T**, Johnson G, Larivière Y, Esanga Longomo E, Matangila J, Maketa V, Lapika B, Mitashi P, Mc Kenna P, De Bie J, Van Geertruyden JP, Van Damme P, Muhindo Mavoko H. Use of Iris Scanning for Biometric Recognition of Healthy Adults Participating in an Ebola Vaccine Trial in the Democratic Republic of the Congo: Mixed Methods Study. J Med Internet Res. 2021 Aug 9;23(8):e28573. doi: 10.2196/28573. PMID: 34378545; PMCID: PMC8386356.
5. Larivière Y, **Zola T**, Stoppie E, Maketa V, Matangila J, Mitashi P, De Bie J, Muhindo-Mavoko H, Van Geertruyden JP, Van Damme P. Open-label, randomised, clinical trial to evaluate the immunogenicity and safety of a prophylactic vaccination of healthcare providers by administration of a heterologous vaccine regimen against Ebola in the Democratic Republic of the Congo: the study protocol. BMJ Open. 2021 Sep 28;11(9):e046835. doi: 10.1136/bmjopen-2020-046835. PMID: 34588237; PMCID: PMC8479954.
6. **Zola Matuvanga T**, Larivière Y, Lemey G, De Bie J, Milolo S, Meta R, Esanga E, Vermeiren PP, Thys S, Van Geertruyden JP, Van Damme P, Maketa V, Matangila J, Mitashi P, Muhindo-Mavoko H. Setting-up an Ebola vaccine trial in a remote area of the Democratic Republic of the Congo: Challenges, mitigations, and lessons learned. Vaccine. 2022 May 31;40(25):3470-3480. doi: 10.1016/j.vaccine.2022.04.094. Epub 2022 May 9. PMID: 35550847..
7. **Zola Matuvanga T**, Doshi RH, Muya A, Cikomola A, Milabyo A, Nasaka P, Mitashi P, Muhindo-Mavoko H, Ahuka S, Nzaji M, Hoff NA, Perry R, Mukamba Musenga E. Challenges to COVID-19 vaccine introduction in the Democratic Republic of the Congo - a commentary. Hum Vaccin Immunother. 2022 Nov 30;18(6):2127272. doi: 10.1080/21645515.2022.2127272. Epub 2022 Sep 27. PMID: 36165731; PMCID: PMC9746480.
8. Johnson GA, Martin S, Aldershot S, **Trésor Zola Matuvanga**, Muhindo Mavoko H, Mulopo PM, et al. "People Are Not Taking the Outbreak Seriously": Interpretations of Religion and Public Health Policy During the COVID-19 Pandemic. Caring on the Frontline during COVID-19: Contributions from Rapid Qualitative Research: Springer; 2022. p. 113-38.
9. Larivière Y, Garcia-Fogeda I, **Zola Matuvanga T**, Isekah Osang'ir B, Milolo S, Meta R, Kimbulu P, Robinson C, Katwere M, McLean C, Hens N, Matangila J, Maketa V, Mitashi P, Muhindo-Mavoko H, Van Geertruyden JP, Van Damme P. Safety and Immunogenicity of the Heterologous 2-Dose Ad26.ZEBOV, MVA-BN-Filo Vaccine Regimen in Health Care Providers and Frontliners of the Democratic Republic of the Congo. J Infect Dis. 2023 Aug 24:jiad350. doi: 10.1093/infdis/jiad350. Epub ahead of print. PMID: 37673423.
10. **Zola Matuvanga T**, Mariën J, Larivière Y, Osang'ir BI, Milolo S, Meta R, Esanga E, Maketa V, Matangila J, Mitashi P, Ahuka Mundeke S, Muhindo-Mavoko H, Muyembe Tamfum JJ, Van Damme P, Van Geertruyden JP. Low seroprevalence of Ebola virus in health care providers in an endemic region (Tshuapa province) of the Democratic Republic of the Congo. PLoS One. 2023 Sep 1;18(9):e0286479. doi: 10.1371/journal.pone.0286479. PMID: 37656725; PMCID: PMC10473486.

11. Lemey G, **Trésor Zola Matuvanga**, Larivière Y, Milolo S, Danoff E, Bakonga L, Esanga E, Vermeiren P, Maketa V, Matangila J, Mitashi P. Researchers' responsibilities in resource-constrained settings: experiences of implementing an ancillary care policy in a vaccine trial in the Democratic Republic of the Congo. Research Ethics. 2023:17470161231194139.
12. Larivière Y, **Matuvanga TZ**, Lemey G, Osang'ir BI, Vermeiren PP, Milolo S, Meta R, Kimbulu P, Esanga E, Matangila J, Van Geertruyden JP, Van Damme P, Maketa V, Muhindo-Mavoko H, Mitashi P. Conducting an Ebola vaccine trial in a remote area of the Democratic Republic of the Congo: Challenges, mitigations, and lessons learned. Vaccine. 2023 Dec 12;41(51):7587-7597. doi: 10.1016/j.vaccine.2023.11.030. Epub 2023 Nov 22. PMID: 37993355.
13. **Zola Matuvanga T**, Larivière Y, Lemey G, Isekah Osang'ir B, Mariën J, Milolo S, Meta R, Matangila J, Maketa V, Mitashi P, Van Geertruyden JP, Muhindo-Mavoko H, Van Damme P. Longitudinal assessment of an Ebola vaccine trial understanding among healthcare providers in the Democratic Republic of the Congo. Vaccine. 2023 Dec 31:S0264-410X(23)01530-X. doi: 10.1016/j.vaccine.2023.12.076. Epub ahead of print. PMID: 38163747.
14. Larivière, Y., **Zola Matuvanga, T**., Osangir, B., Milolo, S., Meta, R., Kimbulu, P., Robinson, C., Katwere, M., McLean, C., Matangila, J., Maketa, V., Mitashi, P., Van geertruyden, J.-P., Van Damme, P., & Muhindo-Mavoko, H. (2024). Long-term immunogenicity of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen and safety of and immune memory response to Ad26.ZEBOV booster vaccination in healthcare providers and frontliners of the Democratic Republic of the Congo: an open-label, randomised, phase 2 trial. The Lancet Infectious Diseases. Advance online publication.
15. Muhindo-Mavoko, H., & **Zola Matuvanga**, T. Gesundheitssystem (pp. 461-470). In, Bobineau, J., Gieg, P., & Lowinger, T. (Eds.). (2024). Handbuch Demokratische Republik Kongo: Geschichte, Politik, Gesellschaft, Kultur. [Number of pages] Seiten gebunden. Frank & Timme. ISBN 978-3-7329-0434-1.
16. **Zola Matuvanga T**, Bikioli Bolombo F, Paviotti A, Larivière Y, Lemey G, Salloum M, Isekah Osang'ir B, Matangila J, Maketa V, Esanga E, Milolo S, Mitashi P, Van Damme P, Muhindo-Mavoko H, Van geertruyden JP. Healthcare providers' and frontline workers' experiences of an Ebola vaccine trial in the Boende Health District of the Democratic Republic of the Congo. Am J Trop Med Hyg. Forthcoming 2024.
17. Kabalu Tshiongo J, **Matuvanga TZ,** Mitashi P, Maketa V, Schallig HDFH, Mens PF, Muhindo Mavoko H, Matangila-Rika J. Prevention of Malaria in Pregnant Women and its Effects on Maternal and Child Health, the Case of Centre Hospitalier de Kingasani II in Democratic Republic of the Congo. Journal of Tropical Medicine and Infectious Disease. In press since April 10, 2024.

I certify that the information provided above is correct.