Improving the measurement of vaccine-attributable impact in the context of concurrent public health interventions in low- and middle-income countries

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

by

Dr Latif Omar Ndeketa

May 2026

Declaration

I, Latif Ndeketa, declare that this thesis is my own work and has been submitted for the degree of Doctor of Philosophy at the University of Liverpool. I declare that no portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or institute of learning.

Except where otherwise stated, the work presented in this thesis was conducted by me during my period of registration at the University of Liverpool. I have acknowledged all sources of information, data, methods, figures and ideas that were not my own. I confirm that I have not knowingly copied, plagiarised, fabricated or falsified any part of this thesis.

I did not use AI tools to generate data, conduct analyses, create results, or replace my own interpretation. I reviewed and edited all outputs, and I take full responsibility for the final content of this thesis. Wherever necessary, I used ChatGPT to support word count refinement and wording suggestions.

Several chapters are written as standalone manuscripts prepared for publication. These chapters were developed through collaborative research and the contributions of the co-authors are acknowledged in the relevant sections of the thesis. In each results chapter, I led or made substantial contributions to the design, analysis, interpretation and writing of the work presented.

I confirm that this thesis has not been previously submitted for a degree or any other qualification at the University of Liverpool or any other institution.

Candidate: Dr Latif Ndeketa

Supervisors: Dr Daniel Hungerford, Prof Peter Dodd and Prof Neil French

Contents

Declaration 2

List of tables 10

List of figures 11

List of abbreviations 12

Supporting papers 17

Conference presentations 18

Acknowledgements 20

Abstract 21

1 Thesis introduction 23

1.1 Infectious Disease Burden in Sub-Saharan Africa 23

1.1.1 Historical trends in infectious burden in low-income settings 23

1.1.2 Transmission dynamics and implications for vaccine evaluation 24

1.2 Immunisation as a Central Public Health Strategy 25

1.2.1 What are vaccines 25

1.2.2 Historical impact of vaccination programmes 26

1.2.3 Innate and adaptive immune responses to vaccination 26

1.2.4 Immune memory and durability of protection 27

1.2.5 Correlates of protection 28

1.3 Vaccine platforms and implications for use 28

1.3.1 Whole-cell vaccines 28

1.3.2 Protein-based/Subunit and antigen-based vaccines 29

1.3.3 Nucleic acid and vector-based vaccines 29

1.4 Population-level effects of vaccination 30

1.4.1 Herd protection and shift in age of infection 30

1.5 Evolution of the Expanded Programme on Immunisation (EPI) 31

1.5.1 Origins and establishment of the EPI 31

1.5.2 Expansion of vaccine schedules 31

1.5.3 Vaccine financing and global partnerships 32

1.5.4 Vaccine introduction in LMIC immunisation programmes 33

1.5.5 Vaccine uptake and schedule timing in Malawi 34

1.5.6 Vaccine hesitancy in Malawi 35
1.6 Implications for evaluation of vaccine impact 35
1.7 Persistent burden of diarrhoeal diseases, cholera, and malaria 36
1.7.1 Rotavirus epidemiology 36
1.7.2 Pathogenesis and clinical presentation 36
1.7.3 Diagnosis and implications for evaluation 38
1.7.4 Management and determinants of severity 38
1.7.5 Rotavirus vaccines 39
1.8 Cholera 40
1.8.1 Cholera aetiology and transmission 40
1.8.2 Pathogenesis and clinical presentation 41
1.8.3 Burden and epidemiological patterns 42
1.8.4 Cholera control and role of vaccination 43
1.8.5 Implications for vaccine evaluation 43
1.9 Malaria 44
1.9.1 Malaria diagnosis and treatment 44
1.9.2 Malaria control interventions 46
1.9.3 Malaria vaccines 46
1.9.4 RTS,S/AS01 47
1.9.5 R21/Matrix-M vaccine 47
1.10 From efficacy to real-world impact 48
1.10.1 Definitions of vaccine efficacy, effectiveness, and impact 48
1.10.2 Limits of randomised controlled trials 49
1.10.3 Study designs used in vaccine evaluation 50
1.11 Concurrent interventions and attribution of vaccine impact 52
1.11.1 Complexity of attribution in routine settings 52
1.11.2 Cluster randomised implementation as an illustrative example 53
1.11.3 Limits of applicability in routine programmes 53
1.11.4 Implications for vaccine evaluation in sub-Saharan Africa 54
1.12 Thesis rationale, aim and objectives 56
1.12.1 Thesis starting point and rationale 56

1.12.2 Thesis aim and objectives: 56
1.12.3 Thesis outline 57
2 Methods used to adjust for non-vaccine interventions in post-licensure vaccine evaluations among children in sub-Saharan Africa: a systematic review 60
2.1 Abstract 60
2.2 Introduction 62
2.2.1 Vaccine-preventable diseases and post-licensure evaluation 62
2.2.2 Overview of post-licensure study designs for vaccine evaluation 62
2.2.3 Challenges in attributing vaccine effects in routine settings 66
2.3 Methods 66
2.3.1 Review design 66
2.3.2 Search strategy 66
2.3.3 Study selection and screening 67
2.3.4 Inclusion and exclusion criteria 67
2.3.5 Data extraction 68
2.3.6 Quality assessment of included studies 68
2.3.7 Data analysis 68
2.4 Results 70
2.4.1 Study selection 70
2.4.2 JBI quality assessment results 71
2.4.3 Study characteristics 71
2.4.4 Vaccine effectiveness and impact 92
2.4.5 Heterogeneity in effectiveness estimates: rotavirus case study 94
2.5 Discussion 96
2.5.1 Summary of key findings 96
2.5.2 Accounting for PHSMs across study designs 97
2.5.3 Recommendations for future studies 99
2.5.4 Implications for policy and practice 100
2.5.5 Strengths and limitations 101
2.6 Conclusion 102

3 The long-term impact and effectiveness of rotavirus vaccination in Malawi: an interrupted time-series and case–control analysis 104
3.1 Abstract 104
3.2 Introduction 106
3.3 Methods 107
3.3.1 Study setting and population 107
3.3.2 Surveillance data 107
3.3.3 Study design 108
3.3.4 Statistical analysis 108
3.3.5 Interrupted time-series analysis of vaccine impact 108
3.3.6 Test-negative case–control study for vaccine effectiveness 109
3.3.7 Analysis of the tOPV to bOPV switch and Rotarix® effectiveness 110
3.3.8 Missing data 110
3.3.9 Ethical approval 110
3.3.10 Patient and public involvement 110
3.4 Results 111
3.4.1 Study population and age distribution of RVGE hospitalisations 111
3.4.2 Long-term impact of Rotarix® on RVGE hospitalisations 111
3.4.3 Variation in Rotarix® impact over time 113
3.4.4 Rotarix® vaccine effectiveness 114
3.4.5 Effect of the tOPV to bOPV switch on Rotarix® effectiveness 115
3.5 Discussion 116
3.5.1 Main findings 116
3.5.2 Interpretation in relation to previous evidence 116
3.5.3 Implications for vaccination policy 117
3.5.4 Oral vaccine interference and the tOPV to bOPV switch 118
3.5.5 Strengths and limitations 118
3.6 Conclusion 119
3.7 Acknowledgements 119
3.7.1 Funding 119
3.7.2 Conflicts of interest 120

3.7.3 Contributions 120
4 Improving estimation of vaccine effectiveness during outbreaks in low-resource settings: A case study of oral cholera vaccination during the 2022-2023 cholera outbreak in Malawi 121
4.1 Abstract 121
4.2 Introduction 123
4.3 Methods 125
4.3.1 Study setting 125
4.3.2 Case data 125
4.3.3 Vaccine data 125
4.3.4 WASH data 126
4.3.5 Statistical analysis 126
4.3.6 Sensitivity analysis 128
4.3.7 Missing data 129
4.4 Results 129
4.4.1 Demographic characteristics of reported cholera cases 129
4.4.2 Spatial distribution of reported cholera cases 130
4.4.3 Outbreak dynamics and timing of interventions 131
4.4.4 Vaccine effectiveness estimates 134
4.4.5 Sensitivity analyses 134
4.5 Discussion 136
4.6 Conclusion 140
4.7 Acknowledgements 140
4.7.1 Funding 140
4.7.2 Conflicts of interest 141
4.7.3 Contributions 141
5 Estimating RTS,S/AS01 malaria vaccine effectiveness in routine programme settings: comparison of cluster-level and individual-level analyses using the Malawi MVIP endline survey 142
5.1 Abstract 142
5.2 Introduction 144
5.3 Methods 147
5.3.1 Study design and setting 147

5.3.2 Household surveys 148
5.3.3 Sampling design and participants 148
5.3.4 Cluster-randomised analysis 149
5.3.5 Matched case–control analysis 151
5.3.6 Unmatched case–control analysis 152
5.3.7 Ethical considerations and consent 152
5.4 Results 153
5.4.1 Cluster-randomised analysis 153
5.4.2 Matched case–control analysis 155
5.4.3 Unmatched case–control analysis 157
5.5 Discussion 158
5.5.1 Summary of findings 158
5.5.2 Why the cluster-level and individual-level estimates differed 158
5.5.3 Interpreting mRDT positivity as an endpoint 159
5.5.4 Adjustment, matching, and comparability across analyses 159
5.5.5 Interpretation within the wider MVIP evidence base 160
5.5.6 Evidence from the EPI-MAL-003 prospective cohort 160
5.5.7 Matched and unmatched individual-level estimates 161
5.5.8 Implications for future evaluation 161
5.5.9 Strengths and limitations 162
5.6 Recommendations for vaccine policy and future evaluations 163
5.6.1 Vaccine policy 163
5.6.2 Future vaccine evaluations 163
5.7 Conclusion 163
5.8 Acknowledgements 164
5.8.1 Funding 164
5.8.2 Conflicts of interest 164
5.8.3 Contributions 164
6 Discussion 166
6.1 Reframing vaccine impact in routine settings 166
6.2 Study design as a determinant of observed effect 167

6.2.1 Divergence across analytical approaches 167
6.2.2 What is being estimated 168
6.3 Analysis approach as a determinant of observed effect 173
6.3.1 Evidence from cholera analyses 173
6.3.2 Parallel evidence from RTS,S 175
6.4 Recommendations from the thesis 177
6.4.1 Interpret estimates in relation to design and outcome 177
6.4.2 Prioritise clinically relevant and incidence-based endpoints 177
6.4.3 Strengthen routine data quality for attribution 178
6.4.4 Account explicitly for concurrent interventions 178
6.4.5 Use design-based approaches carefully 179
6.4.6 Ensure consistency in surveillance before and after introduction 179
6.4.7 Communicate differences in estimates clearly 179
6.4.8 Avoid reliance on a single estimate 180
6.5 Future directions 180
6.6 Conclusion 183
References 184
Supplementary material 225

List of tables

Table 1-1: Licensed oral rotavirus vaccines recommended by WHO 39
Table 1-2: Vaccine evaluation challenges across the three thesis pathogens 55
Table 2-1: Overview of post-licensure study designs for vaccine evaluation 64
Table 2-2: Characteristics of included studies 72
Table 2-3: Descriptive statistics of included studies 90
Table 2-4: Analysis of study designs and public health and social measures 91
Table 3-1: Negative binomial regression results showing the impact of Rotarix® introduction on rotavirus-positive hospitalisations by age group, adjusted for seasonality, secular trends, and test-negative controls 113
Table 3-2: Regression estimates and 95% confidence intervals for models used to estimate Rotarix® vaccine effectiveness across age strata 115
Table 3-3: Results of the interaction analysis between oral poliovirus vaccine (OPV) and Rotarix® showing adjusted estimates of Rotarix® effectiveness among children eligible for both vaccines 115
Table 4-1: Descriptive characteristics of cholera cases in Blantyre District, Malawi, January 2022–April 2023 129
Table 4-2: Posterior estimates of vaccine coverage and WASH effects on R_t 134
Table 4-3: Vaccine effectiveness estimates from the WASH-adjusted model across alternative WASH lag specifications 135
Table 5-1: Baseline demographic characteristics between vaccinating and non-vaccinating clusters 153
Table 5-2: Cluster-level comparison of malaria prevalence between intervention and control clusters 154
Table 5-3: Characteristics of the analytic dataset before matching, stratified by case–control status 155
Table 5-4: Covariate balance before and after matching, expressed as standardised mean differences 156
Table 5-5: Matched case–control estimates of RTS,S effectiveness under primary and alternative matching specifications 156
Table 5-6: Unmatched case–control estimates of RTS,S effectiveness 157

List of figures

Figure 1-1: Under-five mortality rate per 1000 live births. Adapted from UNIGME Child mortality report 2024 (1) 23
Figure 1-2: GAVI co-financing model 2026. Adapted from GAVI co-financing policy (55) 33
Figure 1-3: Structure and proteins of rotavirus. Adapted from Greenberg and Estes (59) 37
Figure 1-4: V. cholerae is classified into serogroups based on the composition of the O antigen of LPS. Adapted from Montero et al. 2023(85) 41
Figure 1-5: Cholera toxin pathogenesis. Adapted from Thiagarajah et al. 2005(89) 42
Figure 1-6: Circumsporozoite protein subunit vaccines RTS,S and R21 structure. Adapted from Tsoumani et al. 2023(117) 48
Figure 1-7: Type of vaccine effect estimates. Adapted from Hungerford et al. 2018(120) [Abbreviations: VE, vaccine effectiveness; λ, hazard rate of infection] 49
Figure 1-8: Conceptual framework for estimating vaccine-attributable effects in routine programme settings. Individual-level risk factors such as age, nutritional status, HIV status, prior infection, socioeconomic status and care-seeking behaviour are not shown 54
Figure 2-1: PRISMA flow chart selection process 70
Figure 2-2: Study locations and number in Sub-Saharan Africa 89
Figure 2-3: Forest plot of rotavirus vaccine effectiveness from case–control studies in children under five years in sub-Saharan Africa 95
Figure 3-1: Sample size flow diagram 111
Figure 3-2: Observed versus counterfactual RVGE cases before and after vaccine introduction 112
Figure 3-3: Annual estimates of rotavirus vaccine impact by age group since RV1 introduction showing year-to-year variation in the reduction of RVGE hospitalisations among children under five years 114
Figure 4-1: Spatial distribution of reported Blantyre cholera cases by health facility catchment area 131
Figure 4-2: Temporal distribution of cholera cases in Blantyre 132
Figure 4-3: Weekly cholera incidence and time-varying transmission dynamics in Blantyre, alongside the timing of vaccination and WASH interventions from November 2022. Panel A shows weekly reported cases. Panel B shows the median R_t with 95% credible intervals. Panel C shows cumulative vaccine coverage. Panel D shows WASH activity intensity, expressed as households reached per 1,000 population. The dashed vertical line marks the start of vaccination 133

List of abbreviations

ACT	Artemisinin-based combination therapy
AGE	Acute gastroenteritis
AL	Artemether-lumefantrine
AS01	Adjuvant system used in RTS,S/AS01
aOR	Adjusted odds ratio
BCG	Bacille Calmette-Guerin vaccine
BDHO	Blantyre District Health Office
bOPV	Bivalent oral poliovirus vaccine
CATI	Case-area targeted intervention
CFR	Case fatality rate or case fatality risk
CI	Confidence interval
CINAHL	Cumulative Index to Nursing and Allied Health Literature
CITS	Controlled interrupted time-series
CrI	Credible interval
CSP	Circumsporozoite protein
CT	Cholera toxin
DHO	District Health Office
DHS	Demographic and Health Survey
DNA	Deoxyribonucleic acid
DTP	Diphtheria-tetanus-pertussis vaccine
EA	Enumeration area
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency

EMBASE	Excerpta Medica Database
EPI	Expanded Programme on Immunisation
EPI-MAL-003	RTS,S/AS01E malaria vaccine implementation study identifier
Gavi	Gavi, the Vaccine Alliance
GTS	Global Technical Strategy
HBsAg	Hepatitis B surface antigen
Hib	Haemophilus influenzae type b
HIC	High-income country
HIV	Human immunodeficiency virus
HR	Hazard ratio
ICC	Intraclass correlation coefficient
IPTp	Intermittent preventive treatment in pregnancy
IRR	Incidence rate ratio
IRS	Indoor residual spraying
ITN	Insecticide-treated net
JBI	Joanna Briggs Institute
LAMP	Loop-mediated isothermal amplification
LLIN	Long-lasting insecticidal net
LMIC	Low- and middle-income country
LPS	Lipopolysaccharide
MAM	Moderate acute malnutrition
MeSH	Medical Subject Headings
MITAG	Malawi Immunization Technical Advisory Group
MLW	Malawi-Liverpool-Wellcome Research Programme
MPL	Monophosphoryl lipid A
MRC	Medical Research Council
MUAC	Mid-upper arm circumference

MVIE	Malaria Vaccine Implementation Evaluation
MVIP	Malaria Vaccine Implementation Programme
NIHR	National Institute for Health and Care Research
NMCP	National Malaria Control Programme
NPO	Non-profit organization
NSO	National Statistical Office
NSP4	Non-structural protein 4
OCV	Oral cholera vaccine
OPV	Oral poliovirus vaccine
OR	Odds ratio
ORS	Oral rehydration salts
PAF	Population attributable fraction
PCR	Polymerase chain reaction
PCV	Pneumococcal conjugate vaccine; in screening-method context, proportion of cases vaccinated
PCV7	7-valent pneumococcal conjugate vaccine
PCV10	10-valent pneumococcal conjugate vaccine
PCV13	13-valent pneumococcal conjugate vaccine
pfhrp2/pfhrp3	Plasmodium falciparum histidine-rich protein 2/3 genes
PHSM	Public health and social measure
PMC	Perennial malaria chemoprevention
PPV	Proportion of the population vaccinated
PR	Prevalence ratio
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QECH	Queen Elizabeth Central Hospital
R21/Matrix-M	R21 malaria vaccine formulated with Matrix-M adjuvant
RDT	Rapid diagnostic test

RNA	Ribonucleic acid
RR	Risk ratio or rate ratio, depending on the model context
RRV-TV	Rhesus-human reassortant tetravalent vaccine
RT-PCR	Reverse transcription polymerase chain reaction
RTS	Fusion protein component of RTS,S
RTS,S/AS01	Recombinant circumsporozoite protein malaria vaccine formulated with AS01 adjuvant
RV1	Monovalent rotavirus vaccine
RV3-BB	Neonatal rotavirus vaccine candidate
RV5	Pentavalent rotavirus vaccine
RVGE	Rotavirus gastroenteritis
R_t	Time-varying reproduction number
SAM	Severe acute malnutrition
SD	Standard deviation
SES	Socioeconomic status
SMC	Seasonal malaria chemoprevention
SMD	Standardised mean difference
SSA	Sub-Saharan Africa
TCP	Toxin-coregulated pilus
TES	Therapeutic efficacy study
tOPV	Trivalent oral poliovirus vaccine
U5MR	Under-five mortality rate
UNICEF	United Nations Children's Fund
VE	Vaccine effectiveness
VLP	Virus-like particle
VP4	Rotavirus outer capsid protein VP4
VP6	Rotavirus inner capsid protein VP6

VP7	Rotavirus outer capsid protein VP7
WASH	Water, sanitation and hygiene
WHO	World Health Organization

Supporting papers

1. **L. Ndeketa**, V. Haine, M. Debois et al., “Effectiveness of the RTS,S/AS01E malaria vaccine in a real-world setting over 1 year of follow-up after the three-dose primary schedule: an interim analysis of a phase 4 study in Ghana, Kenya, and Malawi,” Lancet. Glob. Heal., vol. 14, no. 1, pp. e61–e69, Jan. 2026, doi: 10.1016/S2214-109X(25)00415-2.
2. V. Haine, M. Oneko, M. Debois, **L. Ndeketa**, P. D. Agyapong, O. Boahen, et al. Safety of RTS,S/AS01E malaria vaccine up to 1 year after the third dose in Ghana, Kenya, and Malawi (EPI-MAL-003): a phase 4 cohort event monitoring study. Lancet Glob Health. 2025 Jun;13(6):e995-e1005. DOI: 10.1016/S2214-109X(25)00096-8
3. V. E. Pitzer, **L. Ndeketa**, E. O. Asare, D. Hungerford et al., “Impact of rotavirus vaccination in Malawi from 2012 to 2022 compared to model predictions,” npj Vaccines 2024 91, vol. 9, no. 1, pp. 1–8, Nov. 2024, doi: 10.1038/s41541-024-01008-6
4. **L. Ndeketa**, N. M. Vaselli, V. E. Pitzer, P. J. Dodd, D. Hungerford, and N. French, “Methods of adjustment for non-vaccine interventions in post-licensure vaccine studies in children in sub–Saharan Africa: a systematic review,” medRxiv, p. 2026.04.01.26349767, Apr. 2026, doi: 10.64898/2026.04.01.26349767
5. **L. Ndeketa**, V. E Pitzer, K. C Jere, A. Bennet, N. A. Cunliffe, P. J. Dodd, N. French, D. Hungerford, “The long-term impact and effectiveness of rotavirus vaccination in Malawi: an interrupted time-series and case–control analysis,” medRxiv, p. 2026.02.27.26346681, Mar. 2026, doi: 10.64898/2026.02.27.26346681.
6. **L. Ndeketa**, D. Hungerford, V. E. Pitzer, N. French, P. J. Dodd et al., “Improving estimation of vaccine effectiveness during outbreaks in low-resource settings: A case study of oral cholera vaccination during the 2022-2023 cholera outbreak in Malawi,” medRxiv, p. 2026.03.29.26349659, Mar. 2026, doi: 10.64898/2026.03.29.26349659.
7. C. Baluwa, P. K. Kalonde, U. L Mseka, O. Tsoka, K. Kajanga, F. Chimphondah, P. Aaron, F. Chimbalanga, B. Chiepa, C. Nkolokosa, **L. Ndeketa**, P. Musicha, K. C Jambo, M. Stanton, J. Chirombo, “Updated fine-scale location data for Blantyre district collected using a low-cost approach,” Sci. Data, vol. 12, no. 1, p. 1275, Dec. 2025, doi: 10.1038/S41597-025-05605-5

Conference presentations

1. L. Ndeketa, D. Hungerford, N. French and P. J. Dodd (January 2026). *Improving estimation of vaccine effectiveness during outbreaks in low-resource settings: A case study of oral cholera vaccination during the 2022-2023 cholera outbreak in Malawi*. Abstract driven oral presentation at the **Research & Impact: Combating Infection conference** at the University of Liverpool, UK.

2. L. Ndeketa, D. Hungerford, N. French and P. J. Dodd (November 2025). *Improving estimation of vaccine effectiveness during outbreaks in low-resource settings: A case study of oral cholera vaccination during the 2022-2023 cholera outbreak in Malawi*. Abstract driven oral presentation at the **American Society of Tropical Medicine and Hygiene (ASTMH)** 2025 Annual Meeting. Toronto, Canada.

3. L. Ndeketa, V. E. Pitzer, N. French, P. J. Dodd, D. Hungerford (September 2025). *The effectiveness and impact of rotavirus vaccination in children under the age of five over 7 years and the influence of polio vaccine: an interrupted time-series and case–control analysis for Blantyre, Malawi*. abstract driven poster presentation at the **15th Rotavirus symposium** in Cape Town, South Africa. Awarded best poster presentation

4. L. Ndeketa, D. Hungerford, N. French and P. J. Dodd (November 2025). Improving estimation of vaccine effectiveness during outbreaks in low-resource settings: *A case study of oral cholera vaccination during the 2022-2023 cholera outbreak in Malawi*. Oral presentation at the joint **National Institute for Health and Care Research in Gastrointestinal Infections network meeting for the Health Protection Research Unit** hosted by the University of Liverpool.

5. L. Ndeketa, V. E. Pitzer, N. French, P. J. Dodd, D. Hungerford (October 2024). The effectiveness and impact of rotavirus vaccination in children under the age of five over 7 years and the influence of polio vaccine: an interrupted time-series and case–control analysis for Blantyre, Malawi. Abstract driven oral presentation at the **3rd Kamuzu University of Health Sciences (KUHeS) Research Dissemination Conference.**

6. L. Ndeketa, D. Hungerford, N. French and P. J. Dodd (December 2024). *Descriptive epidemiology of a deadly and persistent outbreak of Vibrio cholerae in Malawi, 2022-2023*. Abstract driven oral presentation at the **57th US-Japan Cooperative Medical Science Program Joint Panel Conference on Cholera and Other Bacterial Enteric Infections** in Okinawa, Japan.

7. L. Ndeketa, V. E. Pitzer, N. French, P. J. Dodd, D. Hungerford (November 2023). *The effectiveness and impact of rotavirus vaccination in children under the age of five over 7 years and the influence of polio vaccine: an interrupted time-series and case–control analysis for Blantyre, Malawi*. Invited oral presentation at the **NIHR Global Health Research Group** in Nairobi, Kenya. Awarded best oral presentation.

8. L. Ndeketa, D. Hungerford, N. French and P. J. Dodd (November 2023). *Descriptive epidemiology of a deadly and persistent outbreak of Vibrio cholerae in Malawi, 2022-2023*. Abstract driven poster presentation at the **North East Postgraduate Conference** in Newcastle, UK.

Acknowledgements

This work was supported by my studentship from the UK Medical Research Council Discovery Medicine North Doctoral Training Programme (DiMeN) (MR/W006944/1).

I would first like to thank everyone who supported the data access, preparation and analysis for the three empirical chapters of this thesis including all co-authors of the manuscripts. For the systematic review, I would like to thank Marcella Vaselli, who was my second reviewer. Thank you for the time, care and patience you put into the screening, data extraction and quality assessment. Your contribution made the review much stronger. For the rotavirus vaccine evaluation, I am grateful to Nigel Cunliffe, Khuzwayo Jere, Virginia Pitzer and Aisleen Bennett, whose work helped establish and sustain the rotavirus surveillance platform at Queen Elizabeth Central Hospital.

For the cholera chapter, I am grateful to Kondwani Jambo for helping gather the case data, and to Chrissy Banda and Nelson Kumwenda from the Blantyre District Health Office for their support with the vaccine and WASH data. I would also like to thank Patrick Kalonde for providing the shapefiles and helping me develop the cholera burden maps. For the RTS,S/AS01 malaria vaccine evaluation, I would like to thank Don Mathanga and Harrison Msuku from the Malaria Alert Centre for their support with access to the MVIP endline survey data.

I am deeply grateful to my supervisory team, Daniel Hungerford, Neil French and Peter Dodd. Thank you for your patience, guidance and constant support throughout this PhD. You created an environment where I could learn, make mistakes, ask questions and grow as a researcher. I am also grateful for the friendship and generosity you showed me throughout this journey.

Finally, I would like to thank my wife, Matina, and our kids, Amirah and Junaid. This PhD took time away from all of you, and I know that came with many sacrifices. Thank you for bearing with the long days, the late nights and the many moments when I was travelling or when I was physically present but mentally still working. Your love, patience and support carried me through this work more than I can properly put into words.

Abstract

Post-licensure vaccine evaluations are usually interpreted as evidence of vaccine impact under routine programme conditions. In low- and middle-income countries, these evaluations often take place in settings where disease burden is also modified by changes in water, sanitation and hygiene (WASH), other vaccines, case management, bed net use, chemoprevention, surveillance practices and healthcare access. This thesis examined how vaccine-attributable impact can be measured more reliably when vaccination is delivered alongside other public health interventions that also affect the disease burden.

I first conducted a systematic review of post-licensure vaccine effectiveness (VE) and impact studies in children under five years in sub-Saharan Africa from 2005 to 2019. Sixty-four studies were included, covering eight vaccine-preventable diseases. Case–control and ecological designs were most common. None of the included studies measured, reported or adjusted for concurrent public health and social measures such as WASH, nutrition, chemoprevention or access to healthcare. This omission had different effects across pathogens. For rotavirus, estimates from comparable case–control studies were relatively consistent across settings. For pneumococcal conjugate vaccines, estimates varied more widely, partly because studies used different clinical endpoints, populations and analytic approaches.

I then evaluated the long-term impact and effectiveness of rotavirus vaccine (Rotarix®) in Blantyre, Malawi using hospital surveillance data from 1997 to 2019. I also assessed whether Malawi's national switch from trivalent to bivalent oral poliovirus vaccine modified Rotarix® VE, given concerns that OPV may interfere with oral rotavirus vaccine performance. Across 7,952 acute gastroenteritis admissions, Rotarix® introduction was associated with a 23% reduction in rotavirus gastroenteritis hospitalisations among children under five years and a 37% reduction among infants. Among children aged ≥1 year, rotavirus-positive admissions increased after vaccine introduction, suggesting delayed primary infection and waning protection. In the test-negative case–control analysis, Rotarix® continued to show protection of 52% against hospitalisation in infants, with no evidence that the OPV switch modified VE.

For cholera, I developed a novel transmission-based approach to estimate vaccine impact using routine outbreak response data from Blantyre during the 2022–2023 epidemic. Oral cholera vaccination was associated with a 53.5% reduction in transmission before adjustment and 62.1% after including lagged WASH activity. This analysis showed that routinely collected intervention data can be used to separate vaccine-attributable reductions from concurrent outbreak response activities when individual vaccination histories are incomplete.

Finally, I compared cluster-level and individual-level approaches for estimating RTS,S/AS01 malaria VE using the Malawi MVIP endline survey data. The cluster-level comparison was imprecise and gave a VE estimate of 8% against mRDT positivity. The unmatched case–control analysis gave a 41% VE and the matched case–control gave a 58.3% VE after matching on cluster and age. These differences reflected endpoint choice, incomplete vaccine uptake, age imbalance and spatial heterogeneity in malaria risk.

These findings show that vaccine impact estimates are influenced by study design, the endpoints measured and the extent to which concurrent interventions are measured and accounted for. This thesis links those differences to the assumptions built into each design and to the structure of the data available. The aim is not to identify one estimate, but to clarify what each estimate represents and how it should be interpreted in practice. Future evaluations need to prospectively record key non-vaccine interventions that may impact on effects estimates and use appropriate endpoints that match the target vaccine effect being estimated. This understanding and evidence is critical for efficient public health decision making under increasingly restricted national and international finances.

1 Thesis introduction

1.1 Infectious Disease Burden in Sub-Saharan Africa

1.1.1 Historical trends in infectious burden in low-income settings

Since the year 2000, the global mortality of children under 5 years of age has declined by more than half. According to United Nations figures, the under-five mortality rate (U5MR) in 2000 was 77 deaths per 1000 live births which declined to 37 deaths per 1000 live births by 2023. This decline in U5MR is attributed to improved public health interventions, improved access to healthcare and broader socioeconomic developments. Despite these notable improvements in U5MR, the period from 2000 to 2023 still witnessed a cumulative 170 million reported deaths among children under five(1). 4.8 million of these deaths were reported in 2023 alone.

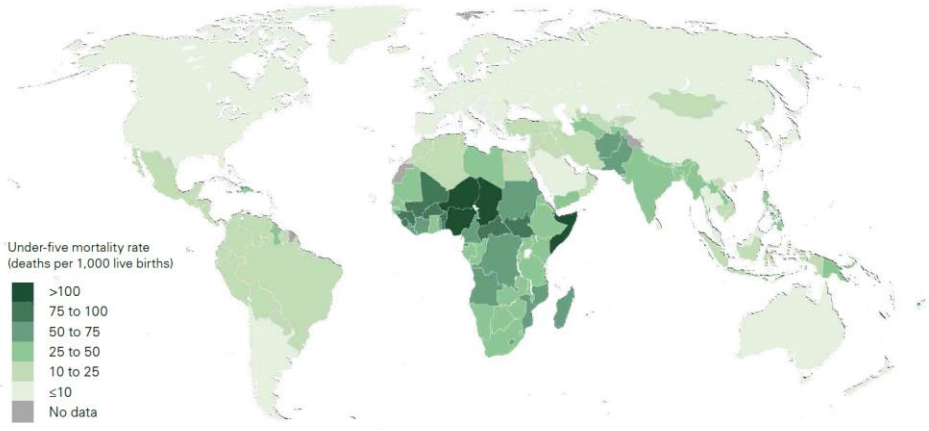


Figure 1-1: Under-five mortality rate per 1000 live births. Adapted from UNIGME Child mortality report 2024 (1)

Sub-Saharan Africa (SSA) remains the region with the highest U5MR at 69 (62-82) deaths per 1000 live births which is 14 times higher than Europe and North America (1). Approximately 58% of all the under-five deaths in the world were reported in Sub-Saharan Africa(2). The leading causes of death in SSA differ markedly from high-income settings with infectious diseases and neonatal complications dominating the burden. Vaccine-preventable infectious diseases such as pneumonia (14–16% of under-five deaths), diarrhoea(9%) and malaria (7-10%)

remain among the leading killers of children aged 1-59 months in SSA(3,4). These patterns point to continuous gaps in vaccine coverage, WASH, and timely access to effective treatment.

HIV infection continues to influence child survival in high-prevalence settings. During the neonatal period, mortality remains largely driven by complications of prematurity, intrapartum events, and sepsis, with these risks often amplified where there is poor access to skilled obstetric care(3,4). Broader socioeconomic factors for U5MR such as poverty, poor maternal education, low birth weight, limited access to healthcare, fragile health systems and malnutrition continue to shape under-five mortality patterns across SSA (5).

This thesis focuses on three infections that remain major public health challenges in Malawi and the wider sub-Saharan African region: rotavirus, cholera, and malaria. These infections differ in their biology, transmission pathways, vaccine platforms, and delivery strategies, but they share a common evaluation problem. Their observed burden is shaped not only by vaccination, but also by concurrent public health and social measures (PHSMs) such as WASH, case management, vector control, chemoprevention, and changes in access to care.

1.1.2 Transmission dynamics and implications for vaccine evaluation

The three pathogens examined in this thesis differ in how they are transmitted and in where vaccines and other interventions act on the disease pathway. Rotavirus is transmitted mainly through the faecal-oral route, through direct contact with infected children or caregivers and through contaminated hands, surfaces and shared objects. Infection occurs early in life, and the first infection is usually more likely to cause severe disease than later infections because immunity builds after repeated exposure(6). Maternal immunity may delay the first infection in young infants, while seasonality, transmission intensity and vaccine response can influence the age at which severe rotavirus gastroenteritis (RVGE) occurs after vaccine introduction(6). Rotavirus vaccines do not fully prevent infection, but they reduce severe RVGE, particularly in infancy. ORS, zinc, water, sanitation and hygiene (WASH) and timely care-seeking act later in the pathway by reducing dehydration, complications and onward contamination(6,7).

Cholera is also transmitted through the faecal-oral route, but outbreaks are shaped by both direct spread around cases and environmental transmission through contaminated water.

Cholera transmission is linked to poverty as people living in high density “slums” with poor WASH are at higher risk of infection. Oral cholera vaccines (OCV) reduce susceptibility to cholera disease, while WASH interventions act on different parts of the transmission pathway: sanitation reduces contamination of water, water treatment increases bacterial removal, point-of-use purification reduces bacterial concentration in drinking water, and alternative safe water sources reduce exposure to contaminated water(8). Case management then reduces severe dehydration and death.

Malaria differs from both of these enteric infections because transmission depends on interactions between humans and *Anopheles* mosquitoes. Transmission is shaped by infectious mosquito abundance, human biting exposure, parasite prevalence, seasonality, rainfall and acquired immunity from repeated exposure. RTS,S/AS01 and R21/Matrix-M are pre-erythrocytic malaria vaccines that target Plasmodium sporozoites and liver-stage infection before blood-stage disease develops. Insecticide treated bed nets and indoor residual spraying (IRS) reduce mosquito exposure, chemoprevention such as intermittent preventive treatment in pregnant women (IPTp), seasonal malaria chemoprophylaxis (SMC), post-discharge malaria chemoprevention (PDMC) and perennial malaria chemoprevention (PMC) reduces blood-stage infection, and diagnosis and treatment reduce progression to severe disease and onward transmission(9,10). Vaccination therefore acts within transmission systems already modified by other interventions, which is why vaccine-attributable effects need to be interpreted alongside changes in PHSMs and routine disease control(10).

1.2 Immunisation as a Central Public Health Strategy

1.2.1 What are vaccines

Vaccination is widely regarded as one of the most impactful public health interventions of the modern era, only improvements in clean water and sanitation have performed better in contribution to population health gains(11). Vaccination is also the most cost-effective health intervention in modern history(12). Vaccines are defined as biological preparations derived from pathogens or their components that are designed to safely stimulate the host immune system and generate protective immunity upon subsequent exposure(11). Through the induction of

immunological memory, vaccination reduces the likelihood of infection, severe disease, and onward transmission(11). Vaccines have been proven to be safer than therapeutics and still undergo broader safety monitoring with public scrutiny because any perceived risk can have disproportionate consequences for uptake(13).

1.2.2 Historical impact of vaccination programmes

The public health impact of vaccination is well illustrated by historical experience. The global eradication of smallpox in 1980 remains one of the most notable achievements of coordinated immunisation efforts(14,15). Smallpox was a substantial global threat and was associated with high case fatality before the introduction of vaccines and it remains the only infectious disease to date that is certified as eradicated. Similarly, the incidence of poliomyelitis has declined by more than 99% since the launch of the Global Polio Eradication Initiative in 1988, preventing millions of cases of paralysis worldwide(16). Measles mortality has also fallen markedly since the introduction and scale-up of measles vaccination programmes, despite the virus remaining highly transmissible. Measles vaccination has been the most cost-effective among the vaccines available to children with US$58 saved for every US$1 invested(17). These examples show how sustained immunisation programmes can reshape the epidemiology of infectious diseases at scale.

1.2.3 Innate and adaptive immune responses to vaccination

Protection following vaccination is initiated at the site of antigen exposure and involves both innate and adaptive immune responses. Vaccines introduce antigens, typically proteins or polysaccharides, which are recognised by the innate immune system(18). Resident antigen-presenting cells, including dendritic cells and macrophages, take up these antigens and process them for presentation to lymphocytes. In killed vaccines, adjuvants – substances included in vaccine formulations to enhance the immune response – stimulate pattern recognition receptors, generating local inflammatory signals that amplify antigen presentation and facilitate the transition from innate to adaptive immunity(19,20).

This initial activation leads to the generation of antigen-specific antibodies by B lymphocytes, which differentiate into plasma cells. These antibodies act through several pathways. They can

bind directly to pathogens or their toxins and neutralise their biological activity, block attachment and entry into host cells, and facilitate clearance through opsonophagocytosis by enhancing the activity of phagocytic cells such as macrophages and neutrophils(20). Antibody binding may also activate the complement cascade, contributing to pathogen lysis and further immune recruitment(18,20). Neutralising antibodies remain the main correlate of protection for many vaccines.

Cellular immune responses also contribute, particularly for intracellular pathogens. Cytotoxic CD8+ T lymphocytes can recognise and eliminate infected cells, while CD4+ T helper cells support B cell maturation and coordinate broader immune responses. In the absence of adequate T cell help, antibody responses are often weaker and less durable(19,20). The relative contribution of these mechanisms varies by pathogen and vaccine platform, although antibody-mediated protection remains central for many established childhood vaccines.

1.2.4 Immune memory and durability of protection

One of the key features of vaccine-induced protection is the generation of immune memory. Following vaccination, antigen-specific memory B and T lymphocytes persist and enable a more rapid and amplified immune response upon subsequent exposure to the pathogen(20). In infections with relatively long incubation periods this memory response may be sufficient to prevent clinical disease even after circulating antibody levels have waned. For example, protection against hepatitis B virus can persist for years despite declining measurable antibody titres, as immunological memory permits the prompt production of protective antibodies upon re-exposure(19).

Some rapidly progressive bacterial infections do require booster doses once circulating antibody concentrations fall below protective thresholds. The durability of vaccine-induced immunity therefore varies according to the nature of the antigen, the vaccine platform, the age at immunisation, and the need for booster doses(21). Understanding these dynamics is central to interpreting patterns of vaccine effectiveness and waning protection observed in real-world programme setting

1.2.5 Correlates of protection

The immune responses induced by vaccination are often assessed using correlates of protection, defined as measurable immunological markers that are associated with protection against infection or disease(22). Correlates are often based on antibody responses and are therefore closely linked to B cell–mediated immunity. Correlates of protection are typically identified through a combination of animal challenge models and clinical efficacy studies, where specific immune responses are shown to be associated with reduced risk of disease (23).

The relationship between immune markers and protection is not uniform across pathogens or clinical endpoints. Mechanisms of protection may differ by site of infection, stage of disease, and population characteristics such as age or prior exposure(23). For example, immune responses that limit infection at mucosal surfaces may differ from those required to prevent severe disease following systemic spread. In addition, vaccine performance may vary across settings, as seen with rotavirus vaccines, where effectiveness differs between high-income and low-income settings(24).

1.3 Vaccine platforms and implications for use

1.3.1 Whole-cell vaccines

Whole-cell vaccines use the entire pathogen, either in a live attenuated form or in an inactivated form. Live attenuated vaccines contain weakened organisms capable of limited replication within the host. This allows them to induce immune responses that resemble natural infection, often resulting in strong and long-lasting immunity after a small number of doses(18). Examples include measles-containing vaccines and rotavirus vaccines(25). Inactivated (killed) vaccines contain pathogens that have been rendered non-replicative. These vaccines are generally safer in immunocompromised individuals but tend to induce weaker immune responses and may require repeated dosing. Oral cholera vaccines are an example of this approach(26).

1.3.2 Protein-based/Subunit and antigen-based vaccines

Subunit vaccines use specific components of a pathogen rather than the whole organism. These components are selected to induce a protective immune response while reducing the risk of adverse reactions. Because they do not replicate, these vaccines are often less immunogenic and typically require adjuvants, which are substances added to enhance the immune response by improving antigen presentation and providing activation signals to immune cells(11).

An example is the acellular pertussis vaccine, which contains purified *Bordetella pertussis* antigens and replaced earlier whole-cell formulations due to an improved safety profile(27).

Polysaccharide vaccines are based on sugar structures found on the surface of bacteria. These vaccines are relatively simple to produce but do not induce strong immune responses in infants, where disease burden is often highest(11). This limitation led to the development of conjugate vaccines, in which polysaccharides are chemically linked to a carrier protein. This modification enables a T cell-dependent immune response and improves immunogenicity in young children. Examples include *Haemophilus influenzae* type b, pneumococcal, and meningococcal conjugate vaccines(28–31). Toxoid vaccines use inactivated bacterial toxins to induce immunity against toxin-mediated disease. Examples include tetanus and diphtheria vaccines(32).

Recombinant vaccines are produced using genetic engineering techniques, allowing antigens to be expressed in heterologous systems. The hepatitis B vaccine is a widely used example(33). Related approaches include virus-like particle vaccines, where structural proteins self-assemble into particles that resemble the native virus but lack genetic material. More recent developments include nanoparticle-based vaccines, which present antigens in highly ordered structures that can enhance immune recognition(34,35).

1.3.3 Nucleic acid and vector-based vaccines

Nucleic acid vaccines deliver genetic material encoding antigenic proteins rather than the antigen itself. DNA vaccines use plasmid DNA to induce antigen expression within host cells, while mRNA vaccines deliver messenger RNA that is translated into antigenic proteins following uptake by host cells(34,36,37). These approaches allow rapid vaccine design and production.

Recombinant vector vaccines use modified viruses to deliver genetic material encoding target antigens. These vectors can infect host cells and induce antigen expression, thereby stimulating both humoral and cellular immune responses(38). Examples include viral vector platforms used in several recent vaccine programmes such as malaria and Ebola vaccines(39,40).

1.4 Population-level effects of vaccination

Vaccination aims to protect those at risk of disease including but not limited to children, the elderly, those that are immunocompromised, travellers to disease endemic areas and people living with chronic diseases(41). Vaccination programmes may be delivered through targeted strategies that focus on high-risk groups or through mass immunisation approaches aimed at interrupting transmission, controlling disease, achieving elimination, and, in rare instances, eradication(11,42). At the population level, vaccines reduce disease burden primarily by lowering the number of susceptible individuals within a community. This reduction in susceptibility decreases the probability of effective contact between infectious and susceptible individuals and can shift the average age of infection to older age groups, where disease outcomes are often less severe(43). These indirect population effects are particularly relevant for infections that disproportionately affect young children.

1.4.1 Herd protection and shift in age of infection

Vaccination affects disease patterns beyond the individual level. As coverage increases, the number of susceptible individuals within a population declines, which can reduce transmission and provide protection to those who are not immunised. This indirect protection, often referred to as herd protection, depends on vaccine effectiveness against infection, coverage levels, contact patterns, the transmissibility of the pathogen and if recovery from the pathogen confers natural immunity.

Changes in transmission can also cause a disease epidemiological shift. As vaccination reduces exposure in early childhood, infection may occur later among susceptible individuals(44,45). This can change the distribution of disease towards age groups with lower risk of severe outcomes, such as in malaria. In some diseases such as rubella, varicella, and hepatitis A,

disease severity increases with age, so a shift in the timing of infection may change the overall burden of severe outcomes(46–48). In pathogens with multiple serotypes, such as pneumococcus, vaccination with pneumococcal conjugate vaccines targeting specific types has led to serotype replacement of non-vaccine serotypes(49). Both direct and indirect effects need to be considered when assessing vaccine impact at the population level.

1.5 Evolution of the Expanded Programme on Immunisation (EPI)

1.5.1 Origins and establishment of the EPI

The Expanded Programme on Immunisation (EPI) was established in 1974 following earlier global efforts to control vaccine-preventable diseases. The programme was shaped by the experience of the smallpox eradication campaign, which demonstrated that vaccines could be delivered at scale through coordinated international efforts and that disease elimination was achievable under sustained implementation(14). These experiences also highlighted the need for a stable and permanent system for vaccine delivery across all WHO member states(50).

The first exploratory meetings on EPI began in 1970 and later the programme was formally adopted at the World Health Assembly in 1974. Its initial focus was on reducing morbidity and mortality from tuberculosis, diphtheria, tetanus, pertussis, poliomyelitis, and measles(51). The aim was to establish basic immunisation infrastructure in all countries and to make these vaccines available to all children. Early expansion of the EPI needed policy commitment from all countries, particularly in low-income countries where competing health priorities existed. There was progress later on by late 1980s as the coverage third dose of diphtheria-tetanus-pertussis (DTP) vaccine increased from approximately 5% in 1974 to over 50%(52).

1.5.2 Expansion of vaccine schedules

The subsequent phases of the EPI involved expansion beyond the original set of vaccines to include those targeting a broader range of diseases. This included the introduction of hepatitis B, *Haemophilus influenzae* type b (Hib), pneumococcal, and rotavirus vaccines. Hepatitis B vaccination was introduced following recognition of its global burden and long-term complications, including liver cirrhosis and hepatocellular carcinoma. Before widespread

vaccination, a large proportion of the global population showed evidence of past or current infection. Hepatitis B vaccination has been successful in reducing the incidence of hepatocellular carcinoma, hepatic failure and liver cirrhosis. (53).

The development of Hib vaccines illustrates how advances in vaccine design influenced programme effectiveness. Early polysaccharide vaccines were safe but did not generate adequate immune responses in infants, who carry the highest burden of disease. Conjugate vaccines, which link polysaccharides to protein carriers, were later introduced and enabled effective protection in early childhood through T cell–dependent immune responses(28). The improved conjugate vaccines proved to be a success and reduced Hib-related invasive disease by >90% in countries that have introduced the vaccine(54). Pneumococcal and rotavirus vaccines were later incorporated into routine schedules following WHO recommendations. Early safety concerns, including the withdrawal of Rotashield which was the first licensed rotavirus vaccine due to intussusception risk, led to more stringent evaluation of subsequent vaccines. Their introduction has been associated with substantial reductions in severe disease outcomes(55,56).

1.5.3 Vaccine financing and global partnerships

Expansion of the EPI in many sub-Saharan African countries has depended on partnerships between ministries of health with organizations such as GAVI, the Vaccine Alliance new vaccine introduction and immunisation system strengthening and UNICEF for support in vaccine procurement(57). Under the current Gavi 6.0 framework, country eligibility and transition status are determined by gross national income per capita, using the World Bank Atlas method(58). Countries with a GNI per capita at or below the World Bank low-income threshold are classified in the initial self-financing phase, while those above this threshold but still within Gavi eligibility enter the preparatory transition phase where they begin with the same $0.20 per dose but in subsequent years countries contribute 15% to 80% of the price(59). Countries that graduate from the preparatory phase enter the accelerated transition phase where they co-finance ≥35% per dose. The last phase is a catalytic phase where countries finance 100% of the price per dose.

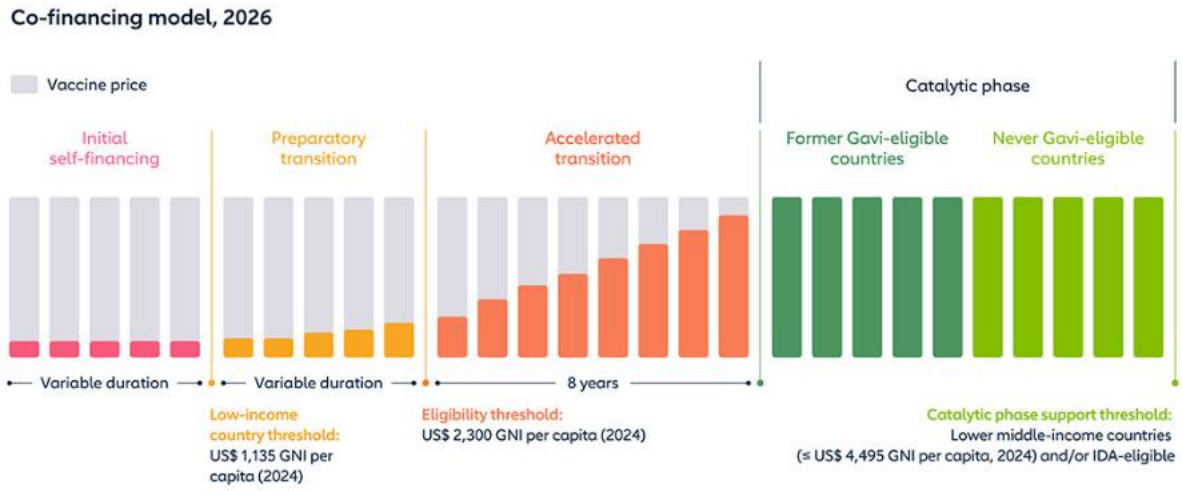


Figure 1-2: GAVI co-financing model 2026. Adapted from GAVI co-financing policy (60)

Beyond vaccine procurement, Gavi 6.0 provides integrated support that combines vaccine financing with funding for health system strengthening, cold chain equipment, technical assistance, and programme delivery(58). This allows countries to plan and prioritise immunisation activities within defined resource envelopes over multi-year periods.

1.5.4 Vaccine introduction in LMIC immunisation programmes

Vaccine introduction in LMICs such as Malawi occurs through a policy and implementation process that extends beyond licensure. The evidence on vaccine safety, efficacy, disease burden and programmatic suitability usually provides the starting point for vaccine introduction. This evidence is generated through individual or cluster randomised-controlled clinical trials during pre-licensure phase. The decision to introduce a new vaccine is then assessed against WHO recommendations, national epidemiology, feasibility, financing and health system readiness. In Malawi, this process is led by the Ministry of Health through the Expanded Programme on Immunisation (EPI), with technical input from the EPI Technical Working Group, the Immunisation Coordination Committee and the Malawi Immunization Technical Advisory Group(61). These structures support policy review, technical guidance, implementation planning and research oversight, while vaccine procurement, storage and distribution are coordinated through national supply-chain systems, including the Central Vaccine Stores(61).

The Malawi National Immunisation Strategy places new vaccine introduction within wider programme priorities that include service delivery, cold-chain capacity, vaccine safety surveillance, advocacy, communication, and data use for decision-making. It also recognises that new vaccines require feasibility assessment, secure supply chains and monitoring systems to support implementation. The introduction of PCV13 provides an example of this process in Malawi. Following the 2007 WHO recommendation for pneumococcal conjugate vaccine use, Malawi introduced PCV13 into the routine EPI schedule in November 2011 with Gavi support(62). Preparatory work included assessment of local pneumococcal serotype coverage, mobilisation of Ministry of Health and partner support, health worker training, cold-chain strengthening and vaccine logistics. The introduction was also linked to post-licensure evaluation of invasive pneumococcal disease, radiological pneumonia and cost-effectiveness. This illustrates that vaccine introduction is not simply the addition of a licensed product to the schedule, but a coordinated programme decision that creates a need for post-licensure evidence on effectiveness, impact, safety and sustainability under routine conditions.

1.5.5 Vaccine uptake and schedule timing in Malawi

Malawi has a high-performing routine childhood immunisation programme especially for vaccines delivered in infancy. The National Immunisation Strategy reports that Malawi's EPI has performed well since its establishment in 1979, with coverage of most antigens sustained above 90% since 2018. Pentavalent dose 3 which is a common benchmark of routine immunisation performance, was reported at 92% in 2018(61). These patterns suggest strong public acceptance of established childhood vaccines and a health system that has been able to deliver infant vaccination at scale. However, coverage is weaker for vaccines scheduled after the first year of life. The second dose of measles-rubella vaccine, given at 15–23 months, has remained below 80% since introduction, indicating a persistent challenge with later childhood contacts rather than poor acceptance of infant vaccination.

The same pattern has been seen with the RTS,S/AS01 malaria vaccine implementation. In Malawi districts where RTS,S has been introduced, median coverage has remained relatively high for the first three doses given at 5, 6 and 7 months, at 81%, 74% and 72%, respectively(63).

Coverage is lower for the fourth dose given at 18–22 months, at 46%. This suggests that reduced uptake of later doses may reflect caregiver familiarity with vaccination as an infancy-based service, missed contacts after the first year of life, and delivery challenges for second-year-of-life vaccination, rather than vaccine hesitancy alone. The current Malawi schedule illustrates this pattern, with most traditional EPI vaccines clustered at birth, 6, 10 and 14 weeks, while MR2 and RTS,S dose 4 require later contacts at 15–23 months and 22 months, respectively.

1.5.6 Vaccine hesitancy in Malawi

Vaccine hesitancy should therefore be distinguished from low coverage caused by programme timing, access or missed opportunities. In Malawi, COVID-19 vaccination provides a clearer example of hesitancy. A scoping review of COVID-19 vaccine hesitancy among Malawians by Nkambule and Mbakaya reported that poor vaccine uptake was strongly linked to misinformation, safety concerns and distrust of the vaccine(64). Specific concerns included beliefs that COVID-19 vaccines caused infertility, blood clots, severe disability or death, and that the vaccines were experimental or ineffective. Religious beliefs, which play a strong part in Malawi, also contributed to the low uptake of the vaccination. This differs from the pattern seen with established infant vaccines such as the pentavalent, where uptake is generally high. The Malawi experience therefore suggests that low coverage should not automatically be interpreted as hesitancy. For routine childhood vaccination, later-dose drop-off may indicate schedule and delivery barriers, while COVID-19 vaccination shows how misinformation, perceived safety concerns, religious narratives and low trust can directly reduce vaccine acceptance. Vaccine fatigue due to the recent increase in the number of vaccines introduced on the Malawi immunisation schedule has been cited as one of the contributing factors to vaccine hesitancy(65). Recently Malawi has introduced the second dose of the measles-rubella vaccine, inactivated polio dose, malaria vaccine, typhoid conjugate vaccine and COVID-19 vaccines(61).

1.6 Implications for evaluation of vaccine impact

Expansion of immunisation programmes has enabled vaccines to be delivered at scale across diverse settings, often alongside other public health interventions. As programmes have

expanded in scope and complexity, attributing observed changes in disease patterns to vaccination alone has become less straightforward. Vaccine effects are realised within routine health systems, where coverage varies, multiple interventions are implemented concurrently, and populations differ in exposure and risk. Under these conditions, evidence from pre-licensure trials is often insufficient to describe vaccine performance in practice. Evaluating the real-world impact of vaccination therefore requires approaches that account for these complexities and that are suited to routine programme settings.

1.7 Persistent burden of diarrhoeal diseases, cholera, and malaria

Despite major global declines in diarrhoeal mortality since 1990, the burden remains disproportionately high among children in sub-Saharan Africa. Diarrhoea is the third leading cause of under-five mortality in the world with over 443,000 deaths (66). The systematic review on global diarrheal mortality by Kyu *et al* reported that diarrhoeal diseases caused 1.2 million deaths worldwide in 2021, down from 2.9 million in 1990, representing a 60% reduction. Rotavirus remains the leading aetiology for severe childhood diarrhoea with a reported 35.2% fatal population attributable fraction (PAF) followed by Shigella *spp* at 24.0% (15.2–37.9) and adenovirus at 23.8% (14.8–36.3)(67).

1.7.1 Rotavirus epidemiology

Rotavirus is the leading cause of severe diarrhoea disease that predominantly affects children <5 years of age. Prior to the licensure and introduction of rotavirus vaccines in 2006, rotavirus was responsible for over 500,000 deaths annually in children under five years of age, with nearly two-thirds of these deaths occurring in LMICs (55). Rotavirus vaccines are estimated to have prevented 140,000 deaths from 2006 to 2019 since their introduction(55). Despite these gains, rotavirus disease is still attributed with 258 million annual cases of diarrhoeal disease (68).

1.7.2 Pathogenesis and clinical presentation

Rotaviruses are non-enveloped viruses with a segmented double-stranded RNA genome consisting of 11 segments, classified within the *Reoviridae* family(69). The viral particle is

arranged in three protein layers. The outer capsid proteins, VP4 and VP7, define the antigenic structure of the virus and are the main targets of neutralising antibodies. The inner capsid is composed of the VP6 protein, which is highly conserved and abundantly expressed. Although it is less immunogenic than the outer capsid proteins, it is commonly used for diagnostic detection(70).

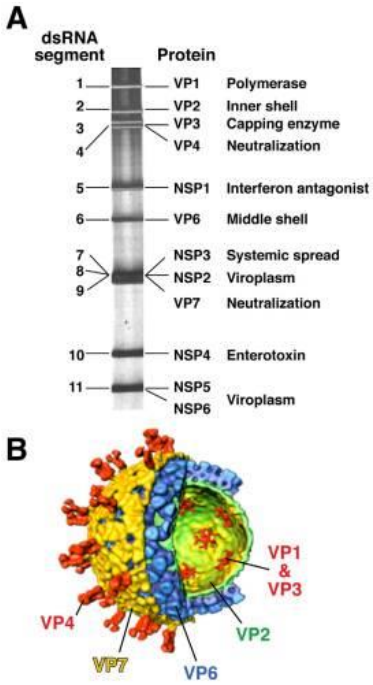


Figure 1-3: Structure and proteins of rotavirus. Adapted from Greenberg and Estes (69)

The VP4 and VP7 proteins form the basis of strain classification into P and G types, which is relevant for understanding strain diversity and how vaccines perform across different settings(70). Several non-structural proteins are produced during infection, although NSP4 is of particular interest as it acts as an enterotoxin and disrupts ion transport in the small intestines leading to secretory diarrhoea.

Rotavirus infects mature enterocytes in the small intestine. Viral replication leads to destruction of these cells, resulting in villous atrophy and reduced absorptive capacity(69). The combination of these processes explains the typical clinical picture of vomiting and diarrhoea, where both malabsorptive and secretory mechanisms are present.

Children with rotavirus infection present with vomiting and watery diarrhoea, often leading to rapid dehydration. Disease severity is highest among children aged 3 to 24 months, reflecting waning maternal antibodies and the gradual development of immunity following repeated exposure(55,70). However, the median age of rotavirus disease varies between LMICs and HICs with, peaking at about 9 months in high-mortality LMICs(71). Prior to vaccine introduction it was estimated that every child would experience a rotavirus infection by the age of 5 years, with subsequent infections likely to be less severe or asymptomatic(72). Passive protection in early life is partly mediated by transplacental antibodies and breastfeeding, while subsequent infections reduce the likelihood of severe disease but are not protective(70).

1.7.3 Diagnosis and implications for evaluation

Enzyme-linked immunosorbent assay (ELISA) has been widely used to detect viral antigen against VP6 in stool. ELISA is central to many surveillance systems and is recommended by WHO for rotavirus diagnosis. Molecular methods such as reverse transcription polymerase chain reaction (RT-PCR) are more sensitive and are increasingly used in multi-pathogen panels(55,70). Detection of viral RNA does not always indicate that rotavirus is the cause of illness, as asymptomatic shedding or recent infection may result in positive tests. For this reason, quantitative thresholds are often applied to improve specificity in epidemiological analyses(70). This distinction matters in VE studies because outcome misclassification can bias estimates.

1.7.4 Management and determinants of severity

There is no specific antiviral treatment for rotavirus infection as with many childhood viral infections. Management relies on supportive care, with fluid replacement as the primary intervention to prevent and treat dehydration. Oral rehydration salts (ORS), particularly low-osmolarity formulations, reduce mortality, while zinc supplementation shortens the duration and severity of illness(55). Continued feeding, including breastfeeding, is recommended. Mortality from rotavirus is therefore largely determined by access to timely care rather than intrinsic differences in viral virulence. In settings where rehydration therapy is not readily available, even moderate dehydration can lead to death.

1.7.5 Rotavirus vaccines

Rotashield was the first rotavirus vaccine licensed in the United States of America in 1998. It was a rhesus-human reassortant tetravalent vaccine manufactured by Wyeth Lederle Vaccine (RRV-TV). Intussusception was detected as a safety signal during pre-licensure clinical trials but causality was not established due to the small sample size(73). Post-licensure, vaccine recipients were found to have a 21.7 times risk of intussusception was reported 3-14 days following administration of the first dose than unvaccinated in post-licensure phase 4 studies which established causality between Rotashield and intussusception(74). Rotashield was subsequently withdrawn in 1999 for the association with intussusception. This event led to more stringent safety evaluations for subsequent vaccines.

There are currently four oral live-attenuated licensed rotavirus vaccines that are recommended by the World Health Organization (WHO) (Table 1-1). Rotarix® (GlaxoSmithKline Biologicals), RotaTeq® (Merck & Co), Rotavac® (Bharat Biotech) and RotaSiil® (Serum Institute) have been introduced in routine childhood immunisation programmes for over 130 countries(75). All are given as two or three primary doses, in the first year of life, with the primary dose recommend as soon as possible after 6 weeks of age(55,76). Each of these vaccines contain difference compositions of rotavirus strains(77).

Table 1-1: Licensed oral rotavirus vaccines recommended by WHO

Name	**Vaccine antigens**	**Efficacy**
Rotarix® (RV1)	Attenuated human strain R1X4414 of G1P[8] strain	HIC settings: 84.7% against severe RVGE(78). LMIC settings: 61.2% pooled efficacy in Malawi/South Africa, with 49.4% in Malawi(79,80)
RotaTeq® (RV5)	Pentavalent rotavirus reassortant with human G1, G2, G3, G4 and P[8]	HIC settings: 98.3% against severe RVGE(81). LMIC settings: 39.3% in African sites and 48.3% in Asian sites over two years(82,83)
Rotavac®	Monovalent vaccine containing live attenuated Rotavirus 116E	LMIC settings: 53.6% against severe RVGE in India(84)
RotaSiil®	Live Attenuated Bovine - Human Rotavirus Reassortant [G1, G2, G3, G4 and G9 grown on vero cells] 10 5.6 FFU / Serotype	LMIC settings: 39.5% against severe RVGE over two years in India and 66.7% in Niger(85,86)

In clinical trials rotavirus vaccine efficacy against severe disease was estimated at over 80% in HICs but efficacy has an inverse relationship with country income and declines in LMICs. These efficacy estimates have translated into the real world. Since their introduction rotavirus vaccines have demonstrated lower protection against severe disease in LMICs (39% – 66%), where disease burden is the highest, compared to high-income countries(>85%)(87,88). The lower performance of rotavirus vaccines in low-income settings has been associated with various factors such as undernutrition, host immunity e.g. HIV infection, higher force of infection, higher gut microbiota diversity, breastfeeding and maternal transplacental antibodies(88,89). The effectiveness and impact estimates for the four rotavirus vaccines are discussed in Chapter 3 of this thesis(90).

1.8 Cholera

1.8.1 Cholera aetiology and transmission

Cholera remains a significant public health concern in low- and middle-income countries (LMICs) where inadequate water, sanitation, and hygiene (WASH) contribute to recurrent outbreaks. It is a rapidly dehydrating diarrhoeal disease caused by toxigenic strains of *Vibrio cholerae*, a Gram-negative bacterium with a curved rod shape(26).

Although more than 200 serogroups exist, epidemic cholera is almost entirely associated with serogroups O1 and O139. There are other serogroups that do not cause outbreaks or pandemics but rather sporadic cases of diarrhoea and are called non-O1-non-O139 V. cholerae(91). The O1 serogroup is further divided into serotypes of Ogawa, hikojima and Inaba based on differences in the O-antigen structure. The O1 serogroup is also be subclassified into biotypes of El Tor and classical based on phenotypic and genetic characteristics(92). O1 Classical biotype is responsible for the first to the sixth pandemics. In recent decades, most large outbreaks, including those in sub-Saharan Africa have been caused by the O1 seventh-pandemic El Tor lineage(93,94). O1 El Tor lineage is recognised by a capsule and a modified lipopolysaccharide (LPS)

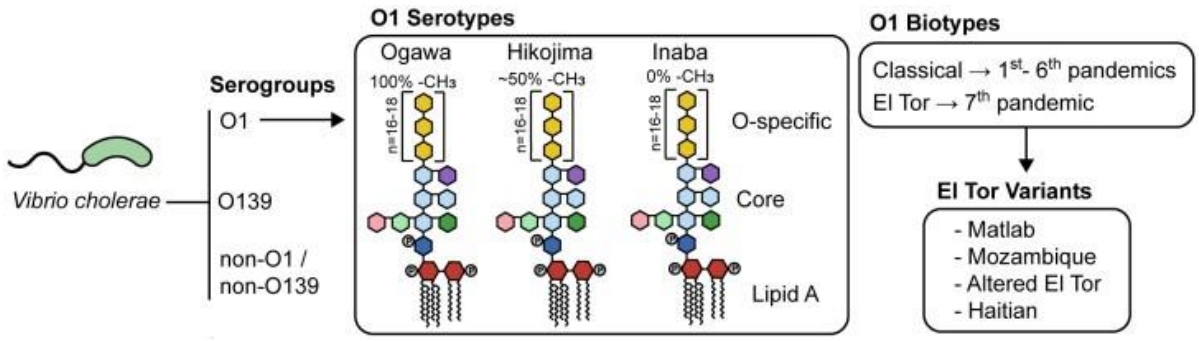


Figure 1-4: V. cholerae is classified into serogroups based on the composition of the O antigen of LPS. Adapted from Montero et al. 2023(95)

Cholera is transmitted through ingestion of contaminated water or food with *V. cholerae* toxins. There are two pathways for cholera transmission. The first is direct faecal-oral transmission within households or communities. The second is environmentally mediated transmission, where *V. cholerae* persists in aquatic reservoirs and is amplified under favourable conditions such as poor sanitation or seasonal rainfall(26). These overlapping pathways help explain why outbreaks often emerge rapidly in urban informal settlements (slums), where high population density coupled with limited access to safe water create conditions for sustained transmission.

1.8.2 Pathogenesis and clinical presentation

Following ingestion, V. cholerae reaches the small intestine, where motility driven by a single flagellum allows it to move through the mucus layer. The toxin-coregulated pilus (TCP) then mediates attachment to the intestinal epithelium, enabling colonisation. The outer membrane lipopolysaccharide (LPS), which defines serogroup and is a key antigen, also contributes to colonisation. Once established, the bacteria release cholera toxin (CT) which contains an A and B subunit toxins. The B subunit binds to GM1 receptors on enterocytes, allowing entry of the A subunit, which triggers a cascade that drives chloride ions and water secretion, leading to profuse diarrhoea(96,97). Vaccine design therefore focuses on LPS to reduce colonisation and on the CT B subunit to block toxin binding and provide antitoxic immunity. Disease severity is driven primarily by fluid loss rather than tissue destruction as seen in invasive enteric disease such as Shigella(98). Clinical presentation ranges from asymptomatic infection to explosive diarrhoea, vomiting, severe dehydration and can rapidly progress to death if patients are not promptly rehydrated. This rapid progression distinguishes cholera from many other causes of

diarrhoeal disease and explains the strong dependence of mortality on timely access to care(91).

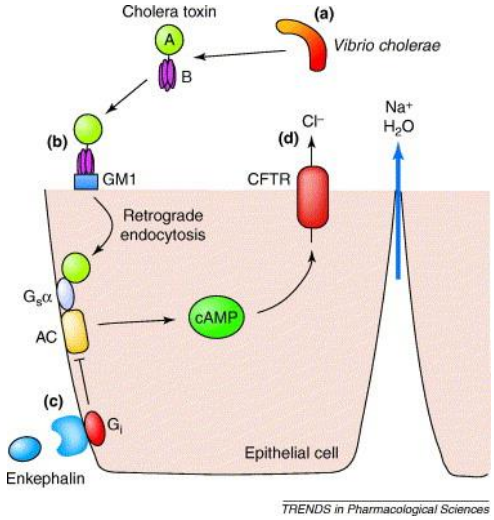


Figure 1-5: Cholera toxin pathogenesis. Adapted from Thiagarajah et al. 2005(99)

1.8.3 Burden and epidemiological patterns

Cholera remains endemic in many parts of South Asia and sub-Saharan Africa with periodic large-scale outbreaks. Global estimates suggest 1.3 to 4.0 million cases and between 21,000 and 143,000 deaths annually, although reported numbers substantially underestimate the true burden(26). There were 535,321 cases reported from 45 countries in 2023 as reported by the World Health Organization. However, during the latest outbreak, reports from January 2023 to July 2024 involving 14 countries in SSA, there were 399,508 cholera cases, including 7,023 deaths. Cholera also remains endemic in parts of sub-Saharan Africa, where recurrent outbreaks continue due to poor WASH infrastructure. The Democratic Republic of the Congo, Ethiopia, Malawi, Mozambique, and Zimbabwe account for 72.2% (288,570) of all cumulative cases and 62.3% (4,375) of the deaths reported(100). These patterns highlight the continued vulnerability of many settings to explosive transmission, even where effective treatment and oral cholera vaccines are available. The recurrent resurgence of cholera in parts of Africa suggests that structural drivers of transmission remain insufficiently addressed.

1.8.4 Cholera control and role of vaccination

Control of cholera relies on a combination of interventions, including case management, improvements in water, sanitation and hygiene (WASH) and vaccination. Oral cholera vaccines (OCVs) are recommended by the World Health Organization for use in both endemic settings and outbreak response, where they are deployed alongside WASH interventions rather than as standalone measures(26). OCVs are typically killed whole-cell vaccines targeting *V. cholerae* O1, with some formulations also providing coverage against O139. These vaccines reduce susceptibility at the individual level and can contribute to reductions in transmission when deployed at scale. In outbreak settings, vaccination is often implemented reactively over short timeframes, targeting high-risk populations. Infection-induced protection develops after symptomatic and asymptomatic V. cholerae infection and lasts for at least 3 years(101).

The WHO recommends three prequalified OCVs for use in endemic and outbreak settings(102). All the vaccines contain killed whole *V. cholerae* O1 cells but they differ slightly in formulation and administration. Dukoral™ (Valneva, Sweden) contains inactivated *V. cholerae* O1 cells together with the recombinant B-subunit of cholera toxin. Shanchol™ (Shantha Biotechnics-Sanofi Pasteur, India) and Euvichol™ (EuBiologics, Republic of Korea) are killed whole-cell vaccines, which simplifies field delivery. These two vaccines share the same antigenic composition and have demonstrated comparable safety and immunogenicity across multiple evaluations(91,103). The vaccine has a reported effectiveness ranging from 37% to 80% in various settings including Zambia, India, Bangladesh and Haiti(104–107). Recently an improved plastic-vial formulation of Euvichol™, Euvichol-Plus®, has been used to improve field delivery and combat cholera outbreaks.

1.8.5 Implications for vaccine evaluation

Cholera presents a distinct set of challenges for vaccine evaluation. Transmission is shaped by environmental conditions, population movement, and infrastructure, leading to rapid changes in incidence over short periods. Vaccination is often introduced during ongoing outbreaks, alongside intensified WASH interventions and case management. During cholera outbreaks, reactive OCV campaigns are implemented alongside case-area targeted WASH interventions.

Disentangling the effect of vaccination from these concurrent interventions is therefore difficult, particularly when routine individual-level data are incomplete.

1.9 Malaria

Malaria is a vector transmitted disease which is caused by protozoan parasites of the genus *Plasmodium*. There are five known species that cause disease in humans following a bite from an infected female *Anopheles* mosquito. The mosquito is infected following a blood meal from an infected human which then proceeds to infect the next human it feeds on. There are four plasmodium species that are transmitted through this method namely: *P. falciparum, P. vivax, P. malariae and P. ovale*(108). The fifth species known as *P. knowlesi* causes malaria between monkeys and only causes malaria in humans when a mosquito bites a human following a blood meal of an infected monkey. The main species that are responsible for the malaria epidemic in sub-Saharan Africa are *P. falciparum*(109). *Plasmodium Vivax* does not cause much disease in Africa because of the lack of the Duffy gene in many African populations which is necessary for the infection of *P. vivax* into human red blood cells.

According to the latest WHO estimations, there were 282 million (256,000 – 313,000) reported cases of malaria in 2024 with about 610,000 deaths (561,000 – 738,000). There was a 25.5% decline in malaria cases from 2000 to 2015 but there was an increase in cases between 2015 to 2024 of 8.5%(109). The malaria case incidence in 2024 was 64 per 1000 population at risk, a 2% increase from the previous year. This increase in incidence highlights the need to increase efforts of malaria interventions. Similarly, there was an increase in malaria related deaths between 2023 and 2024 by 12,000. The African Region continues to bear the greatest burden of malaria, accounting for approximately 94% of malaria cases globally. Additionally, the African Region experiences 95% of the 601,000 estimated deaths attributed to malaria globally with children under the age of 5 bearing 75% of the deaths(109).

1.9.1 Malaria diagnosis and treatment

Accurate diagnosis is central to malaria case management and to surveillance systems used in vaccine and impact evaluations. Current WHO guidance recommends parasitological

confirmation of suspected malaria cases using either microscopy or rapid diagnostic tests (RDTs) before treatment is initiated(108). Microscopy allows quantification of parasite density and species identification, though its accuracy depends on laboratory capacity. RDTs have a sensitivity and specificity of >90% in different settings and detect *P. falciparum*-specific histidine-rich protein 2 genes (pfhrp2) rather than the parasite itself. They are more widely used in routine settings as they provide rapid results without the need for microscopes(109). A systematic review by Ojiniyi *et al*., that pooled data from 48 studies found that the performance of RDTs is influenced by antigen persistence, genetic variation such as pfhrp2 deletions, and parasite density, which leads to both false positive and false negative results(110). These limitations introduce systematic bias into routine surveillance data and complicate the estimation of vaccine effectiveness and impact. Molecular methods, including convectional polymerase chain reaction (PCR), quantitative PCR, loop-mediated isothermal amplification (LAMP), and targeted sequencing, are more sensitive than microscopy and RDTs and can detect low-density or sub-microscopic *Plasmodium* infections(111). They are useful for research and surveillance, including detection of antimalarial resistance markers and pfhrp2/pfhrp3 deletions, but they are not routinely available in routine healthcare settings(109).

The treatment of uncomplicated *Plasmodium falciparum* malaria is based on artemisinin-based combination therapy (ACT) e.g. artemether–lumefantrine (AL), which remains the first-line treatment in most endemic settings(112). ACTs combine a fast-acting artemisinin derivative with a partner drug to reduce parasite burden and delay the emergence of resistance. For severe malaria, parenteral artesunate is recommended, followed by a full course of oral ACT once the patient can tolerate oral medication. In community settings where referral is delayed, pre-referral treatment with rectal artesunate may be used to reduce mortality risk(112).

Access to timely diagnosis and effective treatment has contributed substantially to reductions in malaria mortality over the past two decades. At the same time, emerging resistance to artemisinin and partner drugs in several regions presents an ongoing threat to treatment effectiveness and to broader malaria control efforts(109). In the Africa Region, WHO reported that 8% (15/185) of the therapeutic efficacy studies (TES) showed ACT treatment failure of >10%

which is the threshold that warrants further resistance investigation(109). This reinforces the need for effective malaria vaccines.

1.9.2 Malaria control interventions

Malaria control relies on a combination of interventions that act on different stages of transmission. Vector control remains central. Insecticide-treated nets (ITNs) are widely distributed through both mass campaigns and routine delivery channels and reduce human–vector contact during peak biting times(109). Indoor residual spraying (IRS) provides additional protection by applying insecticides to household walls, though its use has declined in many settings due to the high cost of the chemicals used in IRS(113).

Chemoprevention strategies are used in populations at high risk. These include intermittent preventive treatment in pregnancy (IPTp), seasonal malaria chemoprevention (SMC) in areas with highly seasonal transmission, and perennial malaria chemoprevention (PMC) in settings with year-round transmission(109). These interventions are often implemented simultaneously and vary over time in coverage and intensity. For example, fluctuations in ITN distribution cycles and IRS coverage can lead to changes in population-level protection over short periods. This makes it difficult to attribute observed changes in malaria outcomes to one intervention alone, including vaccination.

1.9.3 Malaria vaccines

In October 2021, WHO recommended the first malaria vaccine, RTS,S/AS01, for use in endemic settings(114). Development of a malaria vaccine has taken over 100 years because of the biological complexity of *Plasmodium falciparum*, which expresses over 5,000 genes across multiple stages of its life cycle. This stage-specific antigen expression, together with antigenic variation, genetic polymorphism, and ongoing parasite evolution, allows the parasite to evade host immune responses and has limited the identification of stable vaccine targets. In October 2023 WHO recommended another malaria vaccine, R21/Matrix-M, for use in endemic settings(115). Both vaccines and are being introduced through routine immunisation programmes in multiple countries(116).

1.9.4 RTS,S/AS01

RTS,S/AS01 is a recombinant protein vaccine targeting the pre-erythrocytic stage of Plasmodium falciparum. The antigen consists of a fusion protein (RTS) combining the central repeat region and C-terminal portion of the circumsporozoite protein (CSP) with the hepatitis B surface antigen (HBsAg). These are combined with another HBsAg (S) and leads to the formation of virus-like particles (VLPs). This configuration enhances antigen presentation(117,118). The vaccine is formulated with a novel AS01 adjuvant system, which contains monophosphoryl lipid A (MPL) and QS-21 in a liposomal formulation(119). This adjuvant induces both humoral and cellular immune responses, with a strong CD4+ T-cell component. The vaccine protection is mediated primarily through antibodies directed against CSP, which act by preventing sporozoite invasion of hepatocytes, thereby interrupting infection before blood-stage replication(120,121). T-cell responses contribute by targeting infected hepatocytes.

In phase 3 trials, RTS,S/AS01 showed 45% efficacy (95% CI: 22–60) against severe malaria(122). In a subsequent phase 4 cluster-randomised implementation study in the same countries, vaccine effectiveness was estimated at 58% (95% CI: 40–70) after accounting for other malaria interventions and patterns of healthcare utilisation(123). This difference reflects how estimates change under routine conditions and highlights the need to evaluate vaccines in real-world settings while accounting for concurrent interventions.

1.9.5 R21/Matrix-M vaccine

R21/Matrix-M is also a recombinant protein vaccine targeting the circumsporozoite protein of *P. falciparum*, but differs structurally from RTS,S in its antigen composition. The vaccine consists of CSP fused to HBsAg without the additional unfused HBsAg, resulting in a higher density of CSP on the surface of the virus-like particle(124). The vaccine is formulated with the Matrix-M adjuvant, a saponin-based adjuvant that enhances antigen presentation and promotes both antibody and cellular immune responses(125). As with RTS,S, protection is primarily mediated by antibodies against CSP, which block sporozoite invasion of hepatocytes. The higher proportion of CSP in the particle is intended to increase the magnitude of the antibody

response. R21 has a reported 75% (95% CI: 71-79) efficacy from a phase 3 trial at sites with high seasonal malaria transmission(126).

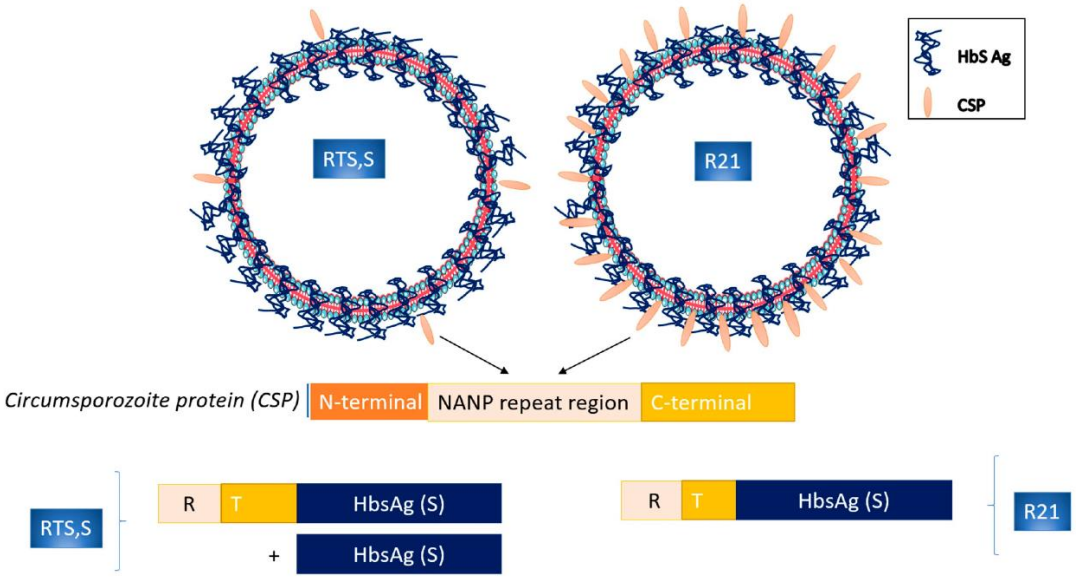


Figure 1-6: Circumsporozoite protein subunit vaccines RTS,S and R21 structure. Adapted from Tsoumani et al. 2023(127)

These two malaria vaccines provide partial protection against clinical malaria and severe disease, with effectiveness varying by vaccine coverage, transmission intensity, age and timing of doses. Unlike many childhood vaccines, they require multiple doses because protection wanes over time.

Implementation has taken different forms. The RTS,S vaccine was initially introduced through a cluster-based pilot in Malawi, Ghana, and Kenya to evaluate safety, feasibility, and impact under routine conditions(116). This approach allowed assessment of vaccine performance alongside other malaria interventions, including vector control and treatment access. More countries have now started introducing the vaccine since the WHO recommendation.

1.10 From efficacy to real-world impact

1.10.1 Definitions of vaccine efficacy, effectiveness, and impact

Vaccine efficacy refers to the reduction in disease risk under controlled trial conditions, while vaccine effectiveness (VE) reflects protection at the individual level under routine use. Vaccine

impact refers to changes in disease burden at the population level attributable to vaccination(128). Effectiveness is typically estimated using relative measures such as risk ratios, odds ratios, or hazard ratios, which can be converted to vaccine effectiveness as (1 – effect measure) × 100%. We can think of vaccine effects as different components that capture protection at both the individual and population level(129). Direct VE measures the reduction in disease rates between vaccinated individuals versus unvaccinated individuals within the same vaccine-exposed population. Indirect VE measures disease rates between vaccinated individuals in a vaccine-exposed population versus unvaccinated individuals in a vaccine-unexposed population. This is also known as herd protection. Total VE is the combination of direct protection and indirect protection, measured by comparing disease rates in vaccinated individuals in a vaccine-exposed population with unvaccinated individuals in a vaccine-unexposed population. The overall VE (vaccine impact) is the average reduction in disease rates at the population level, comparing all individuals (vaccinated and unvaccinated combined) in a vaccine-exposed population with individuals in a vaccine-unexposed population(129).

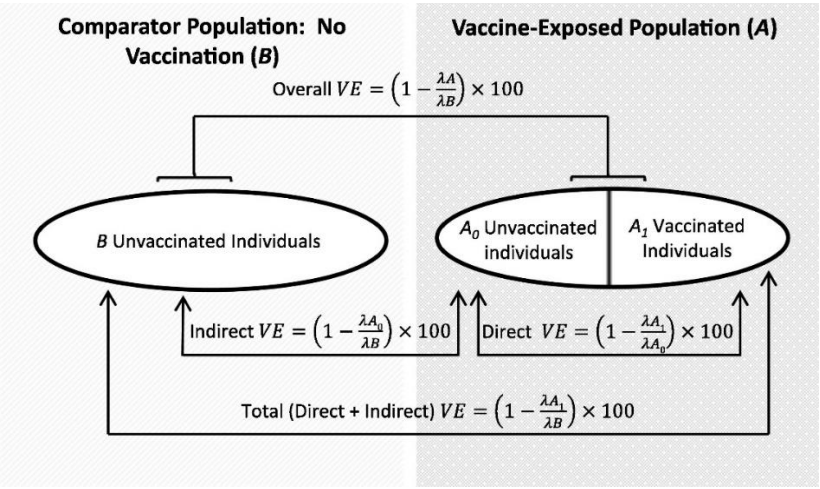


Figure 1-7: Type of vaccine effect estimates. Adapted from Hungerford et al. 2018(130) *[Abbreviations: VE, vaccine effectiveness; λ, hazard rate of infection]*

1.10.2 Limits of randomised controlled trials

Pre-licensure vaccine studies are typically conducted using randomised controlled trials, which are designed to estimate vaccine efficacy under controlled conditions. Randomisation allows for the distribution of measured and unmeasured confounders between study groups, reducing

bias in estimates which makes them the gold standard for generating evidence on vaccine efficacy. These trials are conducted in settings where vaccine delivery, follow-up, and clinical care are closely monitored(131). These conditions differ from routine programme settings. Participants in trials often have better access to healthcare, higher adherence to vaccination schedules, and more consistent follow-up. As a result, trial-based estimates may not reflect vaccine performance under real-world conditions. It is also not ethically acceptable to withhold vaccination from control groups once efficacy has been established which limits the ability to conduct randomised evaluations after licensure(131). Randomised trials are also limited in their ability to assess indirect effects and are often not designed to evaluate long-term protection or waning immunity.

1.10.3 Study designs used in vaccine evaluation

After vaccine candidates have been rolled-out in the population, vaccine impact is assessed through routine programme monitoring, vaccine-preventable disease surveillance and targeted observational studies. These data systems provide the basis for researchers to estimate vaccine effectiveness and impact under routine programme conditions. Specific observational study designs are discussed in more detail in chapter 2 of this thesis.

1.10.3.1 Screening method

The screening method provides a rapid estimate of vaccine effectiveness using the proportion of cases vaccinated (PCV) and the proportion of the population vaccinated (PPV) using the equation $VE = 1 - \frac{PCV}{1-PCV} * \frac{1-PPV}{PPV}$ or 1 minus the odds ratio of vaccination among cases to vaccination among the general population. (132,133). The statistical framework and formal naming were set out by Farrington and colleagues in 1993. This built on earlier work by Orenstein and colleagues in 1985 during measles outbreak investigations, where the approach was used in practice but not developed as a generalisable framework(133,134). It has since been applied across a range of vaccine-preventable diseases including measles, pertussis, rotavirus, influenza and COVID-19 vaccines using surveillance data(132,135–138). It is useful for identifying whether effectiveness estimates are within expected ranges. Its validity depends on accurate measurement of vaccination coverage and case vaccination status. Errors in these

measures can lead to biased estimates(132). Since the screening method measure vaccine effects at a population level, it only measure the overall VE(129,131,139).

1.10.3.2 Case–control studies

Case–control studies were not originally designed for vaccine evaluation. The earliest case–control was conducted by Doll and Hill in 1950, which was the first to establish that smoking was associated with lung cancer(140). The design was later adapted for vaccine effectiveness studies, with early applications in measles vaccine evaluations in the 1970s and 1980s, including work by Orenstein and colleagues in 1985(134). Case–control studies are now one of the most widely used designs to estimate vaccine effectiveness because they can be done more rapidly and are inexpensive compared to other designs. They, however, an only measure direct vaccine effectiveness(129,131). They are efficient for rare outcomes and do not require follow-up. However, they are sensitive to selection bias and misclassification. Controls must represent the population from which cases arise and accurate ascertainment of vaccination status is critical, particularly in settings where records are incomplete(128). Differences in healthcare-seeking behaviour can bias estimates. The test-negative design addresses this by selecting cases and controls from individuals presenting with similar symptoms, although it is not suitable for all pathogens and depends on accurate diagnostic testing(141).

1.10.3.3 Cohort studies

Following their case–control study that identified an association between smoking and lung cancer, Richard Doll and Bradford Hill undertook a cohort study to examine this relationship over time. They recruited 40,701 registered male doctors in the United Kingdom in 1951 and followed them prospectively to compare mortality patterns by smoking status(142). Cohort approaches were used early in vaccine evaluation, particularly in post-licensure studies of inactivated polio and measles vaccines in the 1950 and 1960s(11). Cohort studies estimate vaccine effectiveness by comparing disease incidence between vaccinated and unvaccinated groups over time, either prospectively or retrospectively. Prospective cohorts are less utilised than retrospective cohorts because of the long duration it takes and the cost implications of following up the cohort(143). Although retrospective cohorts are more time efficient, prospective cohorts generally have higher accuracy in measurement of exposures, confounders

and outcomes which makes them stronger(143). Prospective cohorts allow direct estimation of risk and can assess multiple outcomes. One of the main advantages of cohorts studies is that they are able to measure direct, indirect, total and overall vaccine effectiveness(129,131). However, they require large sample sizes and sustained follow-up. Confounding by differences in healthcare access and health-seeking behaviour may bias results. Differential loss to follow-up between vaccine-exposed and vaccine-unexposed participants and changes in vaccination status during follow-up also affect validity due to bias(144).

1.10.3.4 Ecological and time-series designs

Ecological approaches have been widely used to assess vaccine impact at the population level, particularly through analyses of incidence trends before and after vaccine introduction (e.g. polio and measles control programmes)(11). The design is attributed to Willam Farr where he analysed population-level mortality data across different regions and time in England in 1885(145). Ecological designs use aggregated data to assess changes in disease patterns before and after vaccine introduction at a population level and because of this they only measure the overall VE also known population impact(131). They are useful for estimating overall population-level impact, including indirect effects(146). However, they cannot separate the effects of vaccination from other concurrent interventions. Interrupted time-series analysis improves on simple before–after comparisons by estimating changes in level and trend relative to a counterfactual(147). These analyses require stable pre-intervention trends and are sensitive to seasonality and data quality. They are less suitable in outbreak settings where baseline trends are unstable.

1.11 Concurrent interventions and attribution of vaccine impact

1.11.1 Complexity of attribution in routine settings

Different study designs provide complementary evidence on vaccine performance, though each relies on assumptions that may not hold in routine programme settings. In high-burden contexts where multiple public health interventions occur simultaneously, attributing observed changes in disease burden to vaccination alone becomes more complex. For diarrhoeal diseases and cholera, these include improvements in water, sanitation and hygiene(WASH), reactive case-

area interventions, and access to oral rehydration and antibiotics. For malaria, vector control measures such as insecticide-treated nets and indoor residual spraying, together with intermittent preventive treatment in pregnancy (IPTp), seasonal malaria chemoprevention (SMC), perennial malaria chemoprevention (PMC), and prompt treatment with artemisinin-based combination therapies, are delivered alongside vaccination. These interventions vary over time in coverage and intensity and are often introduced in response to changes in transmission.

1.11.2 Cluster randomised implementation as an illustrative example

A recent example shows how study design can be adapted to address some of these challenges. The RTS,S/AS01 malaria vaccine was introduced through a WHO-led pilot implementation in Malawi, Ghana, and Kenya using a cluster randomised design embedded within routine programme delivery(148). The phased introduction of the vaccine allowed for allocation of clusters to earlier and later implementation, while vaccination was delivered through existing health systems rather than under trial conditions. This provides a setting in which both direct and indirect effects can be estimated under programme conditions, while retaining some advantages of randomisation(131,149).

In this context, data on other malaria control measures, including bed net use, parasite prevalence, and healthcare utilisation, were collected and incorporated into the analysis(39). This allows for partial adjustment for concurrent interventions that would otherwise confound estimates of vaccine effectiveness and impact.

1.11.3 Limits of applicability in routine programmes

Such approaches are not routinely feasible. The RTS,S pilot was implemented under specific conditions following a conditional policy recommendation that allowed phased introduction, which made cluster allocation acceptable. For most vaccines introduced into routine programmes, withholding or delaying vaccination for the purpose of evaluation would not be ethically acceptable. In addition, cluster randomised implementation at scale requires substantial resources and coordination, which limits its use in many settings. As a result, most post-licensure evaluations rely on observational designs, where adjustment for concurrent interventions is often incomplete.

1.11.4 Implications for vaccine evaluation in sub-Saharan Africa

These limitations create uncertainty in how vaccine impact is estimated in routine settings, particularly in sub-Saharan Africa, where multiple interventions are implemented alongside vaccination and where data are often incomplete. While a range of study designs are used to evaluate vaccine performance, there remains limited consistency in how these approaches account for concurrent public health interventions and other sources of confounding. This raises questions about the extent to which observed reductions in disease can be attributed to vaccination alone. Across the three pathogens considered in this thesis, vaccine-attributable effects are estimated in settings where disease burden is also shaped by other public health interventions, transmission dynamics, care-seeking, and surveillance practices.

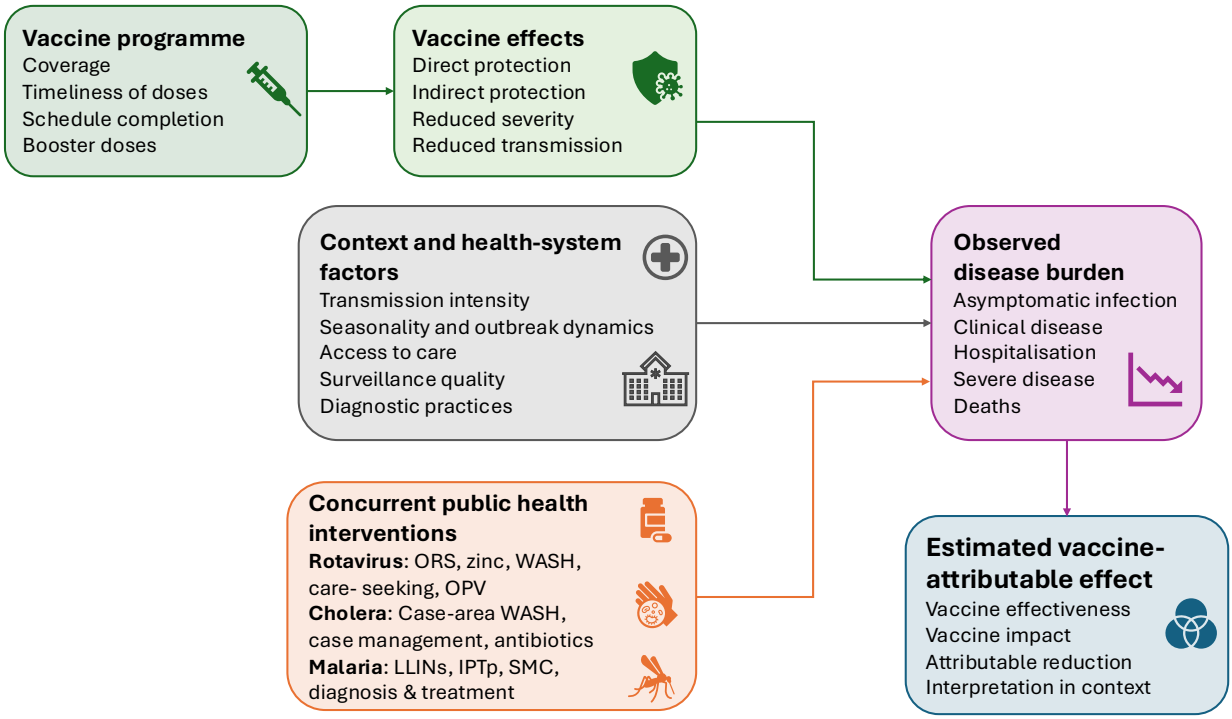


Figure 1-8: Conceptual framework for estimating vaccine-attributable effects in routine programme settings. Individual-level risk factors such as age, nutritional status, HIV status, prior infection, socioeconomic status and care-seeking behaviour are not shown

Figure 1-8 summarises the conceptual framework used in this thesis. The framework starts with the vaccine programme because the effect observed after roll-out depends on how the vaccine is delivered. Coverage, timeliness of doses, schedule completion and booster-dose uptake

determine the proportion of the target population that can benefit from vaccination. These programme inputs then lead to vaccine effects, which may include direct protection in vaccinated individuals, indirect protection through reduced transmission, and reduced disease severity among vaccinated individuals who still become infected. However, the observed disease burden after vaccine introduction is not determined by vaccination alone. It is also influenced by the epidemiology of the disease and by the way disease is detected and managed within the health system. These context and health-system factors include transmission intensity, seasonality, outbreak dynamics, access to care, surveillance quality and diagnostic practice. In addition, other public health interventions may reduce disease burden independently of vaccination. These include ORS, zinc, WASH and care-seeking for rotavirus; case-area WASH, case management and antibiotics for cholera; and long-lasting insecticidal net (LLINs), IPTp, SMC, diagnosis and treatment for malaria. The estimated vaccine-attributable effect therefore needs to be interpreted in relation to programme delivery, epidemiological context, health-system factors and other interventions acting on the same outcome. The implications of this framework differ by pathogen because rotavirus, cholera, and malaria are affected by different concurrent interventions and measurement challenges (Table 1-2).

Vaccine effects may differ by outcome. A vaccine may have a modest effect on infection or mild disease but a larger effect on severe disease, hospitalisation or death if it reduces pathogen replication or limits disease progression. The RTS,S is good example where the EPI-MAL-003 phase 4 analysis estimated 30% effectiveness against any malaria and 58% against severe malaria. This shows why endpoint selection is always important in vaccine evaluation.

Table 1-2: Vaccine evaluation challenges across the three thesis pathogens

Pathogen	**Vaccine/programme context**	**Concurrent interventions**	**Main evaluation challenge**
Rotavirus	Routine infant immunisation	ORS, zinc, breastfeeding, WASH, care-seeking, changes in diarrhoeal admission practice	Separating vaccine impact from secular changes in diarrhoeal care, hospitalisation and child survival

Cholera	Reactive outbreak vaccination and targeted campaigns	Case-area targeted WASH, case management, surveillance changes and population movement	Estimating vaccine effects during rapidly changing outbreaks where multiple interventions are deployed together
Malaria	Routine RTS,S implementation	LLINs, IRS, chemoprevention, diagnosis and treatment, changing transmission intensity	Estimating vaccine contribution when exposure risk and intervention coverage vary spatially and over time

1.12 Thesis rationale, aim and objectives

1.12.1 Thesis starting point and rationale

Over the past decade, my supervisory team and I have been involved in large-scale population-based post-licensure vaccine evaluations in Malawi. These include evaluations of rotavirus vaccine (Rotarix®), pneumococcal conjugate vaccine (PCV13), typhoid conjugate vaccine and the RTS,S/AS01 malaria vaccine, using a variety of study designs. Most post-licensure vaccine evaluations compare disease burden before and after vaccine introduction. Yet vaccines are introduced into immunisation programmes that are increasingly complex and continually changing, often alongside changing PHSMs. Recent vaccine population impact evaluations in Malawi have relied on routine data but this did not capture other changing interventions, e.g. WASH during rotavirus vaccine introduction. Moreover, significant improvements in infant mortality post Rotarix® and PCV13 introductions have raised concern over the level of residual confounding in impact estimates. I examined these challenges using a systematic review and three empirical evaluations of rotavirus, cholera and malaria vaccines in Malawi.

1.12.2 Thesis aim and objectives:

Aim: To examine how vaccine-attributable effects can be measured in routine programme settings where other interventions may also influence disease burden

- Objective 1: To examine the study designs used in post-licensure evaluations and to assess how concurrent interventions are accounted for, addressed through a systematic review of vaccine effectiveness and impact studies conducted in sub-Saharan Africa.
- Objective 2: To evaluate the vaccine effectiveness and impact of the rotavirus vaccine seven years post-introduction
- Objective 3: To evaluate the effectiveness of the oral cholera vaccine during the 2022-2023 cholera outbreak in Blantyre, Malawi
- Objective 4: To compare analytic approaches for estimating RTS,S/AS01 vaccine effects using data from the Malawi cluster-randomised implementation evaluation, and to consider how design and analytic choices influence interpretation.

The final chapter synthesises these findings to consider how vaccine impact can be estimated more reliably in settings where multiple interventions operate simultaneously.

1.12.3 Thesis outline

This thesis is structured around a general introduction, a systematic review chapter, three empirical vaccine evaluation chapters and a final discussion.

- Chapter 1 introduces the burden of vaccine-preventable infectious diseases in sub-Saharan Africa, with specific focus on rotavirus, cholera, and malaria. It also describes vaccine platforms, population-level effects of vaccination, and the challenge of attributing vaccine impact in routine programme settings where multiple public health and social measures are implemented concurrently. Chapter 1 also provides the conceptual and methodological basis for the thesis. It defines vaccine efficacy, effectiveness, and impact, and describes the main study designs used in post-licensure vaccine evaluation. This chapter also sets out the problem of concurrent interventions and explains why estimates of vaccine-attributable impact may differ across study designs.
- Chapter 2 presents a systematic review of post-licensure vaccine evaluations conducted in children in sub-Saharan Africa. This chapter reviews the study designs used in post-licensure vaccine evaluation and whether they measured and adjusted for other

interventions that also influence disease epidemiology. This chapter is submitted to The Lancet Regional Health Africa and available as a preprint in Medrxiv.

- Chapter 3 evaluates the long-term impact and effectiveness of Rotarix® in Malawi using interrupted time-series and test-negative case–control analyses. This chapter estimates population-level impact and individual-level vaccine effectiveness more than seven years after vaccine introduction and examines whether the national switch from trivalent to bivalent oral poliovirus vaccine influenced Rotarix® performance. This chapter is submitted to the BMJ Global Health and available as a preprint in Medrxiv.
- Chapter 4 assesses the effectiveness of oral cholera vaccine during the 2022–2023 cholera outbreak in Blantyre, Malawi. This chapter uses transmission-based modelling to estimate vaccine-attributable reductions in cholera transmission while explicitly incorporating WASH intervention activity. It shows how the estimated vaccine effect changes when WASH activity is included in the model. This chapter is submitted to The Lancet Global Health and available as a preprint in Medrxiv.
- Chapter 5 examines RTS,S/AS01 malaria vaccine effectiveness using data from the Malaria Vaccine Implementation Programme in Malawi. This chapter compares cluster-level, unmatched case–control, and matched case–control approaches applied to the same population to assess how endpoint choice, matching, and study design affect vaccine effect estimates.
- Chapter 6 provides a general discussion that synthesises the findings across the systematic review and empirical chapters. It considers what different study designs estimate, how concurrent interventions influence attribution, and how post-licensure vaccine evidence should be interpreted for policy and programme evaluation. The chapter concludes by setting out future work that will be taken forward in a postdoctoral fellowship focused on developing a framework for interpreting vaccine-attributable impact under concurrent public health interventions.

The results chapters are written as standalone studies for publication, with detailed introduction methods, results, and discussion sections presented within each chapter. The final discussion brings these chapters together to address the central question of the thesis: how vaccine-

attributable effects can be estimated more reliably in routine settings where multiple interventions operate at the same time.

2 Methods used to adjust for non-vaccine interventions in post-licensure vaccine evaluations among children in sub-Saharan Africa: a systematic review

2.1 Abstract

Background

Post-licensure vaccine effectiveness and impact studies provide evidence on how vaccines perform under routine programme conditions in the real world. In sub-Saharan Africa (SSA), vaccine introductions frequently coincide with concurrent public health and social measures that may influence disease risk and transmission. Failure to account for these concurrent interventions may affect the interpretation of vaccine effects.

Methods

We conducted a systematic review of post-licensure vaccine effectiveness and impact studies conducted in children under five years of age in SSA. Electronic databases were searched for peer-reviewed studies published between January 2000 and December 2019. Eligible studies used observational designs to estimate vaccine effectiveness or population-level impact. Two reviewers independently screened studies, extracted data, and assessed methodological quality using Joanna Briggs Institute tools. We examined study designs, vaccines evaluated, outcomes assessed, and whether public health and social measures (PHSMs) were measured or adjusted for. A narrative synthesis was undertaken. In addition, we conducted a meta-analysis for rotavirus and pneumococcal conjugate vaccines where we explored the heterogeneity in individual-level effectiveness estimates where designs and outcomes were comparable.

Results

Sixty-four studies met the inclusion criteria, covering eight vaccine-preventable diseases. Rotavirus vaccines were most frequently evaluated, followed by pneumococcal conjugate vaccines. Case–control and ecological designs were most common, while cohort and time-series analyses were less frequently used. None of the included studies collected, reported, or adjusted for PHSMs such as nutrition, WASH, or access to healthcare. The implications of this

omission varied by pathogen. Rotavirus vaccine effectiveness estimates from comparable individual-level designs were consistent across settings, with no evidence of between-study heterogeneity. Pneumococcal vaccine effectiveness estimates showed substantial heterogeneity, which appeared to reflect differences in outcome definitions, host risk profiles, and study context. Estimates for other vaccines were generally protective in direction, although the magnitude and precision varied across studies.

Conclusions

Post-licensure vaccine effectiveness and impact studies in SSA rarely account for concurrent PHSMs. The consequences of this omission are not uniform across vaccines. For some pathogens, effectiveness estimates appear robust to unmeasured contextual change, while for others they are highly sensitive to outcome choice and setting. Future evaluations should prioritise systematic measurement of key PHSMs and consider study designs that better account for time-varying context. Strengthening routine data systems to capture these factors is needed for generating interpretable evidence to inform immunisation policy.

Funding: MRC Discovery Medicine North (DiMeN) Doctoral Training Partnership (UKRI), National Institute for Health and Care Research (NIHR) Global Health Research Group on Gastrointestinal Infections and Wellcome through the core grant to the Malawi-Liverpool-Wellcome Research Programme.

2.2 Introduction

2.2.1 Vaccine-preventable diseases and post-licensure evaluation

Vaccination remains one of the most effective public health interventions for reducing childhood morbidity and mortality. National immunisation programmes in sub-Saharan Africa (SSA) have progressively introduced vaccines targeting major childhood pathogens including tuberculosis, diphtheria, tetanus, pertussis, poliomyelitis, pneumococcal disease, rotavirus diarrhoea, measles, and more recently typhoid and malaria(150). These vaccines have contributed to substantial declines in vaccine-preventable diseases across the region(12,151–153). Despite this progress, vaccine-preventable infections continue to account for a substantial burden of child mortality, with the majority of deaths occurring in low-income settings where health system constraints and high transmission intensity persist(12). The introduction of many vaccines has occurred alongside broader improvements in child health, including expanded malaria control, improved nutrition, HIV prevention programmes, and water, sanitation and hygiene (WASH) interventions. These concurrent public health and social measures (PHSMs) may influence disease burden and complicate attribution of observed reductions to vaccination alone when evaluating vaccine effectiveness or impact.

2.2.2 Overview of post-licensure study designs for vaccine evaluation

Pre-licensure clinical trials evaluate vaccine safety and efficacy under controlled conditions and are widely regarded as the gold standard for establishing direct protective effects(154). Individually randomised trials estimate vaccine efficacy in vaccinated individuals compared with controls, while cluster-randomised trials can also capture indirect protection by allocating communities or geographical areas to vaccination or control groups(154–156). These trials are designed primarily to support licensure and therefore typically include strict eligibility criteria and clearly defined endpoints, which may not fully reflect vaccine performance in routine use.

Post-licensure studies therefore provide essential evidence on how vaccines perform once introduced into national immunisation programmes across diverse populations and epidemiological settings. These studies can quantify direct protection in vaccinated individuals

as well as broader population effects arising from reduced transmission, including indirect and overall programme impact(129,157). A range of observational designs are used for this purpose, including ecological analyses, time-series approaches, case–control studies, cohort studies and screening methods. Such designs are often necessary because withholding an approved vaccine from populations in need would raise ethical concerns. Observational evaluations nevertheless introduce additional methodological challenges, including confounding from concurrent PHSMs and broader changes in population health that occur alongside vaccine introduction.

Table 2-1: Overview of post-licensure study designs for vaccine evaluation

Study Design	**What it estimates**	**Strengths**	**Limitations**
Ecological (including uncontrolled and controlled interrupted time-series)(147,158–160)	Overall effect or population level impact	Uses routinely collected surveillance data; can capture broad programmatic impact rapidly	Highly vulnerable to confounding from secular trends and concurrent interventions; no individual-level inference
Screening method(134)	Overall vaccine effectiveness (VE) using coverage and case vaccination status	Low cost; relatively quick; minimal data requirements	Dependent on accurate coverage and case ascertainment data; prone to misclassification bias; limited to VE estimation
Case–control (including test-negative and matched designs) (134,161)	Direct VE estimation at individual level	Efficient for rare outcomes; suitable during outbreaks and immediately after new vaccine introductions; requires smaller sample sizes	Susceptible to selection and recall bias; careful adjustment for confounding needed. Challenging if vaccine is highly effective, due to too few cases and / or if vaccine uptake is very high or low, best implemented when uptake is 20-80%.
Cohort studies(148,162–164)	Direct, indirect, total and overall effect	Strong temporal sequence; can estimate incidence; can assess multiple outcomes	Expensive; follow-up losses; residual confounding by health-seeking behaviour or socioeconomic factors
Cluster-randomised trials(155,156,165)	Direct and indirect (herd) protection	Captures community-level as well as individual effects; strong internal validity	Expensive; complex logistics; large sample sizes required; usually impractical post-licensure

Individually randomised trials(155)	Direct protective efficacy at the individual level	Considered the benchmark for causal inference; high internal validity	Often unethical or unfeasible after licensure; restricted generalisability to routine programme conditions

2.2.3 Challenges in attributing vaccine effects in routine settings

There have been observed differences in vaccine effectiveness for particular vaccines in different geographical settings, depending on differing coverage of other interventions for the same disease(166–169). There is no agreed methodological standard for the design of post-licensure vaccine effectiveness and impact studies. The modifying effects of disease-related PHSMs could be important when evaluating impact of vaccination programmes. Furthermore, the epidemiology of other diseases, health interventions, health systems, surveillance efforts and societal developments are continually changing and are often difficult to quantify via the routine surveillance systems common in Sub-Saharan Africa, yet these factors may all confound impact measures. This makes it a necessity to use appropriate methods when estimating vaccine-attributable effects, particularly when studying nonspecific endpoints such as mortality and symptomatic healthcare utilisation. In this review, we examined the principal study designs used in post-licensure vaccine effectiveness and impact studies and assessed the extent to which these studies accounted for concurrent PHSMs.

2.3 Methods

2.3.1 Review design

This systematic review was conducted using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. It aimed to investigate the types of study designs used in post-licensure vaccine evaluations and whether they actively considered PHSMs, and if yes, how they accounted for them in study design and/or adjusted for them in analyses. The review was registered on PROSPERO for systematic reviews (registration number: CRD42023436851).

2.3.2 Search strategy

A comprehensive search was conducted in electronic databases to identify relevant post-licensure vaccine studies that were published in peer-reviewed journals from the year 2000 to 2019. This was done to exclude the post-licensure studies related to COVID-19 vaccines. The electronic databases included in the search were PubMed Central, EMBASE, MEDLINE and CINAHL. In addition, to identify missing papers and non-academic literature, Google Scholar was

searched and citation searching of reference lists of included papers was also conducted. Review articles on vaccine impact and effectiveness studies were also used to search the references for relevant publications. The search was restricted to human studies, in English language and excluded pre-prints and review articles. The search dates were from 24/05/2023 to 27/05/2023.

To develop a comprehensive search query we used Boolean operators “AND”, “OR” and “NOT” to combine Medical Subject Headings (MeSH) terms and keywords to develop a final search string: ((("Vaccination"[MeSH Terms] OR "Immunization"[MeSH Terms] OR "immuni*"[Title/Abstract] OR "vaccin*"[Title/Abstract] OR "vaccination program*"[Title/Abstract] OR "immunization program*"[Title/Abstract] OR "immunisation program*"[Title/Abstract]) AND "Africa South of the Sahara"[MeSH Terms] AND ("Infant"[MeSH Terms] OR "child*"[Title/Abstract] OR "preschool"[Title/Abstract]) AND ("Program Evaluation"[MeSH Terms] OR "Effectiveness"[Title/Abstract] OR "Impact"[Title/Abstract])) NOT "Review"[Publication Type]) AND ((humans[Filter]) AND (english[Filter]) AND (allinfant[Filter] OR preschoolchild[Filter]) AND (2000:2019[pdat]))

2.3.3 Study selection and screening

Studies that were identified using the search strategy were imported into Rayyan AI, a web-based tool for managing systematic reviews(170). Rayyan AI was used to create a database of records and was also instrumental in handling the large number of records from multiple databases and de-duplicating the records.

Two blinded reviewers (LN and MV) carried out the two-stage study selection by (1) screening titles and abstracts and (2) the full text review to select articles that met the inclusion and exclusion criteria. Following the unblinding procedure, discrepancies that arose at both stages of study selection were resolved by discussion, re-examination and consensus (Figure 2-1).

2.3.4 Inclusion and exclusion criteria

Studies were included if they were: (1) Articles published in peer-reviewed journals for vaccine effectiveness and vaccine impact studies; (2) Used observational methods (e.g. case–control, cohort, time series, ecological and cluster-randomised designs); (3) Published between 01

January 2000 and 31 December 2019; (4) Conducted in children living in Africa and (5) were published in English.

Studies were not included if they were: (1) Clinical trials; (2) pre-prints; (3) letters to the editor; (4) conference abstracts; (5) modelling studies that did not use observational data; (6) cost-effectiveness evaluations; (7) systematic reviews; (8) unable to access full articles; (9) serological studies; (10) vaccine coverage studies; (11) carriage studies and (12) pre–post introduction evaluations that used hospital registry data and did not report effect measures.

2.3.5 Data extraction

A data extraction form was developed using Microsoft Excel to systematically collect relevant data from studies including title, year of publication, journal name, author names, geographical location (country), disease of interest, vaccine of interest, other confounding PHSMs, magnitude of effect of PHSMs, adjustment for PHSMs, adjustment for other vaccines, study design, setting (community/hospital), funder, effect estimates, inclusion criteria, exclusion criteria, primary end points, sample size, study period, follow-up period, study population, data collection methods, herd immunity, follow-up procedures, measures of effect and type of funder.

2.3.6 Quality assessment of included studies

The Joanna Briggs Institute (JBI) quality assessment tool was used by two independent reviewers (LN and MV) on the studies that met the inclusion criteria. Any conflicts that arose were resolved through re-examination and advice from a third reviewer (DH).

2.3.7 Data analysis

We undertook a narrative synthesis of the included studies to summarise the outcomes given the heterogeneity of the study designs and other outcomes. The characteristics of studies were described in tables based on variables including, year of publication, vaccine of interest, study design, PHSMs assessed and other risk factors. For measures of vaccine effectiveness and impact we report point estimates and range of point estimates across studies. We have included meta-analyses and forest plots of effectiveness in case–control studies by vaccine type to assess between-study heterogeneity. The objective was not to generate pooled vaccine effectiveness estimates, which have been reported in other systematic reviews of specific vaccines. Instead,

the analysis was intended to assess how reported effectiveness varies across comparable study designs and settings, and to consider whether differences in analytical approaches and contextual factors may contribute to this variation. Adjusted Odds Ratios and confidence intervals reported from case–control studies were included. We used random effects models with inverse variance method, using the DerSimonian-Laird between-study heterogeneity estimator. Heterogeneity was measured using chi-squared ($\chi 2$) heterogeneity p-values and I^2 statistics.

2.4 Results

2.4.1 Study selection

The search across electronic databases yielded 64 studies included in the review. The majority were excluded because the study design and outcome did not meet the inclusion criteria.

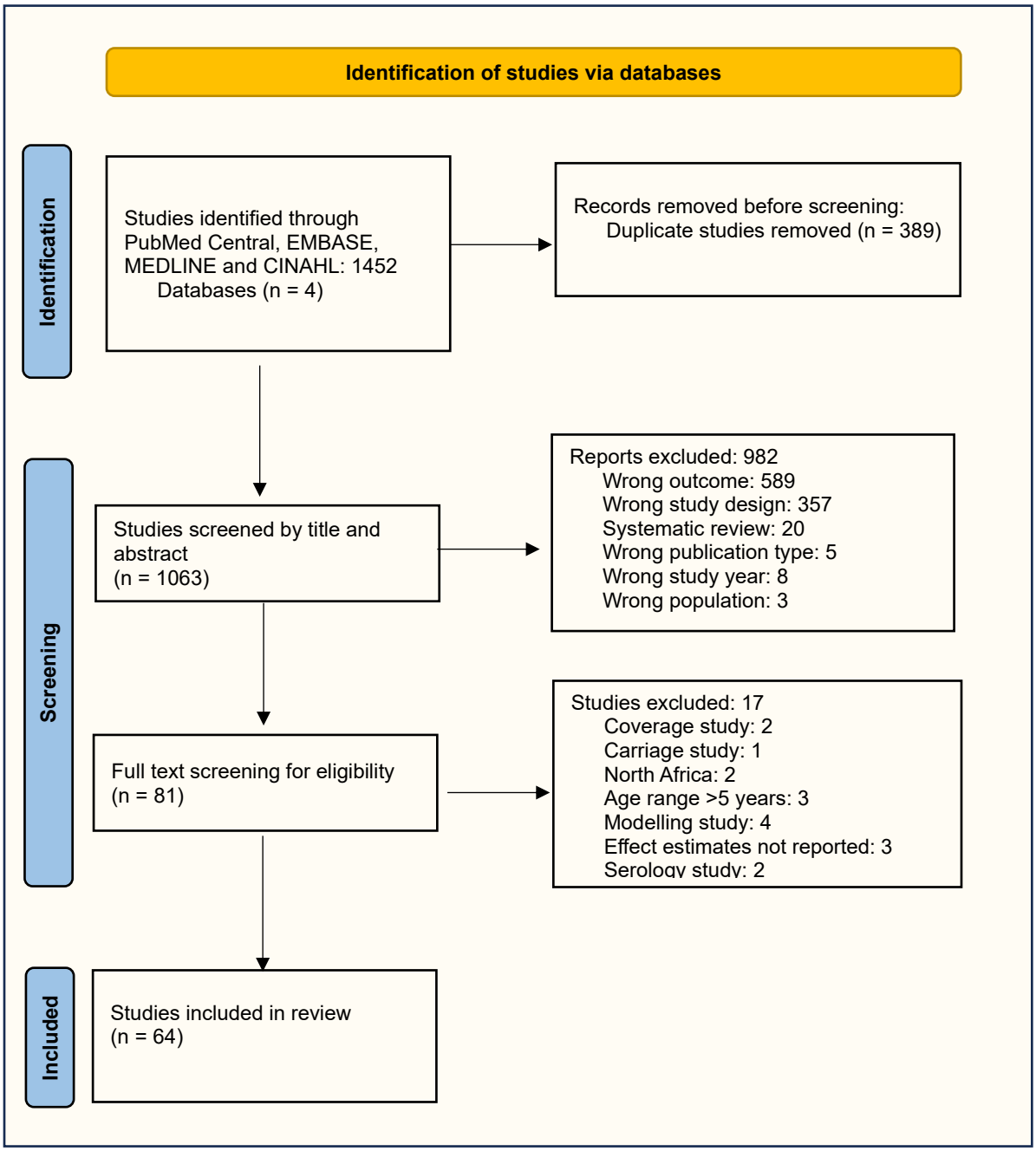


Figure 2-1: PRISMA flow chart selection process

2.4.2 JBI quality assessment results

The quality of the methods of included studies was moderate to high across all study designs. Using the Joanna Briggs Institute appraisal tools, none of the 64 studies were classified as poor quality by either reviewer. Most studies clearly defined their study populations, outcomes, confounders, analytical approaches, and applied appropriate statistical methods for their respective designs. However, despite generally strong internal validity within designs, none collected, reported, or adjusted for concurrent PHSMs. The full JBI quality assessment results are presented in Tables S5–S8, with separate tables for ecological, case-control, cohort and the screening method studies.

2.4.3 Study characteristics

Our review included 64 studies evaluating post-licensure vaccine effectiveness across sub-Saharan Africa, revealing patterns in research approaches and focus areas detailed in Table 2-2.

Rotavirus vaccines were most frequently evaluated, accounting for 31 studies (48.4%), many conducted through the WHO-coordinated African Rotavirus Surveillance Network across multiple countries in Eastern and Southern Africa(171). Pneumococcal conjugate vaccines were assessed in nine studies (14.1%), most commonly PCV13, with fewer studies evaluating PCV7 and PCV10. Measles-containing vaccines were examined in eight studies (12.5%). Influenza and Haemophilus influenzae type b vaccines were less frequently studied, with five and four studies respectively. Meningococcal vaccines were evaluated in three studies, while polio and cholera vaccines were rarely assessed, each represented by two studies (3.1%).

The geographical distribution of studies revealed notable disparities across sub-Saharan Africa. Southern Africa accounted for nearly half of all studies (n = 30, 45.5%), followed by West Africa with 20 studies (30.3%) and East Africa with 12 studies (18.2%). In contrast, Central Africa (6.1%) and Northwest Africa (1.5%) were significantly underrepresented. In terms of publication period, there was a marked increase in study output over the last decade, with 86% (n = 57) of studies published between 2010 and 2019. Only nine studies (13.6%) were published between 2000 and 2009, reflecting both the acceleration of vaccine introductions in the region and an apparent expansion in regional research capacity.

Table 2-2: Characteristics of included studies

Title	**Authors**	**Country**	**Vaccine**	**Study design**	**Outcome**	**Effect measures**
Effectiveness of pentavalent rotavirus vaccine under conditions of routine use in Rwanda(172).	Tate *et al*	Rwanda	RV5	Case–control	Vaccine effectiveness on hospitalisations or health centre visit.	Odds ratio (OR)
Impact and effectiveness of pentavalent rotavirus vaccine in children <5 years of age in Burkina Faso(173).	Bonkoungou *et al*	Burkina Faso	RV5	Ecological	Vaccine impact and effectiveness on hospitalisations	Proportion reduction (%)
Evaluation of the influence of gastrointestinal coinfections on rotavirus vaccine effectiveness in Botswana(174).	Mokomane *et al*	Botswana	RV1	Case–control	Vaccine effectiveness on hospitalisations	Odds ratio (OR)

Title	Authors	Country	Vaccine	Study design	Outcome	Effect measures
Population impact and effectiveness of monovalent rotavirus vaccination in urban Malawian children 3 years after vaccine introduction: ecological and case–control analyses(175).	Bar-Zeev *et al*	Malawi	RV1	Case–control	Vaccine effectiveness in the second year of life and in HIV-exposed and stunted children	Incidence rate ratio (IRR)
A preliminary assessment of rotavirus vaccine effectiveness in Zambia(176).	Beres *et al*	Zambia	RV1	Case–control	Vaccine effectiveness on hospitalisations	Odds ratio (OR)
Vaccine effectiveness against DS-1-like rotavirus strains in infants with acute gastroenteritis, Malawi, 2013-2015(177).	Jere *et al*	Malawi	RV1	Case–control	Vaccine effectiveness on hospitalisations	Odds ratio (OR)
Impact and effectiveness of monovalent rotavirus vaccine against severe rotavirus diarrhea in Ghana(178).	Armah *et al*.	Ghana	RV1	Case–control	Vaccine impact and effectiveness on hospitalisations	Odds ratio (OR)
Effectiveness of monovalent rotavirus vaccine after programmatic implementation in Botswana: a multisite	Gastañaduy *et al*	Botswana	RV1	Case–control	Vaccine effectiveness on hospitalisations	Odds ratio (OR)

Title	Authors	Country	Vaccine	Study design	Outcome	Effect measures
prospective case–control study(179).						
Impact of rotavirus vaccine introduction and post introduction etiology of diarrhea requiring hospital admission in Haydom, Tanzania, a rural African setting(180).	Platts-Mills *et al*	Tanzania	RV1	Case–control	Vaccine impact on hospitalisations	Odds ratio (OR)
Effectiveness of monovalent human rotavirus vaccine against admission to hospital for acute rotavirus diarrhoea in South African children: a case–control study(181).	Groome *et al*	South Africa	RV1	Case–control	Vaccine effectiveness on hospitalisations	Odds ratio (OR)
Effectiveness of a monovalent rotavirus vaccine in infants in Malawi after programmatic roll-out: an observational and case–control study(182).	Bar-Zeev *et al*	Malawi	RV1	Case–control	Vaccine impact and effectiveness on hospitalisations	Odds ratio (OR)
Monovalent rotavirus vaccine effectiveness and impact on rotavirus hospitalizations in Zanzibar, Tanzania: data from	Abeid *et al*	Tanzania	RV1	Case–control	Vaccine impact and effectiveness on hospitalisations	Odds ratio (OR)

Title	Authors	Country	Vaccine	Study design	Outcome	Effect measures
the first 3 years after introduction(183).						
Impact of monovalent rotavirus vaccine on diarrhoea-associated post-neonatal infant mortality in rural communities in Malawi: a population-based birth cohort study(184).	Bar-Zeev *et al*	Malawi	RV1	Cohort	Vaccine impact on hospitalisations	Hazard ratio (HR)
Impact of rotavirus vaccination on hospitalizations and deaths from childhood gastroenteritis in Botswana(185).	Enane *et al*	Botswana	RV1	Ecological	Vaccine impact on hospitalisations	Proportion reduction (%)
Evidence of the impact of monovalent rotavirus vaccine on childhood acute gastroenteritis hospitalization in Togo(186).	Tsolenyanu *et al*	Togo	RV1	Ecological	Vaccine impact on hospitalisations	Proportion reduction (%)

Title	Authors	Country	Vaccine	Study design	Outcome	Effect measures
Early impact of rotavirus vaccination in children less than five years of age in Mozambique(187).	de Deus *et al*	Mozambique	RV1	Ecological	Vaccine impact on hospitalisations	Proportion reduction (%)
Impact of rotavirus vaccine on all-cause diarrhea and rotavirus hospitalizations in Madagascar(188).	Rahajamanana *et al*	Madagascar	RV1	Ecological	Vaccine impact on hospitalisations	Proportion reduction (%)
Early evidence of impact of monovalent rotavirus vaccine in Togo(189).	Tsolenyanu *et al*	Togo	RV1	Ecological	Vaccine impact on hospitalisations	Proportion reduction (%)
Detection of rotavirus before and after monovalent rotavirus vaccine introduction and vaccine effectiveness among children in mainland Tanzania(190).	Jani *et al*	Tanzania	RV1	Ecological	Vaccine impact on hospitalisations	Prevalence ratio

Title	Authors	Country	Vaccine	Study design	Outcome	Effect measures
Impact of rotavirus vaccine introduction and genotypic characteristics of rotavirus strains in children less than 5 years of age with gastroenteritis in Ethiopia: 2011-2016(191).	Abebe *et al*	Ethiopia	RV1	Ecological	Vaccine impact on hospitalisations and genotypic characteristics of rotavirus strains	Proportion reduction (%)
Impact of routine rotavirus vaccination on all-cause and rotavirus hospitalizations during the first four years following vaccine introduction in Rwanda(192).	Sibomana *et al*	Rwanda	RV1	Ecological	Vaccine impact on all-cause and rotavirus hospitalisations	Proportion reduction (%)
Impact of rotavirus vaccination on diarrheal hospitalizations in children aged <5 years in Lusaka, Zambia(193).	Mpabalwani *et al*	Zambia	RV1	Ecological	Vaccine impact on hospitalisations	Proportion reduction (%)
Sustained impact of rotavirus vaccine introduction on rotavirus gastroenteritis hospitalizations in children <5 years of age, Ghana, 2009-2016(194).	Enweronu-Laryea *et al*	Ghana	RV1	Ecological	Vaccine impact against rotavirus hospitalisations	Proportion reduction (%)

Title	Authors	Country	Vaccine	Study design	Outcome	Effect measures
Reduction of hospitalizations with diarrhea among children aged 0-5 years in Nouakchott, Mauritania, following the introduction of rotavirus vaccine(195).	Ahmed *et al*	Mauritania	RV1	Ecological	Vaccine impact against rotavirus hospitalisations	Proportion reduction (%)
Impact of rotavirus vaccine on acute gastroenteritis in children under 5 years in Senegal: Experience of sentinel site of the Albert Royer Children's Hospital in Dakar(196).	Diop *et al*	Senegal	RV1	Ecological	Vaccine impact against rotavirus hospitalisations	Proportion reduction (%)
Sustained impact of rotavirus vaccine on rotavirus hospitalisations in Lusaka, Zambia, 2009-2016(197).	Mpabalwani *et al*	Zambia	RV1	Ecological	Vaccine impact against rotavirus hospitalisations	Proportion reduction (%)
Early impact of rotavirus vaccine in under 5 year old children hospitalized due to diarrhea, Swaziland(198).	Maphalala *et al*	Swaziland	RV1	Ecological	Vaccine impact against rotavirus hospitalisations	Proportion reduction (%)

Title	Authors	Country	Vaccine	Study design	Outcome	Effect measures
Direct and possible indirect effects of vaccination on rotavirus hospitalisations among children in Malawi four years after programmatic introduction(199).	Bennett *et al*	Malawi	RV1	Interrupted time series analysis and case–control study	Vaccine effectiveness on hospitalisations and indirect effects	Incidence rate ratio (IRR)
Monovalent rotavirus vaccine effectiveness against rotavirus hospitalizations among children in Zimbabwe(200).	Mujuru *et al*	Zimbabwe	RV1	Test-negative case–control	Vaccine effectiveness on hospitalisations	Odds ratio (OR)
Temporal association of rotavirus vaccine introduction and reduction in all-cause childhood diarrheal hospitalizations in South Africa(201).	Groome *et al*	South Africa	RV1	Time series	Vaccine impact on hospitalisations	Incidence rate reduction (%)

Title	Authors	Country	Vaccine	Study design	Outcome	Effect measures
Impact of rotavirus vaccination on rotavirus hospitalisation rates among a resource-limited rural population in Mbita, Western Kenya(202).	Wandera *et al*	Kenya	RV1	Time series	Vaccine impact on hospitalisations	Incidence reduction (%)
Effectiveness of pneumococcal conjugate vaccine against presumed bacterial pneumonia hospitalisation in HIV-uninfected South African children: a case–control study(203).	Madhi *et al*	South Africa	PCV7 and PCV13	Matched case–control	Vaccine effectiveness on hospitalisations	Odds ratio (OR)
Pneumococcal conjugate vaccines and hospitalization of children for pneumonia: a time-series analysis, South Africa, 2006-2014(204).	Izu *et al*	South Africa	PCV7 and PCV13	Time series	Vaccine impact on hospitalisations	Incidence rate reduction (%)

Title	Authors	Country	Vaccine	Study design	Outcome	Effect measures
Effectiveness of 7-valent pneumococcal conjugate vaccine against invasive pneumococcal disease in HIV-infected and -uninfected children in South Africa: a matched case–control study(205).	Cohen *et al*	South Africa	PCV7	Case–control	Vaccine effectiveness against IPD in HIV infected and uninfected children	Odds ratio (OR)
Impact of the introduction of pneumococcal conjugate vaccination on pneumonia in The Gambia: population-based surveillance and case–control studies(206).	Mackenzie *et al*	Gambia	PCV13	Ecological	Vaccine impact on IPD and radiological pneumonia with consolidation	Incidence rate ratio (IRR)
Impact of 13-valent pneumococcal conjugate vaccine on pneumococcal meningitis, Burkina Faso, 2016-2017(207).	Soeters *et al*	Burkina Faso	PCV13	Ecological	Vaccine impact against hospitalization	Rate ratio (RR)

Title	Authors	Country	Vaccine	Study design	Outcome	Effect measures
Impact of 13-valent pneumococcal conjugate vaccine on meningitis and pneumonia hospitalizations in children aged <5 years in Senegal, 2010–2016(208).	Faye *et al*	Senegal	PCV13	Interrupted time-series analysis	Vaccine impact on pneumonia and meningitis hospitalisations	Prevalence ratio (PR)
Effectiveness of the 13-valent pneumococcal conjugate vaccine against invasive pneumococcal disease in South African children: a case–control study(209).	Cohen *et al*	South Africa	PCV13	Matched case–control	Vaccine effectiveness on hospitalisations in HIV positive and negative children	Odds ratio (OR)
Pneumococcal conjugate vaccine impact on meningitis and pneumonia among children aged <5 years-Zimbabwe, 2010-2016(210).	Dondo *et al*	Zimbabwe	PCV13	Time series	Vaccine impact on pneumonia and meningitis hospitalisations	Incidence rate reduction (%)
Surveillance of impact of PCV-10 vaccine on pneumococcal meningitis in Mozambique, 2013 – 2015(211).	Nhantumbo *et al*	Mozambique	PCV10	Ecological	Vaccine impact against hospitalisations	Proportion reduction (%)

Title	Authors	Country	Vaccine	Study design	Outcome	Effect measures
Effectiveness of immunization against paralytic poliomyelitis in Nigeria(212).	Jenkins *et al*	Nigeria	Oral polio vaccine	Matched case–control	Efficacies of monovalent type 1 oral poliovirus vaccine and trivalent oral poliovirus vaccine	Odds ratio (OR)
Effectiveness of oral polio vaccination against paralytic poliomyelitis: a matched case–control study in Somalia(213).	Mahamud *et al*	Somalia	Oral Polio vaccine	Retrospective case–control	Vaccine effectiveness of oral polio vaccine on at preventing WPV1	Odds ratio (OR)
Impact of previous immunisation on the incidence of meningococcal disease during an outbreak in a Sahelian area of Senegal(214).	Chippaux *et al*	Senegal	Meningitis vaccine (Polysaccharide)	Ecological	Vaccine impact on meningitis hospitalisations	Incidence rate ratio (IRR)
Impact of the serogroup A meningococcal conjugate vaccine, MenAfriVac, on carriage and herd immunity(215).	Kristiansen *et al*	Burkina Faso	Meningitis vaccine (conjugate)	Ecological	Vaccine impact on carriage	Prevalence ratio (PR)

Title	Authors	Country	Vaccine	Study design	Outcome	Effect measures
Continuing effectiveness of serogroup a meningococcal conjugate vaccine, Chad, 2013(216).	Gamougam *et al*	Chad	Meningitis vaccine (conjugate)	Interrupted time series analysis	Vaccine impact on meningitis hospitalisations	Incidence rate ratio (IRR)
Preplanned national measles vaccination campaign at the beginning of a measles outbreak--Sierra Leone, 2009-2010(217).	Sugerman *et al*	Sierra Leone	Measles vaccine	Screening method	Vaccine effectiveness against disease	Vaccine effectiveness (screening method)
Measles outbreak in Burkina Faso, 2009: a case–control study to determine risk factors and estimate vaccine effectiveness(218).	Kidd *et al*	Burkina Faso	Measles vaccine	Case–control	Vaccine effectiveness against disease	Odds ratio (OR)
Measles outbreak propagated by children congregating at water collection points in Mayuge District, eastern Uganda, July - October, 2016(219).	Majwala *et al*	Uganda	Measles vaccine	Case–control	Vaccine effectiveness against disease	Odds ratio (OR)
Measles vaccination effectiveness among children under 5 years of age in Kampala, Uganda(220).	Mupere *et al*	Uganda	Measles vaccine	Cohort	Vaccine effectiveness against disease	Risk ratio (attack rate ratio)

Title	Authors	Country	Vaccine	Study design	Outcome	Effect measures
Impact of measles outbreak response vaccination campaign in Dar es Salaam, Tanzania(221).	Goodson *et al*	Tanzania	Measles vaccine	Ecological	Vaccine effectiveness against disease and mortality	Incidence rate ratio (IRR)
The effect of immunization on measles incidence in the Democratic Republic of Congo: Results from a model of surveillance data(222).	Doshi *et al*	Democratic Republic of Congo	Measles vaccine	Ecological	Vaccine effectiveness against disease	Incidence rate ratio (IRR)
Lessons and challenges for measles control from unexpected large outbreak, Malawi(223).	Minetti *et al*	Malawi	Measles vaccine	Ecological study	Vaccine effectiveness against disease	Risk ratio (RR)
Field evaluation of measles vaccine effectiveness among children in the Democratic Republic of Congo(224).	Doshi *et al*	Democratic Republic of Congo	Measles vaccine	Test-negative case–control	Vaccine effectiveness against disease	Odds ratio (OR)
Uptake and effectiveness of a trivalent inactivated influenza vaccine in children in urban and rural Kenya, 2010 to 2012(225).	Katz *et al*	Kenya	Influenza vaccine	Case–control	Vaccine coverage and effectiveness	Odds ratio (OR)

Title	Authors	Country	Vaccine	Study design	Outcome	Effect measures
Influenza epidemiology and vaccine effectiveness among patients with influenza-like illness, viral watch sentinel sites, South Africa, 2005-2009(226).	Ntshoe *et al*	South Africa	Influenza vaccine	Case–control	Vaccine effectiveness against disease	Odds ratio (OR)
Impact of H1N1 influenza vaccination on child morbidity in Guinea-Bissau(227).	Hansen *et al*	Guinea-Bissau	Influenza vaccine	Cohort & ecological	Vaccine effectiveness against all-cause consultation rates	Hazard ratio (HR)
Estimating vaccine effectiveness in preventing laboratory-confirmed influenza in outpatient settings in South Africa, 2015(228).	McAnerney *et al*	South Africa	Influenza vaccine	Test-negative case–control	Vaccine effectiveness against disease	Odds ratio (OR)
Effectiveness and knowledge, attitudes and practices of seasonal influenza vaccine in primary healthcare settings in South Africa, 2010-2013(229).	McAnerney *et al*	South Africa	Influenza vaccine	Test-negative case–control design	Vaccine effectiveness against disease	Odds ratio (OR)
Haemophilus influenzae type b conjugate vaccine is highly effective in the Ugandan routine immunization program: a case–control study(230).	Lee *et al*	Uganda	Hib	Case–control	Vaccine effectiveness against disease	Odds ratio (OR)

Title	Authors	Country	Vaccine	Study design	Outcome	Effect measures
Reduced effectiveness of Haemophilus influenzae type b conjugate vaccine in children with a high prevalence of human immunodeficiency virus type 1 infection(231).	Madhi *et al*	South Africa	Hib	Cohort	Vaccine effectiveness against disease invasive HIV disease in children with HIV	Odds ratio (OR)
Effectiveness of haemophilus influenzae type B conjugate vaccine for prevention of meningitis in Senegal(232).	Fleming *et al*	Senegal	Hib	Matched, case–control	Vaccine effectiveness against Hib meningitis	Odds ratio (OR)
Effectiveness of Haemophilus influenzae type b conjugate vaccine introduction into routine childhood immunization in Kenya(233).	Cowgill *et al*	Kenya	Hib	Surveillance study	Vaccine effectiveness against invasive disease	Incidence rate ratio (IRR)
Effectiveness of mass oral cholera vaccination in Beira, Mozambique(234).	Lucas *et al*	Mozambique	Cholera vaccine (rBS-WC vaccine)	Case–control	Vaccine effectiveness against disease	Odds ratio (OR)

Title	Authors	Country	Vaccine	Study design	Outcome	Effect measures
Use of Vibrio cholerae vaccine in an outbreak in Guinea(235).	Luquero *et al*	Guinea	Cholera vaccine	Matched, case–control	Vaccine effectiveness against disease	Odds ratio (OR)

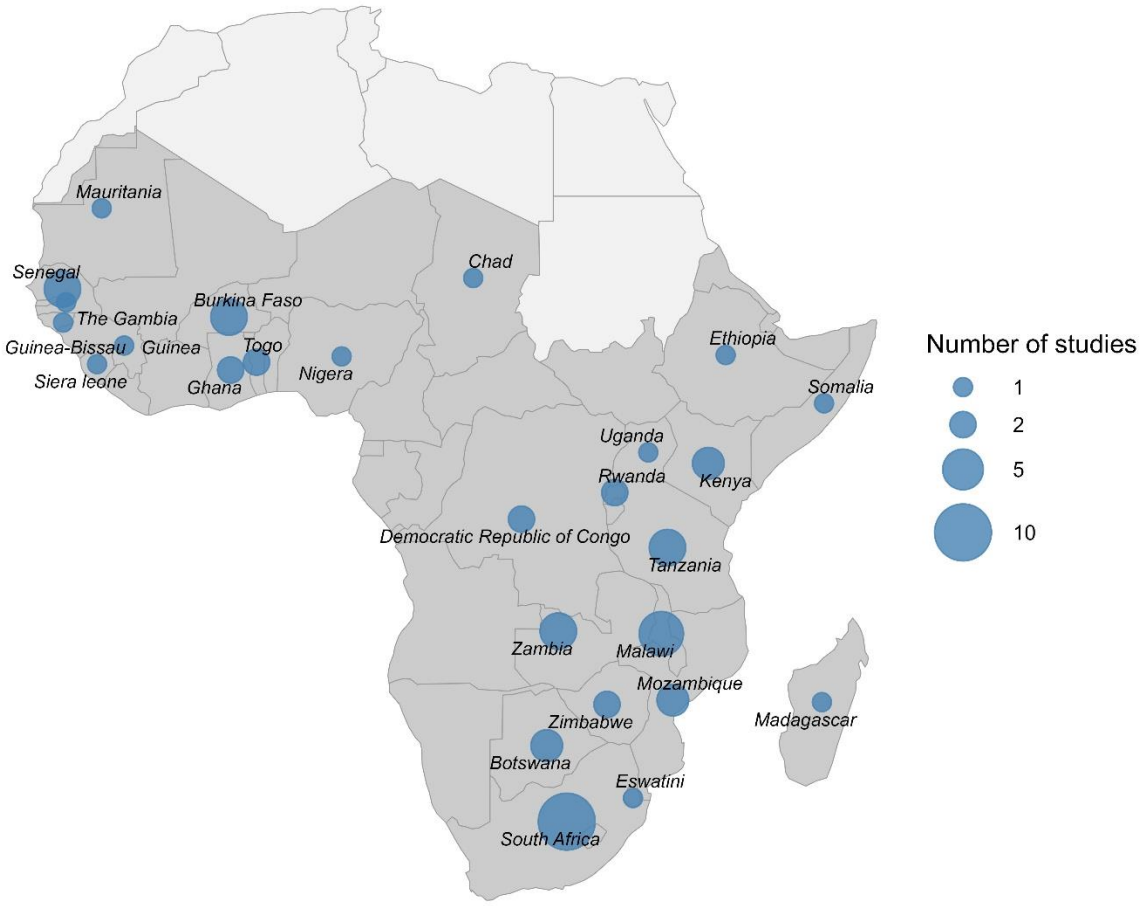


Figure 2-2: Study locations and number in Sub-Saharan Africa

Over half of the studies (53%) were funded by non-profit organisations such as WHO AFRO, Gavi, the Vaccine Alliance, and the Bill and Melinda Gates Foundation often in collaboration with public institutions (15%). Public sector funding alone accounted for 12%, while support from the pharmaceutical industry was limited, representing less than 5% of included studies.

Table 2-3: Descriptive statistics of included studies

Characteristic	**Number of studies**	**Percentage**
Vaccine of interest		
Rotavirus vaccine	31	48.4
Pneumococcal vaccine	9	14.1
Measles vaccine	8	12.5
Influenza Vaccine	5	7.8
Haemophilus Influenza type B Vaccine	4	6.3
Meningococcal vaccine	3	4.7
Cholera vaccine	2	3.1
Polio vaccine	2	3.1
Geographical location		
Southern Africa	29	45.3
West Africa	19	29.7
East Africa	12	18.8
Central Africa	3	4.7
Northwest Africa	1	1.6
Study period		
2000 - 2009	9	14.1
2010 - 2019	55	85.9
Funder		
NPO	35	54.7
NPO and Public	10	15.6
Public	6	9.4
Pharma and NPO	3	4.7
Pharma	1	1.6

Pharma and Public	1	1.6
No funding	5	7.8
Not reported	3	4.7

Table 2-4 summarises the study designs used across the 64 included studies and their consideration of public health and social measures. None of the studies collected or reported data on interventions such as WASH, nutritional improvements, or access to healthcare. Ecological studies were the most common design, comprising 30 studies (46.7%), followed by case–control design (43.8%). Neither design accounted for these measures. Cohort studies were used in five studies (7.8%), while the screening method was used only used once (1.6%). Two studies combined designs, and classification was based on the primary analytical approach. Although time-series and cohort designs may offer greater scope for causal interpretation, none incorporated information on concurrent public health and social measures. No studies used the screening method or cluster-randomised designs.

Table 2-4: Analysis of study designs and public health and social measures

Study Design	**Number of Studies (%)**	**Studies Reporting PHSMs**	**Studies Adjusting for PHSMs**	**Key Limitations**
Ecological	30 (46.9%)	0	0	Cannot assess individual-level PHSMs effects
Case–control	28 (43.8%)	0	0	Reliance on retrospective data. No control for PHSMs
Cohort	5 (7.8%)	0	0	PHSMs not measured prospectively
Screening method	1 (1.6%)			
Cluster Randomised	0 (0%)	0	0	Can adjust for PHSMs but not used
Total	64 (100%)	0	0	Overall, no PHSMs adjusted for

2.4.4 Vaccine effectiveness and impact

2.4.4.1 Rotavirus vaccines

Rotavirus vaccines effect estimates were consistently protective in direction, although the magnitude of both effectiveness and impact varied across study designs, outcome measures and population. Case–control and test-negative studies most often reported VE of 52% – 78% against severe or hospitalised laboratory-confirmed rotavirus(87,174,176,177,236–239). Ecological studies, including interrupted time-series designs, reported similar declines in diarrhoeal hospitalisations following vaccine introduction with reductions in rotavirus positivity ranging from 35% - 81%(173,189,190,193,196,198,201,240–248). Two studies found no evidence that malnutrition or HIV exposure altered rotavirus vaccine effectiveness(181,249). There was one cohort study that reported a VE of 34% against rotavirus-associated diarrhoea mortality in Malawi(184). These findings suggest that the direction of rotavirus vaccine effects were consistent across study designs. Differences in reported effectiveness and impact may reflect variation in outcomes and epidemiological context.

2.4.4.2 Pneumococcal conjugate vaccines

Pneumococcal conjugate vaccine studies reported variable effects across outcomes and study designs. Ecological analyses generally indicated substantial reductions in invasive pneumococcal disease and meningitis following vaccine introduction, with reported effectiveness estimates often exceeding 75%(206,211,250). Individual-level case–control studies showed lower effectiveness against broader clinical pneumonia outcomes but higher protection against invasive pneumococcal disease, with estimates ranging from around 57% to 85% depending on HIV status and outcome definition(203,205,209). Time-series analyses also showed heterogeneous population-level impacts. While some settings reported moderate reductions in pneumonia hospitalisations in both HIV infected and uninfected(204), others reported smaller or non-significant changes(251). Larger declines in meningitis and pneumonia hospitalisations were observed in Zimbabwe (252). Overall, variation in reported PCV effects appears to reflect differences in outcomes, HIV status and vaccine valency. These estimates were not compared directly across study designs because they used different outcomes and effect measures. Comparisons were interpreted only within similar designs, endpoints and effect measures.

2.4.4.3 Measles vaccines

Measles vaccine studies consistently reported high levels of protection across study designs. Case–control evaluations from Uganda estimated a VE of around 74–75%(219,253). A screening method analysis conducted during an outbreak investigation in Sierra Leone reported a similar effectiveness of 74%(254). Ecological analysis from Malawi also indicated strong protection, with effectiveness estimated at 83.9% for a single dose and 90.5% for two-dose schedules. Together these findings indicate that protection from measles vaccination remains strong across different countries in SSA. Estimates were more variable in outbreak settings.

2.4.4.4 Influenza vaccines

Influenza vaccine studies showed much more variability in effect estimates across seasons and settings. Case–control studies in South Africa reported effectiveness estimates ranging from −14% to 67% across five influenza seasons, with wide confidence intervals in several years(228,255,256). A study from Kenya reported more consistent protection. Vaccine effectiveness ranged from 39% to 57% depending on follow-up duration(225). One cohort analysis from Guinea-Bissau examining non-specific effects found no evidence of reduced consultation rates among vaccinated children and suggested a weaker decline compared with unvaccinated children(227). Together these findings indicate that influenza vaccine effectiveness varied substantially across seasons and contexts, with estimates ranging from negligible or uncertain protection to moderate effectiveness depending on circulating strains, follow-up period and study design.

2.4.4.5 Meningitis vaccines

In contrast, Hib vaccine studies consistently reported high effectiveness against invasive disease and meningitis. Case–control evaluations estimated effectiveness of around 96–99% in Uganda and Senegal(230,232). A cohort study from South Africa reported effectiveness of 83% among fully vaccinated children, with substantially lower protection in HIV-infected children(231). Surveillance data from Kenya also indicated high effectiveness of around 88%(233). Meningococcal vaccine studies reported contrasting findings by vaccine type. Ecological analysis from Senegal evaluating the polysaccharide vaccine found no significant change in meningitis incidence among children under five years(257). In contrast, ecological evaluation of the

MenAfriVac conjugate vaccine in Chad reported high effectiveness of approximately 89.6%(258). These findings suggest substantially stronger population-level impact for the conjugate vaccine compared with earlier polysaccharide formulations.

2.4.4.6 Cholera vaccines

Evidence for cholera vaccines was limited but consistently indicated high protection against disease. Case–control studies evaluating oral cholera vaccines reported effectiveness of 78% for the recombinant cholera toxin B-subunit killed whole-cell vaccine (Dukoral™) in Mozambique and 86.6% for the killed whole-cell vaccine Shanchol™ in Guinea. These findings suggest substantial protection across settings, although the small number of studies limits broader conclusions about variability in vaccine impact across epidemiological contexts(234,235).

2.4.4.7 *Polio vaccines*

Evidence for polio vaccines was limited to two case–control studies but indicated moderate to high effectiveness against paralytic disease. Estimates varied by vaccine formulation and dose number. In Nigeria, monovalent oral polio vaccine (mOPV1) showed substantially higher effectiveness (67%) against type 1 poliovirus than the trivalent formulation (16%). Trivalent vaccine effectiveness remained low (18%) against both type 1 and type 3 disease(212). A matched case–control study from Somalia reported overall effectiveness of around 70%, with higher protection observed among children receiving four or more doses. These findings highlight the importance of vaccine formulation and cumulative dosing in determining observed protection against poliovirus infection(259). As with the other vaccines, none of these studies explicitly measured or adjusted for concurrent public health and social measures, despite many evaluations being conducted in settings undergoing rapid contextual change.

2.4.5 Heterogeneity in effectiveness estimates: rotavirus case study

For rotavirus, 12 case–control and test-negative studies reported ratio-based effectiveness estimates against hospitalised or severe laboratory-confirmed rotavirus gastroenteritis in children under five. These studies used comparable individual-level designs and outcomes. Effect estimates were consistent across settings. In a random-effects meta-analysis, the pooled odds ratio was 0.42 (95% CI 0.35–0.51). No between-study heterogeneity was detected ($I^2 = 0\%$, $\tau^2 = 0$; X^2 $p = 0.67$). Several small studies reported wide confidence intervals. This uncertainty

reflected limited precision within individual studies rather than differences between studies once design and outcome definitions were aligned.

Variation in post-licensure vaccine effectiveness and impact was not uniform across vaccines. It reflected differences in pathogen biology, outcome specificity, and contextual modifiers (Supplementary Table S1). In settings where public health and social measures change over time yet remain unmeasured, this distinction shapes how vaccine impact evidence should be interpreted and compared across studies.

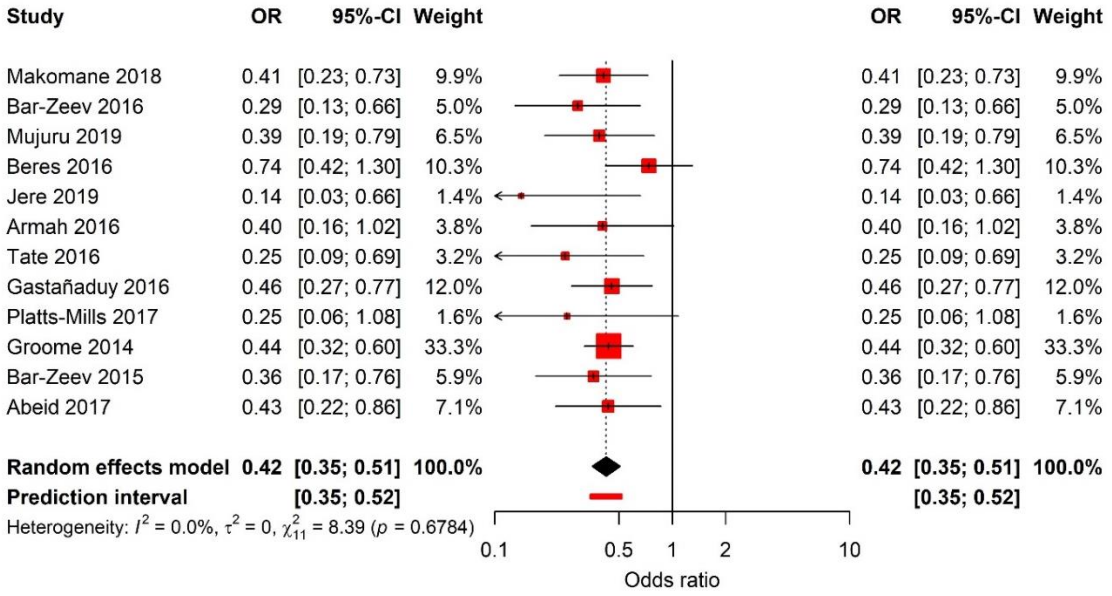


Figure 2-3: Forest plot of rotavirus vaccine effectiveness from case–control studies in children under five years in sub-Saharan Africa

2.5 Discussion

2.5.1 Summary of key findings

To the best of our knowledge, this is the first systematic review to assess the extent to which post-licensure vaccine effectiveness and impact studies conducted in children under five in sub-Saharan Africa account for PHSMs in study design and/or analysis. The key finding of this review is that none of the 64 studies across eight vaccine-preventable diseases reported or adjusted for the effects of PHSMs such as improved WASH practices, nutritional supplementation, or improved healthcare access. This represents a methodological gap for vaccine evaluations as PHSMs are known to modify vaccine effect estimates (260).

All studies included in this review used observational designs, most commonly case–control and ecological approaches. Case–control studies allow adjustment for individual-level confounders, though this relies on the assumption that cases and controls are comparable with respect to unmeasured exposures or that relevant confounders are adequately captured. This assumption may not hold for PHSMs, which are often not measured at the individual level and may vary over time and across populations. Time-series and cohort designs were less frequently used, which may reflect limited availability of pre-vaccine data, constraints in routine data systems, and the resources required to implement longitudinal studies.

The absence of adjustment for public health and social measures did not have uniform implications across vaccines. In the Results, rotavirus effectiveness estimates from comparable individual-level designs and outcomes were stable across settings, despite the absence of measured PHSMs. Pneumococcal conjugate vaccine estimates varied across outcomes and contexts, even within similar case–control designs. Influenza estimates showed pronounced seasonal and strain-related variation, while Haemophilus influenzae type b and meningococcal conjugate vaccines were associated with large and sustained reductions in disease in high-coverage settings. These patterns suggest that unmeasured PHSMs may influence vaccine effects in ways that differ by pathogen, rather than acting as a uniform source of variability.

2.5.2 Accounting for PHSMs across study designs

PHSMs should be measured in post-licensure vaccine evaluations because they may reduce the same disease outcomes as vaccination, but through different pathways. Without measuring both, reductions in disease burden may be incorrectly attributed to the vaccine alone, or true vaccine effects may be missed when background disease control is changing.

Case–control studies collect individual-level data on vaccination status and disease outcomes, which in principle provides a statistical framework that allows adjustment for confounding factors. Their relatively low cost and operational feasibility stem from reliance on retrospective data and their ability to be implemented rapidly(134). They were the most common study design in this review (47%). Interpretation of vaccine effectiveness estimates from case–control studies nevertheless rely on an assumption that cases and controls arise from the same underlying population and experience comparable exposure to contextual interventions that influence disease risk. When exposure to public health and social measures differs between cases and controls, or when these exposures are not measured, residual confounding may arise. As observed in this review, case–control studies did not report or adjust for PHSMs. Data on such measures are often not collected at the individual level and may be prone to recall bias. Their retrospective nature further limits the availability of reliable information on PHSMs that vary over time.

A case–control study in Malawi stratified rotavirus VE by nutritional status and reported substantially higher effectiveness (VE 78.1% [95% CI: 5.6%–94.9%]) in well-nourished children than in stunted children (VE 27.8% [95% CI: –99.5% to 73.9%])(261). This pattern is consistent with nutritional status acting as an effect modifier rather than a confounder. Stratified estimates are therefore informative. The challenge arises when individual-level anthropometric data are not available while nutritional status shifts at the population level over time. Under these conditions, the overall vaccine effect becomes difficult to interpret, as the underlying distribution of nutritional status changes in ways that are not captured in the analysis. This may introduce variability in effectiveness and impact estimates even within individual-level case–control designs. Collection of anthropometric data alongside vaccination status would

strengthen future rotavirus evaluations. Ecological analyses would require corresponding adjustment for population-level indicators of nutritional status.

Ecological studies remain one of the most used designs for evaluating vaccines accounting for approximately 36.4% of studies in this review. They compare disease trends before and after vaccine introduction using population aggregates and are unable to measure individual-level uptake or exposure to PHSMs(158). They report disease trends from routine surveillance data which do not include data on PHSMs, and if they do, it is incomplete and inconsistently reported. Vaccine introductions in sub-Saharan Africa are usually accompanied by introduction of PHSMs such was WASH, bed nets and other disease-control interventions(262). Without granular data, it is impossible to disentangle the effects of PHSMs which potentially leads to overestimation or underestimation of effect estimates. Additionally, the temporal sequence of changes in PHSMs relative to vaccine introduction is often unclear, further complicating causal inference.

Cohort studies offer a methodological advantage for adjusting for PHSMs. They collect exposure data (vaccination status) at baseline and follow-up individuals over time allowing comparison of disease incidence between vaccinated and unvaccinated children(263). Compared to ecological designs they provide individual-level data ideal for adjusting for time-varying PHSM-related confounders. For instance, data on improved PHSMs access in a particular township mid-study can be collected and adjusted for accordingly. Despite this potential, none of the four cohort studies in this review adjusted for PHSMs which is likely because of the lack of data on PHSMs source, nutrition and hygiene behaviours. This can lead to residual confounding, particularly when PHSMs such as WASH are unequally distributed between vaccinated and unvaccinated children. Moreover, cohort studies may suffer from confounding by indication, as children who receive vaccines may differ systematically from those who do not in terms of maternal education, health-seeking behaviours, and other factors linked to PHSMS exposure.

Interrupted time series (ITS) analysis are quasi-experimental designs that assess vaccine impact using aggregated surveillance data across multiple time points. These models can adjust for trend and seasonality to provide vaccine effect estimates following vaccine introduction.

However, despite this potential, ITS rarely adjust for time-varying PHSMs due to data limitations or methodological constraints which introduces confounding. For instance, a South African pneumococcal time-series study showed 33% (50% credible interval, CrI: 6 to 52) reduction in pneumonia hospitalisations post-introduction of pneumococcal conjugate vaccines but lacked data on coincident improvements in HIV treatment access from 2006 to 2014(264). Controlled ITS have a control group which allows researchers to control for time-varying PHSMs for vaccine impact estimates(160). There is a need for researchers to link time series data to demographic health systems data to enhance the ability of ITS to control for co-occurring interventions and strengthen impact estimates.

2.5.3 Recommendations for future studies

Future post-licensure vaccine effectiveness and impact studies in sub-Saharan Africa should prioritise improved measurement of PHSMs that plausibly modify vaccine effects and change over short time scales. Future evaluations could also stratify or adjust analyses by measured PHSM coverage, such as comparing vaccine effects across areas with different levels of WASH access, bed-net use, chemoprevention, case management or healthcare access, provided these measures are collected consistently and are not simply proxies for broader socioeconomic differences.

Case–control studies should collect individual-level data on nutrition, WASH exposure, malaria prevention, and other contextually relevant factors that are known to influence susceptibility or immune response. These variables should be incorporated for matching in design or in analytical models to allow estimation of effect modification rather than treated solely as sources of confounding.

Matching or adjustment for broader factors such as geography, seasonality may provide partial control for contextual variation and can be considered as complementary strategies. However, these approaches do not replace direct measurement of rapidly evolving PHSMs. In ecological and time-series evaluations, strengthening routine data systems such as District Health Information Systems is crucial for capturing population-level indicators of nutrition, WASH and other key interventions, enabling better interpretation of vaccine impact alongside changes in

risk factors. As climate variability affects disease seasonality and risk, improving the capture of environmental and social indicators will become more important.

Cohort studies should prioritise the collection of time-varying PHSMs data and adjust for PHSMs accordingly. For interrupted time-series analyses, linking aggregated health data with routine sources such as WASH databases or HIV programme records can improve adjustment for concurrent interventions and reduce residual confounding. Ministries of Health should be supported to strengthen surveillance platforms by incorporating indicators of PHSMs into routine health information systems to improve monitoring and vaccine impact assessment. Where PHSMs change rapidly and are not captured at the individual level, alternative evaluation designs may be required.

One such approach is cluster-randomised evaluation designs as used in the RTS,S/AS01 malaria vaccine pilot implementation and evaluation (MVIE) in Malawi, Ghana and Kenya (265). This allowed unmeasured contextual factors such as health system strengthening, care-seeking behaviour, and concurrent malaria interventions such as bed net usage and indoor residual spraying to be balanced across study arms by design. While this design doesn't eliminate effect modification at the individual level, it allows estimation of population-average treatment effects that are less sensitive to confounding by time-varying and unmeasured PHSMs. While cluster randomised post-licensure evaluations are not routinely feasible, the RTS,S experience illustrates that such designs can be justified where safety, effectiveness, and programme impact need to be assessed under real-world conditions and where conventional observational designs are likely to yield unstable estimates.

2.5.4 Implications for policy and practice

Design limitations can lead to underestimation or overestimation of vaccine effect estimates. This has implications for immunisation programmes in low-resource settings, where competing priorities for limited resources may lead to missed opportunities to identify and prioritise synergistic interventions that could enhance the overall impact of vaccination. For example, governments could combine immunisation campaigns with nutrition interventions or distribution of insecticide treated bed nets. For diarrhoeal disease such as cholera, reactive

vaccination campaigns should be implemented alongside WASH interventions. It is crucial to collect and adjust for data on PHSMs implemented alongside vaccination programmes during evaluations.

Neglecting PHSMs may lead health authorities in LMIC settings to place excessive emphasis on vaccination alone, sidelining complementary interventions such as nutrition programmes, WASH improvements, and maternal health education. At the same time, underestimation of vaccine effects may result in deprioritising effective vaccines, risking robust coverage and outbreak control. Vaccination remains among the most cost-effective public health interventions across all settings(266). Delaying or abandoning vaccine introduction based on biased effect estimates may increase costs for both households and health systems while leaving populations vulnerable to preventable disease.

Underestimated vaccine impact may undermine public and political confidence in vaccines, especially in the face of rising vaccine hesitancy as experienced with COVID-19 vaccines(267). Adjusting for PHSMs for post-licensure studies is also an opportunity for LMICs to expand national data platforms like DHIS to routinely capture PHSMs indicators. Health facility registers and survey questionnaires could be updated to include nutrition, WASH, and other co-interventions to improve evidence quality and support integrated programme planning.

2.5.5 Strengths and limitations

The review employed a comprehensive and reproducible search strategy across multiple databases including PubMed Central, EMBASE, MEDLINE, and CINAHL and adhered to PRISMA guidelines. We included a wide range of observational study designs, funding sources and vaccine types which allowed for a comprehensive appraisal of the methodological approaches employed in post-licensure vaccine evaluations. Furthermore, our dual-reviewer approach to screening, extraction, and quality assessment enhanced the rigour and reliability of the review process.

However, our systematic review had several limitations. The review was restricted to English-language publications, which may have led to the exclusion of relevant studies conducted in francophone and lusophone countries within the region which also have strong immunisation

programmes. The review was also limited to countries in sub-Saharan Africa, which excludes evidence from other regions and may limit the generalisability of the findings beyond this context. We relied on published data where analyses were constrained by what was reported in the articles. It is possible that some researchers measured PHSMs but simply did not report them. Lastly, we limited our review to 2019 which excluded the evaluation of COVID-19 vaccines.

This scope was chosen to focus on post-licensure evaluation under routine immunisation conditions. COVID-19 vaccine studies were conducted in a markedly different context, with rapidly changing and large-scale PHSMs introduced alongside vaccine rollout. These features create strong co-interventions and secular shifts that are not comparable with the settings in which most established childhood vaccines are evaluated. The rapid expansion of COVID-19–related publications would also have dominated the evidence base and shifted our review away from its primary objectives. Despite these limitations, our review highlights a critical methodological gap in post-licensure vaccine studies and underscores the urgent need for future evaluations to systematically account for PHSMs in both design and analysis.

2.6 Conclusion

This systematic review highlights a critical and under-addressed gap in post-licensure vaccine effectiveness and impact studies conducted in children under five years in sub-Saharan Africa which is the lack of adjustment for PHSMs such as improved nutrition, WASH, malaria control, and HIV-related services. None of the 64 studies included in this review collected, reported, or adjusted for PHSMs in their analysis despite the known confounding effects of these interventions on disease burden and health outcomes. With increasing number of vaccines and broader demands on health budgets it is necessary to provide the most accurate assessments of vaccine contributions to public health in rapidly changing settings.

It is critical to improve the design and reporting of post-licensure vaccine studies in LMIC settings. This includes routine collection of PHSM-related data, use of stepped-wedge cluster randomised design and incorporation of such variables into multivariate regression models. Additionally, national health information systems should be strengthened to facilitate the

routine capture of relevant PHSM indicators. Integrating these improvements into surveillance and evaluation frameworks will improve accuracy and provide context-specific evidence to guide immunisation policies and maximise public health gains from vaccination programmes.

3 The long-term impact and effectiveness of rotavirus vaccination in Malawi: an interrupted time-series and case–control analysis

3.1 Abstract

Background

Rotavirus remains a leading cause of childhood diarrhoeal hospitalisation globally. Malawi introduced the monovalent G1P8 rotavirus vaccine (Rotarix®) in October 2012 and in April 2016 switched from trivalent to bivalent oral poliovirus vaccine (tOPV to bOPV). More than a decade after Rotarix® introduction, evidence on sustained vaccine effectiveness and population-level impact in high-transmission, low-income settings remains limited, and it is uncertain whether programme changes such as the OPV formulation switch have influenced Rotarix® performance over time.

Methods

We estimated the long-term impact and effectiveness of Rotarix® on diarrhoea hospitalisation in Malawi and explored whether OPV type modifies Rotarix® effectiveness. We used interrupted time-series and test-negative case–control analyses to assess the impact and effectiveness of Rotarix®, respectively, over seven years post vaccine introduction using data from diarrhoeal surveillance studies in children under five years hospitalised with acute gastroenteritis at Queen Elizabeth Central Hospital, in Blantyre, Malawi, between 1997 and 2019. Stool samples from these children were collected and tested for rotavirus A antigen using enzyme immunoassay (EIA). To assess the effect of concurrent vaccination with OPV, we used a test-negative case–control study to estimate the interaction between the two vaccines.

Findings

The interrupted time-series was based on 7,952 hospitalisations and showed a 23% (95% confidence interval (CI): 10 – 34%) reduction in rotavirus hospitalisation rates among children

under five years in the post-vaccine introduction period (January 2013 – December 2019) compared with the pre-vaccine period (July 1997 – December 2012). There was a stronger effect among infants under one year (37%; 95% CI: 25 – 47%). Protection declined with age, with little measurable impact beyond infancy. The test-negative analysis enrolled 1,909 children and Rotarix® effectiveness for two doses was 52% (95% CI: 18 – 71%) overall, 67% (95% CI: 36 – 82%) among infants and 29% (95% CI: −136 – 74%) in those older than one year. Analyses of the tOPV to bOPV switch (n = 1,622) showed no measurable interaction with Rotarix® performance (aOR 1.07; 95% CI: 0.85 – 1.34).

Interpretation

Rotarix® provides moderate protection for Malawian infants, and the transition from tOPV to bOPV did not influence vaccine effectiveness. The lower effectiveness of rotavirus vaccination with increasing child age highlights the need to evaluate alternative vaccination strategies alongside strengthened WASH interventions to sustain vaccine impact in LMICs.

Funding: MRC Discovery Medicine North (DiMeN) Doctoral Training Partnership (UKRI) and National Institute for Health and Care Research (NIHR)

Data availability statement: Data are available upon reasonable request. De-identified surveillance data used in this study are not publicly available due to institutional and ethical restrictions but may be obtained from the corresponding author subject to approval by the relevant ethics committee.

3.2 Introduction

Rotavirus infection is the leading cause of severe childhood diarrhoea globally. Prior to the licensure and introduction of rotavirus vaccines, rotavirus was responsible for over 500,000 deaths annually in children under five years of age, with nearly two-thirds of these deaths occurring in low- and middle-income countries (LMICs)(55,268). Children in LMICs tend to experience rotavirus infection earlier in life when the risk of severe disease is greatest, with peak incidence typically between 9 and 15 months(269). At the same time these settings face higher case-fatality rates because of limited access to healthcare(270,271).

Four oral live-attenuated licensed rotavirus vaccines are currently recommended by the World Health Organization (WHO). Rotarix® (GlaxoSmithKline Biologicals), RotaTeq® (Merck & Co), Rotavac® (Bharat Biotech) and RotaSiil® (Serum Institute) have been introduced in routine childhood immunisation programmes for over 130 countries(75). Following introduction, rotavirus vaccines are estimated to have prevented 140,000 deaths from 2006 to 2019(56).

Malawi introduced the Rotarix® vaccine into its Extended Programme for Immunisation (EPI) in October 2012, with a recommended two-dose schedule at 6 weeks and 10 weeks of age. A study conducted two years after vaccine introduction in Malawi estimated a vaccine effectiveness (VE) of 70.6% in children aged <1 year and 31.7% in those aged 1-<2 years(261). Another study reported 61.89% VE for Rotarix® four years post-introduction in the first year of life(244). Most recently, Pitzer. *et al* estimated the population-level impact of Rotarix® introduction to be a 36% reduction in the incidence of rotavirus diarrhoea in children <5 years and a 52.5% reduction in children <1 year in Malawi(272).

While these estimates are similar to vaccine efficacy estimated from a phase III randomised clinical trial from Malawi, they remain lower than the VE observed in high-income countries HIC(24,80). This highlights the need to better understand factors influencing impact and VE in LMICs. One potential factor is the possible interference of oral polio vaccines (OPV) with rotavirus vaccine immunogenicity. Trivalent OPV (tOPV) has been shown to interfere with the immune response to rotavirus vaccines(273–275). This interference is thought to be due to rapid replication of the type 2 poliovirus component of tOPV in the gut at the time of the first

tOPV dose, which is administered concurrently with the first Rotarix® dose at 6 weeks of age(276,277). Malawi switched from tOPV to a bivalent OPV (bOPV) on 25 April 2016, which includes only type 1 and 3 poliovirus(278). However, the implications of this switch for rotavirus VE remain unknown.

Our study measures the long-term direct effectiveness (VE) of Rotarix® and impact (overall effectiveness) on rotavirus hospitalisations in children under 5 years in Malawi, and the influence of the tOPV to bOPV transition on Rotarix® VE.

3.3 Methods

3.3.1 Study setting and population

Blantyre is the largest commercial district in the southern region of Malawi. Healthcare is delivered through a network of private and government hospitals, and government health centres. Queen Elizabeth Central Hospital (QECH) is the largest government referral hospital, providing free medical care to the 1.3 million urban, peri-urban and rural residents of Blantyre district.

3.3.2 Surveillance data

The Malawi Liverpool Wellcome Research Programme (MLW) in partnership with the Ministry of Health established a surveillance programme for children with acute gastroenteritis (AGE) as part of the effectiveness and impact evaluation of Rotarix® following its introduction in the EPI. The surveillance programme enrolled children under 5 years old who presented at QECH with diarrhoea lasting less than 14 days and had ≥3 episodes in 24 hours. Stool samples were collected and tested for rotavirus A antigen using RotaClone™ ELISA (Meridian Bioscience, USA)(279). The surveillance had three periods: July 1997 to June 2007, January 2008 to December 2009, and from January 2012 onwards(280–283). There was a period of no surveillance between December 2009 and November 2011 which was excluded from the analysis. Population denominators were obtained from the National Statistics Office publications of the demographic surveys and their population projections covering the defined period.

3.3.3 Study design

3.3.4 Statistical analysis

The study sample size was determined by the number of eligible children identified through surveillance during the study period. No formal sample size calculation was performed. The participant flowchart is presented in the supplementary.

3.3.5 Interrupted time-series analysis of vaccine impact

We used an interrupted time-series design to assess the population-level impact of Rotarix® introduction on rotavirus gastroenteritis (RVGE) cases observed from July 1997 to October 2019. We set the change point for the interrupted time-series analysis on 01 January 2013 when Rotarix® vaccine coverage exceeded 80% following the introduction in October 2012. Hospitalisations occurring between October and December 2012 were included in the pre-vaccine period. Age-specific RVGE hospitalisation rates were calculated using rotavirus-positive AGE admissions captured through QECH surveillance as the numerator and the corresponding age-specific Blantyre population as the denominator. Monthly aggregates of rotavirus confirmed diarrhoeal admissions were analysed before and after vaccine introduction. To explore changes over time, a fitted time-series model was used to assess changes in both the gradient (slope) and level (intercept) of diarrhoeal cases post-vaccine introduction. We used generalised linear regression models with negative binomial distributions to account for the overdispersion in the data. The models included terms for seasonality (seasonal harmonics) and secular trends (linear year term). Counterfactual monthly counts were generated from the fitted negative binomial interrupted time-series model by setting the post-vaccine indicator to zero for the post-introduction period while keeping all other covariates unchanged(160).

To adjust for changes in healthcare seeking and surveillance intensity over time, we included test-negative diarrhoeal cases as a proxy for background admission trends independent of vaccination. Yearly census data for all the age categories were obtained from the National Statistics Office of Malawi and provided a robust population offset for the analysis. The vaccine impact on the hospitalisation rate was calculated as $100 \times (1 - Incidience\ Rate\ Ratio)$ with associated 95% confidence intervals (95% CIs)

For the counterfactual analysis, we used our primary analytic model to predict hospitalisation trends in the absence of vaccination. The factual series reflected observed vaccination effects while the counterfactual series was generated by setting the vaccine indicator variable to zero and keeping all other covariates unchanged. Monthly observed case counts were visualised alongside the factual and counterfactual predictions with solid and dashed lines used to distinguish the two. We performed all analyses separately for children aged <1 year, ≥1 to <5 years, and <5 years.

3.3.6 Test-negative case–control study for vaccine effectiveness

We conducted a test-negative case–control study to evaluate Rotarix® effectiveness, comparing the odds of vaccination among cases and controls from October 2012 to December 2019. We included children born on or after 17 September 2012 as they would have been 6 weeks old when the vaccine was introduced and eligible for vaccination for the first Rotarix® dose. Children were classified as vaccinated only if they had completed the full two-dose schedule. For consistency with the interrupted time-series analysis, we estimated vaccine impact separately for children aged <1 year, ≥1 to <5 years, and <5 years.

Vaccine status was obtained through hand-held patient medical records (health passports). Children who were verbally reported to be vaccinated but had no record in the health passport were excluded from the analysis. Cases were defined as children who presented with AGE and tested rotavirus EIA positive (RVGE), while controls were children who presented with AGE and tested rotavirus EIA negative. We measured the odds of vaccination between cases and controls adjusting for sex, age at admission, and month and year of birth using logistic regression. Vaccine effectiveness was calculated as $(1 - Odds\ Ratio) \times 100\%$ and presented with 95% CIs.

We considered potential sources of bias including misclassification of vaccination status, differences in healthcare-seeking behaviour between cases and controls, and unmeasured confounding due to factors such as WASH and socioeconomic status. Restricting vaccination status to documented records aimed to reduce misclassification.

3.3.7 Analysis of the tOPV to bOPV switch and Rotarix® effectiveness

We used a test-negative case–control design to assess the relative VE of Rotarix® following the switch from tOPV to bOPV. Specifically, we analysed if children vaccinated with dose one of bOPV (administered at 6 weeks concurrently with Rotarix®) were at a lower risk of laboratory-confirmed rotavirus hospitalisation than children vaccinated with tOPV and Rotarix®. The analysis utilised a subset of the same surveillance data previously described and covered the post-Rotarix® introduction period from January 2013 to December 2019. Only children who received both Rotarix® and OPV were included in the analysis.

The exposure variable was vaccination with bOPV and included all infants who were vaccinated with oral polio vaccine after 31 May 2016. The unexposed group represented infants who received their oral polio vaccine before 1 April 2016 (tOPV vaccinated). The months of April and May 2016 were excluded from the analyses to reduce the risk of cross-contamination in the exposure groups related to lags in vaccine switch. The adjusted odds ratio (aOR) was calculated using logistic regression with adjustments for age at admission, month of birth, sex and nutritional status measured by mid upper arm circumference (MUAC). Vaccine effectiveness was calculated as $(1 - aOR) \times 100\%$ and presented with 95% CIs. All statistical analyses were performed in R Version 4.2.3.

3.3.8 Missing data

Data completeness for key variables was assessed, and analyses were restricted to individuals with complete data on vaccination status and covariates. The extent of missing data was <5% and not expected to bias estimates.

3.3.9 Ethical approval

Ethical approval was obtained from the National Health Sciences Research Committee, Lilongwe, Malawi (number 867) and the Research Ethics Committee of the University of Liverpool, United Kingdom (000490).

3.3.10 Patient and public involvement

Patients and members of the public were not involved in the design, conduct, reporting, or dissemination plans of this study. The analysis used routinely collected diarrhoeal surveillance

data from children admitted to Queen Elizabeth Central Hospital, Blantyre, Malawi and did not involve prospective recruitment for the purpose of this analysis.

3.4 Results

3.4.1 Study population and age distribution of RVGE hospitalisations

The interrupted time-series analysis examined 7,952 AGE cases across the periods before and after Rotarix® introduction. The test-negative case–control study included 1,909 children, from which a subset of 1,622 was used for the OPV-Rotarix® interaction analysis (Figure 3-1)

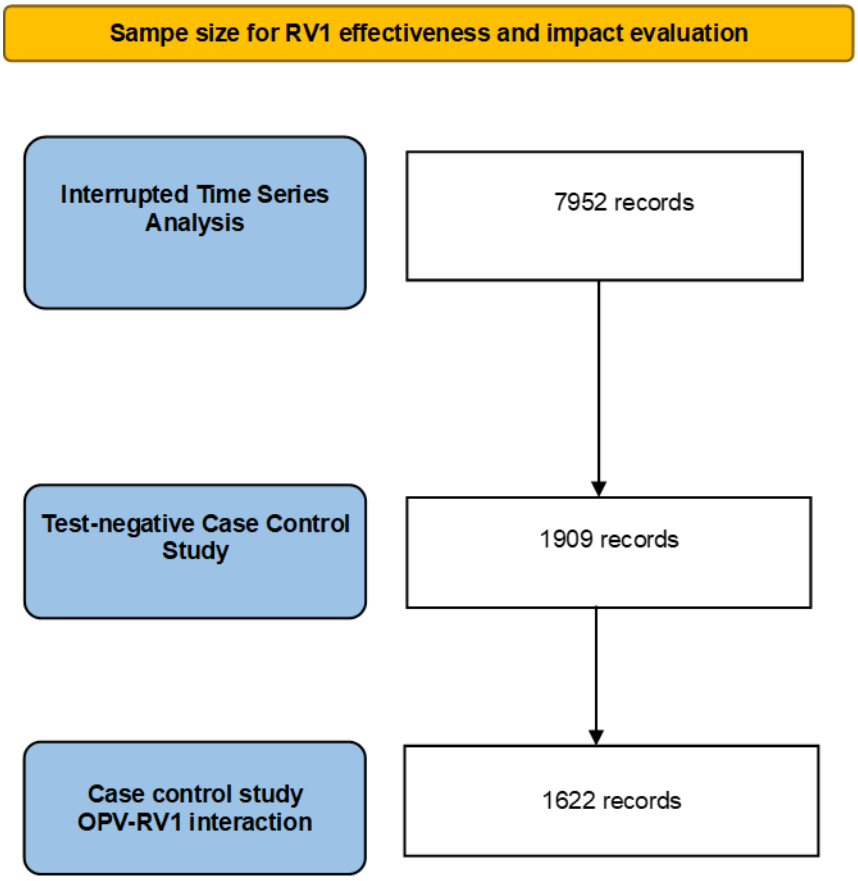


Figure 3-1: Sample size flow diagram

3.4.2 Long-term impact of Rotarix® on RVGE hospitalisations

We observed a shift in the age distribution of rotavirus-confirmed hospitalisations towards older children following Rotarix® introduction. In children aged ≥12 months, the mean annual number of RVGE hospitalisations increased from 22.7 cases per year in the pre-vaccine period to 51.3 cases per year after January 2013. Over the same period, mean annual RVGE hospitalisations in

infants aged <12 months declined modestly, from 86.8 to 76.6 cases per year. These patterns indicate that, while RVGE burden in infancy decreased following Rotarix® introduction, a greater share of hospitalised disease occurred among older children. Figure 3-2 illustrates the modelled trend of rotavirus-positive cases before vaccine introduction extended to show the expected pattern had Rotarix® not been introduced (counterfactual). The monthly trend of rotavirus-positive and negative AGE cases before and after Rotarix® introduction among children <5 years is shown in Supplementary Figure S1.

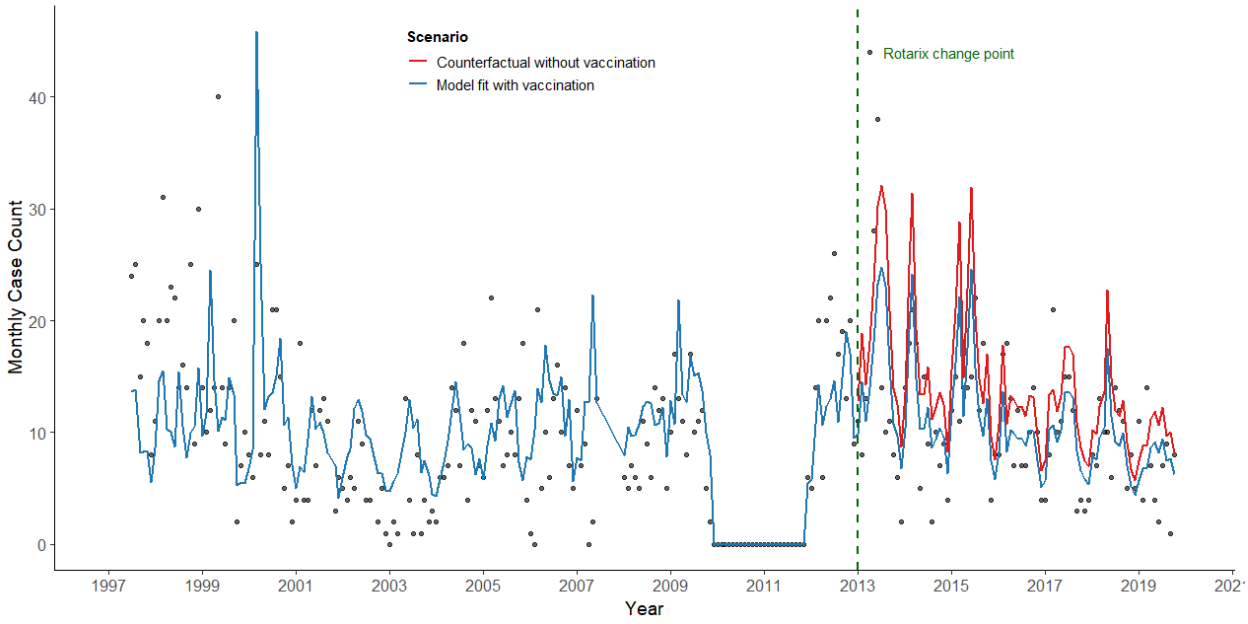


Figure 3-2: Observed versus counterfactual RVGE cases before and after vaccine introduction

Our analysis revealed age-specific patterns in Rotarix® impact on hospitalisations. There was an absolute 3.5% reduction in RVGE prevalence among children aged <5 years (from 33.2% to 29.7%) and an overall vaccine impact of 23% (95% CI 10 – 34%, p = 0.001) (Table 3-1). Among infants <1-year, post-vaccine RVGE prevalence was reduced by 9.7% (from 39.3% to 30.4%), corresponding to a 37% vaccine impact (95% CI 25 – 47%, p<0.001). In contrast, we observed an increased burden of RVGE to older age groups; RVGE prevalence increased by 7.5% in children ≥1 to 5 years old (from 23.9% to 31.4%) with a non-significant effect estimate of -39% (95% CI -70 – 24%, p=0.52).

Table 3-1: Negative binomial regression results showing the impact of Rotarix® introduction on rotavirus-positive hospitalisations by age group, adjusted for seasonality, secular trends, and test-negative controls

Age group	**Pre-vaccine RVGE prevalence**	**Post-vaccine RVGE prevalence**	**Adjusted IRR (95% CI)**	**Vaccine Impact % (95 % CI)**	**P-value**
<5 years	33.2% (1,643/4,944)	29.7% (895/3,008)	0.77 (0.66 – 0.90)	23 (10 – 34)	0.001
<1 year	39.3% (1,302/3,310)	30.4% (536/1,764)	0.63 (0.53 – 0.75)	37 (25 – 47)	<0.001
≥ 1 year	23.9% (304/1273)	31.4% (310/987)	1.39 (1.13 – 1.70)	-39 (-70 – -13)	0.002

3.4.3 Variation in Rotarix® impact over time

Yearly estimates across seven years after Rotarix® introduction also showed age-dependent variation in impact, but vaccine impact remained fairly consistent over time (Figure 3-3). Among children <5 years, the overall vaccine impact was modest, with only the second year after introduction showing a significant reduction in RVGE hospitalisations. The IRR in children aged <1 year varied between 0.56 and 0.74 and was highest in 2016. The IRR was >1 among children aged ≥1 to <5 years, indicating that an increased incidence in older children mostly occurred right after vaccine introduction. More detailed results are presented in the supplementary table S1.

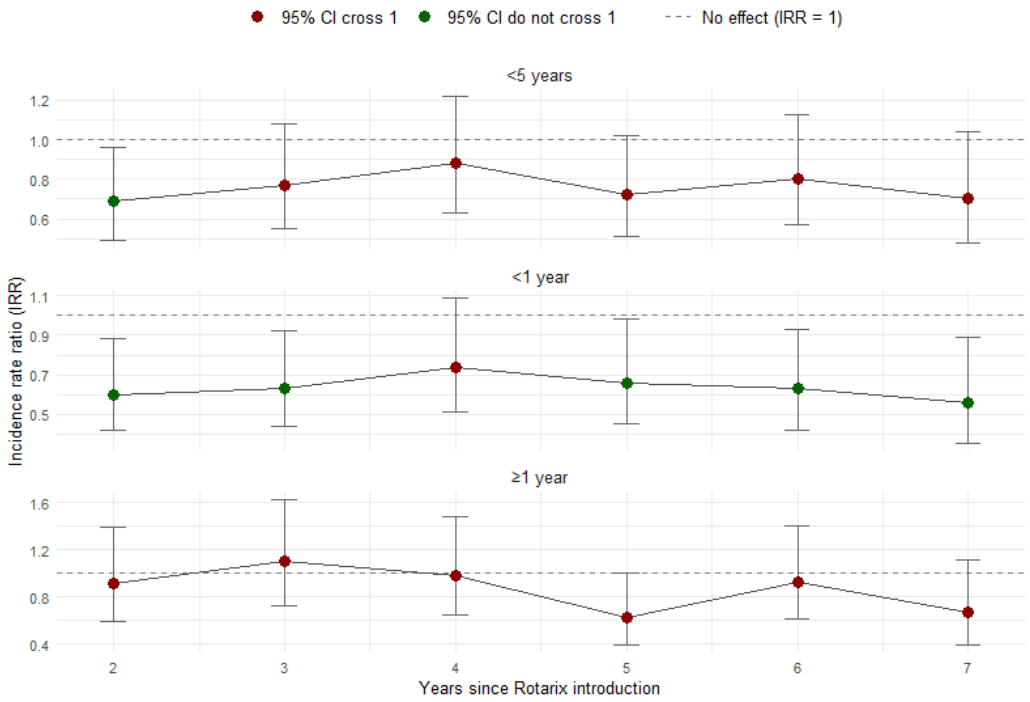


Figure 3-3: Annual estimates of rotavirus vaccine impact by age group since RV1 introduction showing year-to-year variation in the reduction of RVGE hospitalisations among children under five years

3.4.4 Rotarix® vaccine effectiveness

The test-negative case–control study included 1,909 children hospitalised with AGE between January 2013 and December 2019, comprising 551 rotavirus-positive cases (28.9%) and 1,358 test-negative controls (71.1%). The median age was 10.6 months (interquartile range: 8 - 14.8 months), with 64.4% of cases occurring in infants <1 year. More details on the baseline characteristics are presented in Supplementary Table S3. Complete two-dose Rotarix® vaccination demonstrated 52% protection against rotavirus hospitalisation in children <5 years (aOR 0.45, 95% CI 0.29-0.82; p = 0.006). Vaccine effectiveness differed across age groups as shown in Table 3-2. Among children <1 year, protection reached 67% (aOR 0.33; 95% CI 0.18–0.64; p = 0.001). Vaccine effectiveness in children older than 1 year was lower at 29% (aOR 0.78; 95% CI 0.26–2.32; p = 0.50).

Table 3-2: Regression estimates and 95% confidence intervals for models used to estimate Rotarix® vaccine effectiveness across age strata

Age Group	Cases/Controls	Unadjusted OR (95% CI)	Adjusted OR† (95% CI)	Vaccine Effectiveness‡ (95% CI)	P-value
<5 Years	551/1,358	0.49 (0.30–0.81)	0.48 (0.29–0.82)	52% (18–71)	0.006
<1 year	355/822	0.40 (0.22–0.73)	0.33 (0.18–0.64)	67% (36–82)	0.001
>1–<5 years	196/536	0.83 (0.33–2.43)	0.78 (0.26–2.32)	29% (−136 to 74)	0.500

†Adjusted for age (months), birth month, birth year
‡Calculated as (1 - aOR) × 100%

3.4.5 Effect of the tOPV to bOPV switch on Rotarix® effectiveness

The analysis included 1,622 children who received both OPV and Rotarix®, with 504 rotavirus cases and 1,118 test-negative controls. The proportion receiving bOPV was similar between cases (32.3%) and controls (30.3%). Age, sex and nutritional status were evenly distributed across the two groups, and most participants were <1 year of age (64.7% of cases and 65.0% of controls). More details of the descriptive statistics are presented in Supplementary Table S3.

In our crude analyses, the odds of laboratory-confirmed rotavirus hospitalisation were similar among children who received bOPV alongside Rotarix® and those who received tOPV alongside Rotarix® (OR 0.87; 95% CI 0.70–1.08; p = 0.21). We found no evidence of an association between OPV formulation and Rotarix® vaccine effectiveness (Table 3-3). After adjusting for age, sex and nutritional status, the association remained non-significant (aOR 1.07; 95% CI 0.85–1.34; p = 0.58). Children with severe acute malnutrition had significantly lower odds of RVGE compared with well-nourished peers (aOR 0.60, 95% CI 0.42–0.85; p=0.005), whereas no association was observed among those with moderate malnutrition.

Table 3-3: Results of the interaction analysis between oral poliovirus vaccine (OPV) and Rotarix® showing adjusted estimates of Rotarix® effectiveness among children eligible for both vaccines

Variable	Adjusted Odds Ratio† (95% CI)	p-value
bOPV (vs. tOPV)	1.07 (0.85–1.34)	0.58
Male (vs. Female)	0.96 (0.78–1.19)	0.74
Nutritional status (vs. Well Nourished)		
MAM	1.12 (0.85–1.47)	0.42

SAM	0.60 (0.42–0.85)	**0.005**
†Model adjusted for age, sex, and nutritional status. MAM = Moderate acute malnutrition; SAM = Severe acute malnutrition		

3.5 Discussion

3.5.1 Main findings

Rotarix® continues to provide modest protection against RVGE hospitalisation in Malawi more than seven years after its introduction. Vaccine effectiveness and impact analyses showed sustained benefit in infancy, with protection highest in the first year of life. Protection declined with age, and among children >1 year neither analysis demonstrated clear benefit, with effectiveness estimates overlapping the null and impact estimates indicating increased admissions.

We observed a shift in the age distribution of rotavirus-confirmed hospitalisations towards older children following Rotarix® introduction. Mean annual RVGE hospitalisations among children aged ≥12 months increased in the post-vaccine period, while admissions in infants declined modestly, indicating that protection was concentrated in the first year of life. This pattern likely reflects delayed primary infection alongside waning vaccine-derived protection, with child age acting as a proxy for time since vaccination. The year 1 estimate in children aged ≥1 year is difficult to attribute to vaccination, since most children in this stratum would not have been vaccine eligible during the initial rollout period. Consistent with this interpretation, we observed no evidence that the switch from tOPV to bOPV influenced Rotarix® effectiveness in this population.

3.5.2 Interpretation in relation to previous evidence

Our findings align with those of Pitzer *et al*., who estimated the impact of Rotarix® in Malawian children to be 36% (95 % prediction interval 33.6–39.9) in those <5 years and 52.5% (95% prediction interval 50.1–54.9) in infants <1 year(272). The authors used validated transmission models to predict the incidence of RVGE hospitalisation over the 10-year period following vaccine introduction. Here, we took a more traditional interrupted time-series-based approach

to predict the counterfactual incidence of RVGE in the absence of vaccination. Our interrupted time-series analysis partially controlled for the effect of long-term underlying trends which could potentially explain the decrease in rotavirus hospitalisations due to improved access to water, sanitation and hygiene (WASH) over the analysis time period if it coincided with the introduction of Rotarix® in Malawi(284). Our model also adjusted for time-varying confounders and year-to-year seasonal variation of the RVGE cases over time. We included rotavirus test-negative cases as a covariate in our analysis to adjust for any effect on the vaccine impact that could be introduced by the changes in surveillance methods over time, differences in healthcare seeking behaviour among children and any improvements in WASH interventions over time.

Figure 3.2 should not be interpreted by visual inspection alone and cannot be presented to a policy maker alone. The figure is a visual presentation of the observed and modelled counterfactual monthly RVGE hospitalisations from the interrupted time-series analysis. The interpretation is based on the fitted negative binomial model, which accounts for seasonality, secular trend, test-negative admissions and population size, and compares observed hospitalisations with the counterfactual burden expected in the absence of vaccine introduction.

3.5.3 Implications for vaccination policy

Both studies indicated reduced or negative impact estimates in children older than 1 year. The observed upward age shift in RVGE epidemiology is commonly observed in vaccination programmes and is largely attributed to waning of vaccine-induced immunity and delayed exposure to primary infection following high vaccination coverage(285). Our findings indicate that protection declines beyond infancy suggesting the need for a booster dose of Rotarix® to sustain protection in older children, which aligns with other studies on Rotarix® performance in Malawi(249,286). Mandolo *et al*. recommend 6 to 8 months as the optimal window for administering a booster in an LMIC, high-disease-burden setting(287). Whilst our results support the provision of a booster in children older than 1 year, policy implementers must consider the programmatic feasibility and cost-effectiveness of a booster dose. Previous analyses suggest the provision of an additional dose at 14 weeks of age may be cost-effective in Malawi and could be integrated within the EPI schedule(288). A further dose administered at 14

weeks of age or nine months concurrently with the measles vaccine could be considered to strengthen rotavirus protection without requiring an extra visit on the immunisation schedule(289,290).

3.5.4 Oral vaccine interference and the tOPV to bOPV switch

We found no evidence that the switch from tOPV to bOPV altered the effectiveness of Rotarix®. This aligns with immunogenicity data from Bangladesh showing reduced Rotarix® seroconversion when Rotarix® and OPV are administered on the same day, with similar interference observed across monovalent, bivalent, and trivalent OPV formulations[15]. These findings suggest that changes in OPV formulation alone may not remove oral vaccine interference. Other mechanisms may contribute to reduced oral vaccine performance in low-income settings. For example, higher gut microbiota diversity in early infancy has been associated with lower Rotarix® seroconversion in several LMIC studies suggesting that biological and environmental factors beyond OPV formulation remain important(89). The association we observed between severe malnutrition and RVGE hospitalisation should also be interpreted cautiously, as similar studies in India, Nigeria, and Pakistan have not reported consistent evidence of this relationship(291–293).

A neonatal schedule could also be an alternative to a booster dose to optimise protection in the face of interference from other oral vaccines. RV3-BB, a neonatal strain rotavirus vaccine (G3P[6]) currently in Phase III trials, has shown improved replication in the newborn gut and may be less affected by maternal antibodies, potentially offering an effective option for more durable protection(294,295). Use of a neonatal vaccine has been predicted to be the most cost-effective option for Malawi in a recent modelling study(288). This is supported by clinical data showing that the neonatal rotavirus vaccine RV3-BB is safe and immunogenic when administered at birth or in early infancy in Malawi, with serological responses comparable to those achieved with current schedules(296).

3.5.5 Strengths and limitations

There were several limitations to our data and analysis. Although our surveillance data were robust, vaccine coverage data for Blantyre were unavailable to include in our negative binomial

model for vaccine impact. Rotarix® coverage has exceeded 80% since January 2013, so calendar time was used as a proxy for vaccine exposure, which is likely to be a reasonable approximation in this setting(297). Finally, QECH caters to the Blantyre urban catchment area, where improved WASH may lead to a higher estimated vaccine impact, resembling what is observed in high-income countries with strong WASH infrastructure. As a result, impact estimates from this analysis may be higher than those that would be observed in more rural parts of Blantyre, where poorer baseline WASH conditions may sustain a higher underlying burden of disease. In addition, confidence intervals around vaccine effectiveness estimates in children aged >1 to <5 years were wide, reflecting limited precision as RVGE admissions declined in older age groups. We could not examine the VE within each 1-year age category because the offset age-specific Blantyre population needed for the negative binomial model was not available from the National Statistics Office. In addition, it would not have been possible even if the population denominator was available because the sparse case data in children >1 would have made the model unstable and unreliable.

3.6 Conclusion

Our study provides a comprehensive evaluation of Rotarix®'s long-term effectiveness and impact in Blantyre, Malawi. This analysis demonstrated strong protection among infants. Vaccine protection declined with age and led to a gradual shift in disease burden towards older children. Despite these shifts, Rotarix® maintained a significant overall impact through reduced RVGE incidence in the vaccine-eligible population. The transition from tOPV to bOPV did not appear to influence vaccine effectiveness, suggesting minimal interference with the immune response. Evaluation of neonatal vaccination or booster dosing alongside strengthened WASH interventions should be prioritised to sustain vaccine impact in LMICs.

3.7 Acknowledgements

3.7.1 Funding

This work was supported by Latif Ndeketa's studentship from the MRC Discovery Medicine North (DiMeN) Doctoral Training Partnership (MR/W006944/1), the UK National Institute for Health and Care Research (NIHR) Global Health Research Group on Gastrointestinal Infections at

the University of Liverpool using UK aid from the UK Government to support global health research (NIHR133066) and the US National Institutes of Health/National Institute of Allergy and Infectious Diseases (R01AI112970). Nigel Cunliffe is a NIHR Senior Investigator (NIHR203756). Nigel Cunliffe and Daniel Hungerford are also affiliated with the NIHR Health Protection Research Unit in Gastrointestinal Infections at the University of Liverpool, a partnership with the UK Health Security Agency in collaboration with the University of Warwick. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health, the NIHR, the Department of Health and Social Care, the UK government or the UK Health Security Agency.

3.7.2 Conflicts of interest

D.H. and N.F are currently receiving grant support from Seqirus UK Ltd for the evaluation of influenza vaccines. DH has received grant support and personal consultancy fees from Merck & Co (Kenilworth, New Jersey, USA) for rotavirus strain surveillance and honoraria for a presentation at a Merck Sharp & Dohme (UK) Limited symposium on vaccines. K.C.J. has received research grant support from GlaxoSmithKline Biologicals for work on rotavirus vaccines. All other authors declare no competing interests. N.F has received research grant support from GlaxoSmithKline Biologicals for work on malaria vaccine.

3.7.3 Contributions

L.N., D.H., N.F, N.A.C, and K.C.J conceived of the study and secured the funding. L.N. performed the analysis, wrote the manuscript. N.F., V.E.P, D.H., P.D assisted with the analysis and interpretation of results and edited the manuscript. K.C.J. A.B., and N.A.C. oversaw the data collection. All authors assisted with the interpretation of results and edited the manuscript.

4 Improving estimation of vaccine effectiveness during outbreaks in low-resource settings: A case study of oral cholera vaccination during the 2022-2023 cholera outbreak in Malawi

4.1 Abstract

Background

Use of oral cholera vaccine (OCV) is globally recommended as a public health response to cholera outbreaks, alongside water, sanitation and hygiene (WASH) interventions. Estimating vaccine effectiveness during emergencies in low- and middle-income countries is challenging because vaccination campaigns are often implemented over short time frames, while individual-level data are frequently incomplete due to constraints in infrastructure, resources and data systems. There is a need for pragmatic approaches that can generate timely, policy-relevant evidence using routinely collected data.

Methods

We analysed routine surveillance data from a large 2022–2023 cholera outbreak in Blantyre District, Malawi. The EpiEstim framework was used to generate estimates of the time-varying reproduction number (R_t) from line-listed case data. We modelled changes in R_t as a function of cumulative OCV coverage using a log-linear framework and propagated uncertainty through posterior sampling. Lagged WASH exposure variables were incorporated in the model to generate adjusted vaccine effectiveness estimates and to explore potential interaction effects. Sensitivity analyses assessed robustness to alternative lag structures.

Findings

The Blantyre outbreak was characterised by an initial period of low-level transmission followed by a sharp increase in cases from late November 2022, after which transmission declined steadily through April 2023. This decline coincided with the implementation of a reactive OCV campaign. The majority of the cases were among middle-aged men living in urban Blantyre. The

unadjusted vaccine-associated reduction in transmission was estimated at 53.52% (95% credible interval (CrI): 42.5–64.1%). After adjusting for a 7-day rolling average WASH activity, total vaccine effectiveness increased to 62.1% (95% CrI: 49.3–74.9%). Sensitivity analyses using alternative lag structures for WASH exposure produced comparable adjusted estimates.

Interpretation

Implementation of OCV contributed to a substantial reduction in cholera transmission during the outbreak. This study demonstrates a feasible approach for estimating vaccine-attributable impact whilst accounting for public health and social measures, such as WASH interventions. The methods described will be useful in outbreaks where classical observational designs are not possible, providing actionable evidence to policy makers for outbreak response in resource-limited settings.

Funding: MRC Discovery Medicine North (DiMeN) Doctoral Training Partnership (UKRI), National Institute for Health and Care Research (NIHR) Global Health Research Group on Gastrointestinal Infections and Wellcome through the core grant to the Malawi-Liverpool-Wellcome Research Programme.

4.2 Introduction

Cholera remains a significant public health concern in low- and middle-income countries (LMICs) where inadequate water, sanitation, and hygiene (WASH) contribute to recurrent outbreaks(26). Globally, there are an estimated 1.3–4.0 million cases and 143,000 cholera-attributable deaths each year(298). Cholera is caused by *Vibrio cholerae* (*V. cholerae*), a Gram-negative bacterium with a distinctive curved rod shape. Infection with the bacterium results in a severe and potentially deadly acute diarrhoeal illness(91). *V. cholerae* includes many serogroups, though epidemic cholera has historically been associated with serogroups O1 and O139. In recent decades, however, nearly all reported outbreaks have been caused by the O1 seventh-pandemic El Tor lineage (7PET) (93,94). Oral cholera vaccines (OCVs) are recommended by the World Health Organization (WHO) for use in addition to other public health and social measures (PHSMs) such as case management and improved WASH(102,299).

Cholera is endemic in Malawi, a low-income African country with recurrent seasonal outbreaks typically occurring during the rainy season between November and May. The number of reported cholera-attributable deaths during seasonal outbreaks in Malawi rarely exceeds 100 deaths nationwide. The largest recorded cholera outbreak occurred between January 2022 and May 2023, resulting in over 58,000 cases and over 1,700 deaths(94,300). This outbreak had a recorded case fatality risk (CFR) of 3.1%, exceeding the CFR observed in neighbouring countries(100). A genomic analysis of the 2022-2023 outbreak revealed the 7PET lineage, O1 Ogawa serotype as the primary driver of the outbreak in Malawi(94). The Ministry of Health of Malawi responded to the cholera outbreak through a combination of reactive vaccination with oral cholera vaccine (OCV) and targeted WASH interventions(301). Vaccination was implemented as part of the national outbreak response strategy. WASH activities were delivered using a case-area targeted intervention (CATI) approach, which focuses on improving hygiene measures around detected cases and within outbreak hotspots(302,303). While OCV deployment aimed to reduce individual susceptibility, WASH measures aimed to limit environmental transmission.

The WHO recommends three prequalified OCVs for use in endemic and outbreak settings(102). All the vaccines contain killed whole *V. cholerae* O1 cells but they differ slightly in formulation and administration. Dukoral™ (Valneva, Sweden) contains inactivated *V. cholerae* O1 cells together with the recombinant B-subunit of cholera toxin. Shanchol™ (Shantha Biotechnics-Sanofi Pasteur, India) and Euvichol™ (EuBiologics, Republic of Korea) are killed whole-cell vaccines, which simplifies field delivery. These two vaccines share the same antigenic composition and have demonstrated comparable safety and immunogenicity across multiple evaluations(91,103). During the 2022–2023 outbreak response in Malawi, Euvichol-Plus®, an improved plastic-vial formulation of Euvichol™, was deployed to facilitate large-scale field delivery. The vaccine has a reported effectiveness ranging from 37% to 80% in various settings including Zambia, India, Bangladesh and Haiti(104–107).

Evaluating the effectiveness of OCV during large-scale outbreaks presents practical and methodological challenges. Rapid vaccination campaigns deployed during an ongoing crisis often rely on routine surveillance that is frequently incomplete or delayed. This lack of timely and high-quality data makes traditional study designs such as case–control or cohort studies difficult to implement, particularly when very few vaccinated cases are observed and when transmission dynamics change faster than recruitment or follow-up periods. Therefore, there is an urgent need for more pragmatic approaches to enable timely measurement of vaccine effectiveness (VE) during emergencies, particularly when other PHSMs, such as WASH, are also introduced.

To address this gap, our study uses a novel approach that leverages the time-varying reproduction number (R_t) to estimate the vaccine-attributable effect of OCV in the presence of concurrent WASH interventions. R_t, which represents the average number of secondary infections generated by a primary case occurring at time t, provides evidence for the combined influence of population susceptibility, human behaviour and public health interventions such as vaccination and WASH on cholera transmission, offering a more dynamic and pragmatic way to evaluate VE during emergency responses(304). By adopting this approach using routinely collected data, we aim to pragmatically improve estimation of VE in outbreak settings and provide crucial information for future outbreak response.

4.3 Methods

4.3.1 Study setting

The analysis focused on the January 2022 to April 2023 cholera outbreak in Blantyre district, Malawi. The district measures 240 km² with a population of approximately 1,400,000 people; healthcare is delivered through a network of private and government health facilities(305,306). These health facilities also serve as fixed points for routine and reactive immunisation for the expanded programme on immunisation (EPI). Blantyre is divided into five geographically based clusters, each encompassing several health facilities and their catchment areas. This clustering approach is routinely used by district health authorities to plan and monitor public health interventions such as responsive vaccination campaigns and WASH activities.

4.3.2 Case data

We obtained line-listed anonymised cholera case data from the Blantyre District Health Office (DHO) surveillance system which aggregated case reports from 32 health facilities within Blantyre. Each entry included the date of symptom onset and diagnostic confirmation. A confirmed case was defined as *V. cholerae* confirmed by rapid diagnostic testing of stools plus the clinical case definition of cholera of at least three episodes of watery diarrhoea within the last 24 hours, with or without dehydration or vomiting. A probable cholera case was defined as any clinical case definition plus confirmed contact with a case. Probable case definitions were used when the health system was overwhelmed with high incidence of cases and stool testing was not possible.

4.3.3 Vaccine data

The Ministry of health conducted a reactive oral cholera vaccination campaign from December 2022. Administrative reports indicated coverage of approximately 76% among the eligible population, although the underlying data were not available for verification. For the purposes of this analysis, vaccine coverage was derived from the available time-series data used in the model, which reached a maximum of approximately 24%. Although the vaccine is administered as a two-dose regimen given two weeks apart, constraints in supply meant that most recipients received a single dose. Vaccination counts were reported daily at facility level as doses

administered against doses allocated, and dose allocation was estimated through catchment area populations. All the cases in the analysis were hospital-attended cases and recoded through the routine lise-list. Coverage was calculated as cumulative doses administered in the district per eligible population over time. The vaccine was offered to all individuals aged one year and older and was administered through a hybrid strategy of routine administration at health facilities and campaigns conducted door-to-door and in populous areas like markets and religious gatherings.

4.3.4 WASH data

Health authorities adopted the CATI strategy to guide WASH interventions. From December 2022, they disinfected the homes and latrines of confirmed *V. cholerae* cases. Each affected household received two buckets (one for safe water storage and one for hand hygiene) and chlorine-based water treatment agents. Neighbouring households within a defined ring around each case also received the same WASH package. Chlorine tablets were distributed in disease hotspot areas reporting sustained transmission. The WASH intervention teams also conducted environmental surveillance, including water quality testing for faecal contamination and chlorine residual levels. WASH activities were recorded as the number of households the Blantyre DHO teams reached per day.

4.3.5 Statistical analysis

4.3.5.1 Estimation of time-varying reproduction number (R_t)

We estimated R_t from daily *V. cholerae* case counts using the *EpiEstim* package in R, which incorporates a user-defined serial interval distribution. We assumed a gamma-distributed serial interval with a mean of 5 days and standard deviation of 8 days(307). The serial interval is defined as the time between symptom onset in successive cases and was obtained from previous Malawi studies(308). EpiEstim returned a posterior distribution for each day representing plausible values of R_t given case incidence and the assumed serial interval.

4.3.5.2 Breakpoint identification and analytic window

We used the *strucchange* package in R to identify structural breaks in the weekly time series. This approach was used to identify the earliest significant shift in transmission dynamics, which we interpreted as the transition from the endemic/sporadic phase to the epidemic phase of the

outbreak(309). The primary breakpoint was identified on 11 December 2022 (95% CI: 04 December – 18 December). Consequently, all regression analyses for vaccine effectiveness and WASH impact were restricted to the period from 11 December 2022 onwards to focus on the period of sustained transmission.

4.3.5.3 Unadjusted vaccine effectiveness model

We expect on mechanistic grounds that R_t will vary in proportion to the fraction of the population that is susceptible, i.e.

$$R_t = R_0 \times (1 - coverage_t \times VE)$$

This neglects depletion of susceptibles via natural immunity. Regressing R_t against coverage allows us to extract VE but has the potential to generate negative values of VE for sampled R_t trajectories. To avoid this problem, we assumed that $(1 - coverage_t \times VE) \approx exp(-coverage_t \times VE)$, which is true when coverage times VE is low:

$$log\ (R_t)\ \approx log\ (R_0)\ - VE\ \times\ coverage_t$$

For each of 1,000 R_t trajectories, we then fitted a regression of the above form and estimated VE based on the coefficient of the time-varying vaccine coverage. Under this approximation, the slope coefficient provides a direct estimate of vaccine effectiveness, which we express as a percentage. Coverage at time t was defined as the cumulative proportion of the district-level population vaccinated prior to time t (range 0–1). To reflect the delay between vaccination and the development of protective immunity, we applied a seven-day biological lag to vaccine coverage, such that coverage at time t represents the proportion vaccinated at time t−7. The distribution of VE across all posterior samples yielded a median estimate and 95% credible interval (CrI), reflecting uncertainty in both R_t estimation and model fit.

4.3.5.4 WASH and vaccine exposure variables

As stated above, WASH activities were recorded as the number of households the Blantyre DHO teams reached per day. Dates were irregular due to operational deployment patterns. Daily household counts were then aggregated and joined to the cholera time series. Days with no recorded intervention activity were retained as zero events to avoid missing data and to ensure a complete time series. We converted daily WASH counts to households reached per 1,000

population to capture the temporal effect of WASH on transmission. From this, we derived two exposure measures: a 7-day rolling average representing short-term intensity of WASH activity and a cumulative exposure measure capturing the total number of households reached up to each day. We phenomenologically modelled WASH using the short-term measure rather than a cumulative exposure. This reflects the nature of the multiple components involved in WASH activities, where some components are transient while others may persist. A cumulative formulation assumes sustained impact across all components and, in this setting, was highly correlated with vaccine coverage. This reduces the ability of the model to separate their independent effects on transmission. The WASH variable was then lagged by seven days because WASH activities improve environmental conditions more gradually(306,307). In addition, we applied a seven-day biological lag to vaccine coverage to reflect the delay between vaccination and the development of protective immunity.

4.3.5.5 WASH-adjusted vaccine effectiveness model

To derive a VE estimate adjusted for the concurrent WASH intervention, we modified our regression to represent a phenomenological effect of WASH coverage on R_t:

$$log\ (R_t)\ = log\ (R_0)\ - VE_{adj} \times coverage_t + \beta \times wash_t$$

Under the same low-coverage approximation, the coefficient on time-varying coverage provides a direct estimate of VE after adjustment for WASH intensity. This model estimates the vaccine-attributable effect independent of variation in WASH activity, rather than the combined effect of concurrent interventions. The 7-day rolling average WASH variable represents the short-term intensity of households reached over time at district level. However, it does not necessarily represent continuous protection of households already reached. All the variables in the analysis are described in detail in Supplementary Table S4.

4.3.6 Sensitivity analysis

We assessed the robustness of the results by varying assumptions about the timing of intervention effects. Exposure variables for vaccine coverage and WASH intensity were reconstructed using alternative lag structures of 0 and 14 days. These estimates were compared with the primary specification using a 7-day biological lag. The adjusted vaccine effectiveness

model was refitted to all 1,000 R_t trajectories under each lag specification. Vaccine effectiveness estimates obtained under the alternative lags were compared with those from the primary 7-day lag model.

4.3.7 Missing data

Cholera case data with missing RDT results were treated as not tested and included as probable cases. A small number of RDT-negative cases were excluded from the analysis. These decisions did not affect the results. Days without WASH activity were retained as true zero exposure. Vaccine coverage and case reporting were complete at district level for the analytic period. R_t estimation naturally accommodates stochastic noise in case series, and posterior sampling ensures that uncertainty arising from day-to-day variability is propagated into all downstream estimates. No imputation was performed.

4.4 Results

4.4.1 Demographic characteristics of reported cholera cases

Between January 2022 and April 2023, a total of 8,013 cholera cases were reported in Blantyre District (Table 4-1), amounting to 0.6% of the population of which 7,992 were included in the analysis. Cases were concentrated in urban areas and occurred more frequently among males. The age distribution was typical of cholera epidemics, with the highest burden observed among young adults and children and adolescents. Most cases were classified as probable on the basis of epidemiological linkage, with laboratory confirmation limited by testing capacity during the outbreak. The outbreak resulted in 201 deaths, corresponding to a CFR of 2.5%. Vaccination history was rarely documented among reported cases, with fewer than one percent having a recorded history of oral cholera vaccination. The classification of cases by vaccination status is shown in Supplementary Table 4.

Table 4-1: Descriptive characteristics of cholera cases in Blantyre District, Malawi, January 2022–April 2023

Characteristic	**n (%) or summary**
Total cases	7,992
Residence	

Urban	6,404 (80.1%)
Rural	1,588 (19.9%)
Sex	
Male	4,799 (60%)
Female	3,193 (40%)
Age	
Median age (range)	23 years (1 month–97 years)
0–19 years	3,137 (39.3%)
20–39 years	3,568 (44.6%)
≥40 years	1,287 (16.1%)
Case classification	
Confirmed (RDT-positive)	1,107 (13.9%)
Probable	6,885 (86.1%)
Laboratory confirmation	
RDT-positive	1,107
Culture-confirmed	50
Outcomes	
Deaths	201
Case fatality rate	2.5%
Vaccination status	
Vaccinated	34 (0.4%)
Unvaccinated	7,891 (98.7%)
Missing vaccination data	67 (0.8%)

4.4.2 Spatial distribution of reported cholera cases

Reported cholera cases were geographically heterogeneous across Blantyre, with high burdens observed across multiple health-facility catchments rather than being confined to a single focal

area (Figure 4-1). This pattern indicates that transmission was distributed across several parts of the district rather than being driven by one dominant hotspot.

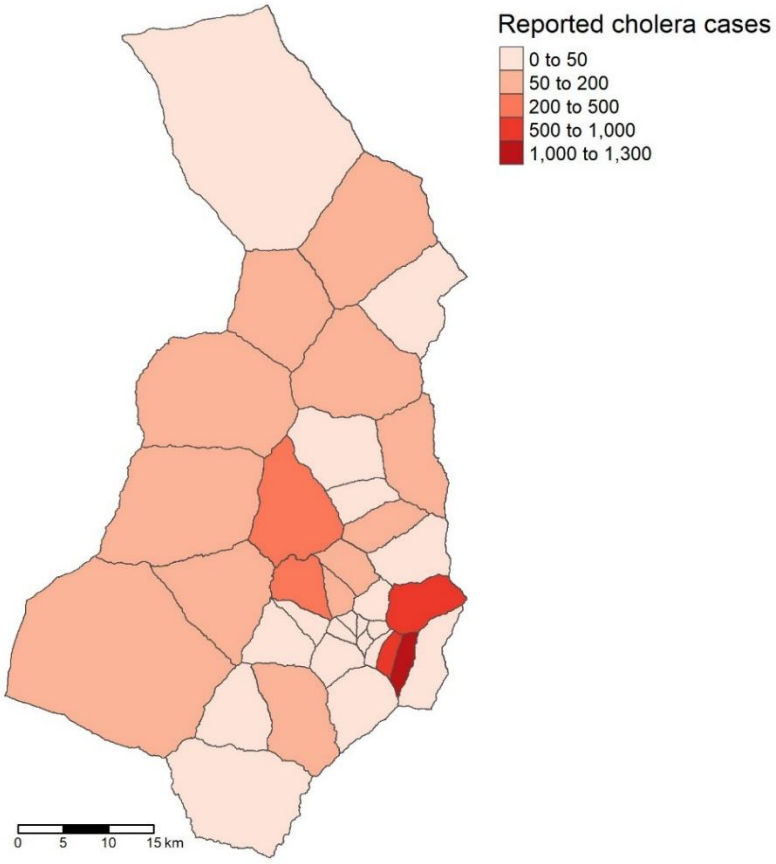


Figure 4-1: Spatial distribution of reported Blantyre cholera cases by health facility catchment area

4.4.3 Outbreak dynamics and timing of interventions

The epidemic began with a period of sporadic cases from January 2022 to April 2022 (Figure 4-2). During this phase, the median R_t was 1.74 (95% CrI: 0.12 – 4.30), and 77.6% of weeks were above the epidemic threshold of R_t >1. From May to November 2022, cholera cases occurred consistently, although incidence remained low. The median R_t during this period was 1.03 (95% CrI: 0.68 – 1.50), with 62.1% of weeks above the epidemic threshold. This reflects sustained but low-level transmission. The outbreak reached its peak in December 2022, corresponding to the weeks with the highest number of reported cases (Figure 4-2). Following this peak, incidence

declined gradually until April 2023. During the vaccination period (December 2022–April 2023), the median R_t was 1.0 (95% CrI: 0.92–1.08).

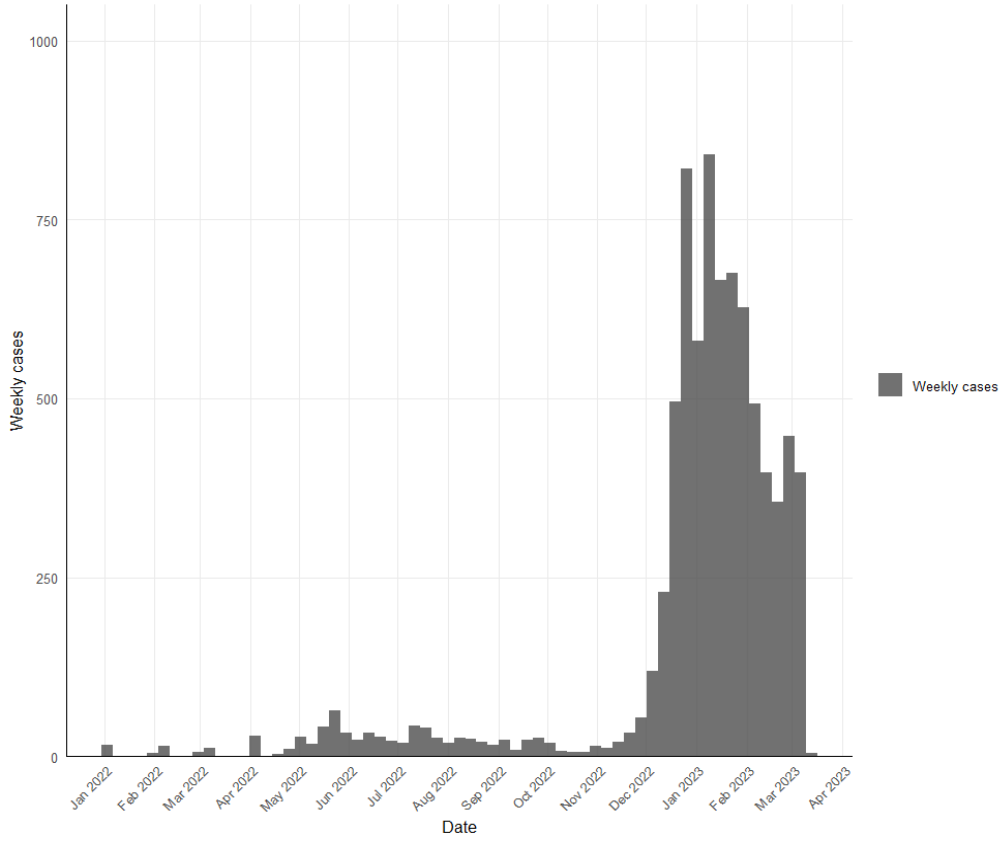


Figure 4-2: Temporal distribution of cholera cases in Blantyre

Breakpoint analysis identified 11 December 2022 (95% CI 04 – 18 December) as the point marking the onset of sustained transmission where the weekly number of cases rose from approximately 22.7 to 529.1. Figure 4-3 illustrates how transmission shifted as vaccination and WASH activities were introduced. Responsive vaccination started in early December 2022 and scaled up rapidly through January as cumulative doses increased across the district (Figure 4-3C). R_t was already beginning to decline at the outset of this rollout, with the subsequent drop coinciding with sharp gains in vaccine coverage. WASH interventions were initiated later, deployed intermittently from mid-December 2022, and most intensively between January and March 2023 (Figure 4-3D).

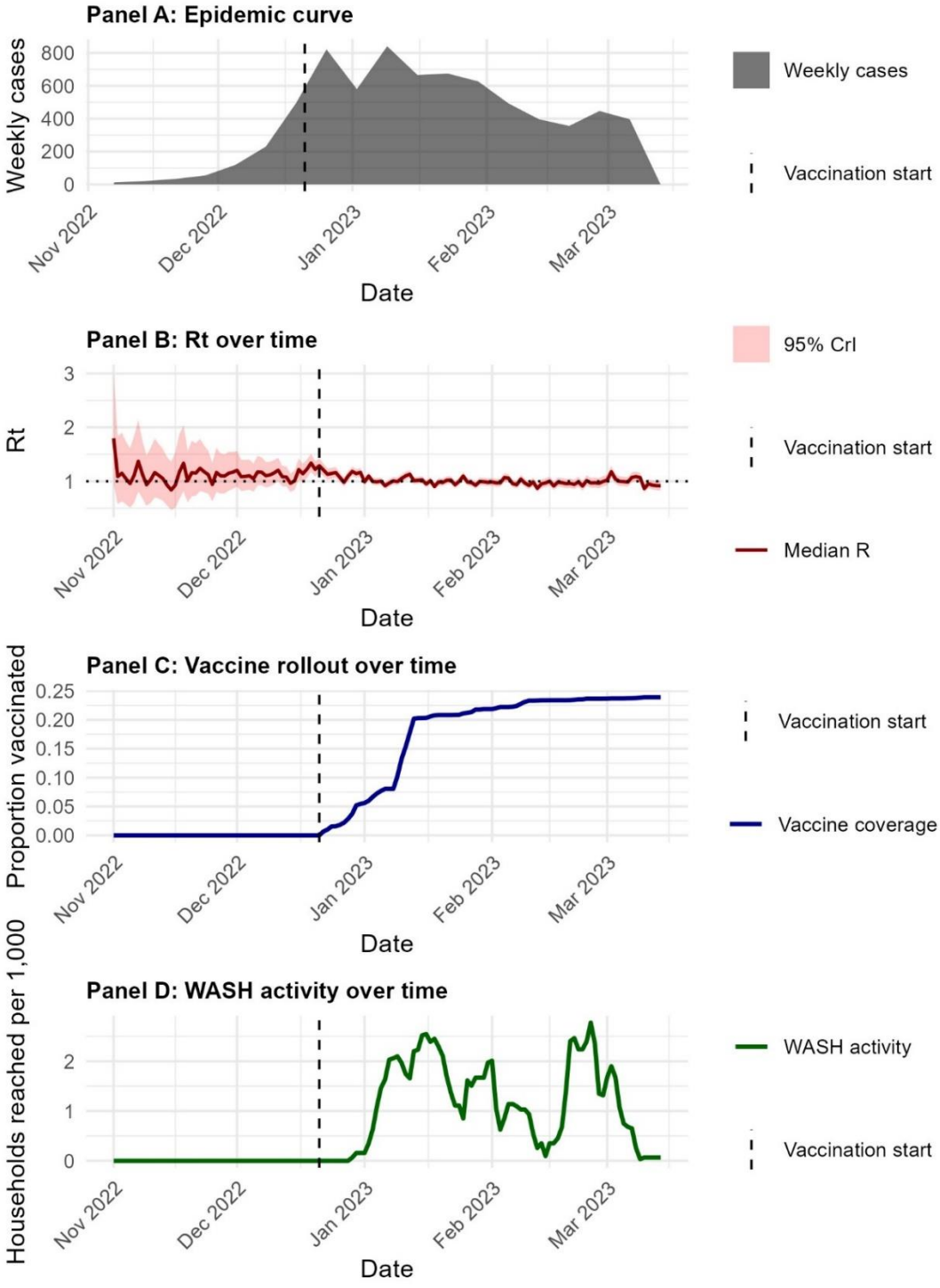


Figure 4-3: Weekly cholera incidence and time-varying transmission dynamics in Blantyre, alongside the timing of vaccination and WASH interventions from November 2022. Panel A shows weekly reported cases. Panel B shows the median R_t with 95% credible intervals. Panel C shows cumulative vaccine coverage. Panel D shows WASH activity intensity, expressed as households reached per 1,000 population. The dashed vertical line marks the start of vaccination

4.4.4 Vaccine effectiveness estimates

Using 1,000 posterior draws from the R_t distribution, the unadjusted VE was estimated to be 53.5% (95% CrI: 42.5 – 64.1), as shown in Table 4-2. This represents the estimated reduction in transmission potential associated with full vaccine coverage under the low-coverage approximation, independent of other interventions. When adjusting for WASH activity, the VE estimates increased to 62.1% (95% CrI: 49.3 – 74.9) which suggested that vaccination retained a strong independent effect even after accounting for concurrent WASH interventions.

In addition, we explored the effect of WASH activities on R_t and found that WASH interventions were associated with a small reduction in transmission intensity. Using the 7-day rolling WASH exposure lagged by seven days, the posterior distribution of the hazard ratio was consistently below unity, with a median estimate of 0.993 (95% CrI: 0.990–0.996).

Table 4-2: Posterior estimates of vaccine coverage and WASH effects on R_t

Model	β (log-HR) median	95% CrI (β)	HR median	95% CrI (HR)	VE (%) median	95% CrI (VE)
Vaccine effect only (lagged 7 days)	-0.535	-0.641 – -0.425	0.59	0.53 − 0.65	**53.5**	42.5 – 64.1
WASH only (lagged 7 days)	-0.009	-0.011 − -0.007	0.991	0.989 − 0.993	**—**	—
Vaccine + WASH (lagged 7 days)	-0.621	-0.749 − -0.493	0.54	0.47 − 0.61	**62.1**	49.3 – 74.9

4.4.5 Sensitivity analyses

The sensitivity analyses using alternative lag assumptions and WASH specifications showed variation in estimates. The median VE was 57.1% (95% CrI: 44.7–70.5) with no lag, 62.1% (95% CrI: 49.3–74.9) with a 7-day lag, and 35.9% (95% CrI: 25.5–49.4) with a 14-day lag. Using cumulative WASH exposure in place of the rolling WASH variable produced a higher VE estimate of 83.0% with uncertainty bounds exceeding 100% (95% CrI: 59.5–106.3).

Table 4-3: Vaccine effectiveness estimates from the WASH-adjusted model across alternative WASH lag specifications

Vaccine + WASH Effect	**HR median**	**95% CrI (HR)**	**VE (%) median**	**95% CrI (%)**
0 days lag	0.56	0.49 – 0.64	57.1	44.7 – 70.5
7 days lag (primary)	0.54	0.47 − 0.61	62.1	49.3 – 74.9
14 days lag	0.70	0.61 – 0.80	35.9	25.5 – 49.4
7 days lag & cumulative WASH	0.43	0.35 – 0.55	83	59.5 – 106.3

4.5 Discussion

We developed a pragmatic method for estimating vaccine performance during a fast-moving cholera outbreak, with concurrent PHSMs, such as WASH interventions, where traditional epidemiological designs were not feasible. Using routinely collected surveillance data and a framework that links temporal changes in R_t to changes in vaccine coverage, we found in unadjusted analysis that OCV was associated with a reduction of 53.5% in cholera disease transmission. Vaccine effectiveness increased to 62.1% once a lagged 7-day rolling WASH variable was included in the model. The similarity between unadjusted and adjusted estimates suggests that the decline in transmission was not primarily driven by the reported WASH interventions and that vaccination contributed independently to the epidemic control during the response period.

Our VE estimates align with the broader evidence base for killed OCVs, despite differences in design and setting. A recent systematic review and meta-analysis by Xu *et al*. reported VE of 69% for two doses and 60% for one dose at 12 months following vaccination(310). This review synthesised data from 10 observational studies worldwide (two from Africa), most using case–control or cohort designs to measure individual-level protection against *V. cholerae*. None of the studies in this review reported adjusting for WASH in their analysis. In contrast, our analysis estimates changes in transmission at the population level during an outbreak, using R_t , while accounting for WASH activity(129). This reflects a total vaccine effectiveness, incorporating changes in susceptibility, infectiousness, and indirect protection, rather than individual-level protection against disease measured in case–control or cohort studies.

Our findings are also consistent with evidence from sub-Saharan Africa. A matched case–control study in the Democratic Republic of Congo, evaluating a single dose of Euvichol-Plus, reported a VE of 52.7% (95% CI 31.4–67.4) 12–17 months after vaccination(311). A similar design for Shanchol vaccine in Guinea reported a VE of 86.6% (95% CI 56.7 – 95.8%)(235). These estimates fall within the range observed here.

Our study uses a similar methodology to a study by Hulland and colleagues (2024) who used EpiEstim and the VaxEstim extension to estimate single-dose OCV effectiveness in Haiti and

Cameroon(312). However, there are important distinctions to be noted. Their analysis did not have access to full surveillance line-listing and instead reconstructed weekly case counts by digitising epidemic curves from situation reports and outbreak bulletins. Their estimates suggested OCV1 effectiveness of approximately 75.3% in Haiti and 54.9% in Cameroon without adjusting for other interventions such as WASH activities(312). In contrast, our analysis was at the district-level rather than national and adjusted for WASH activity. In addition, we applied a log-linear regression of R_t on cumulative coverage and WASH rather than imposing an assumed fixed relationship between R_t, coverage and WASH.

A key contribution of this study lies in the method itself. Case–control and cohort designs were not feasible in this outbreak because only 34 vaccinated cases were identified, resources were unavailable for establishing a control population and a prospective cohort design was incompatible with the duration of the epidemic wave. Time-series approaches requiring extended pre- and post-intervention periods were also unsuitable because there was no clear post-vaccination period in our dataset. The posterior sampling framework instead draws information directly from the epidemic curve, propagates uncertainty from R_t estimation and exploits temporal variation in vaccination coverage to generate population-level estimates of VE(307). This approach does not replace classical designs but offers a feasible alternative for Ministries of Health during emergencies, particularly in low-resource settings where rapid evaluation is crucial and field data collection is constrained.

The analysis also showed important limitations in the outbreak surveillance data. Case data were reported from 32 health facilities to the Blantyre DHO but facility names were not always recorded in the same way, some line-list entries were duplicated, and dates of symptom onset, facility attendance and testing were often recorded as the same date. Vaccination status was largely incomplete in the line-list so vaccine exposure was analysed using ecological coverage rather than vaccination history linked to individual cases. WASH data recorded households reached but the data did not specify the intervention components delivered to each household. The data also did not show whether households were revisited or whether CATI was repeated when additional cases occurred nearby or in the same household. These gaps highlight the need for standardised line-list fields, unique case identifiers, clearer date recording, linkage between

case and vaccination data, and more detailed recording of WASH activities during outbreak response.

Interpretation of our findings must also consider the role of naturally acquired immunity. Infection-induced protection develops after symptomatic and asymptomatic *V. cholerae* infection and lasts for at least 3 years(101). In this outbreak, however, the scope for natural immunity to drive large reductions in transmission at district level was probably limited. The 7,992 reported cases represent well under 1% of Blantyre's population of about 1.4 million, and much of the burden occurred during the peak rather than during the earlier sporadic and low-incidence phases. Even allowing for underreporting and asymptomatic infections, the proportion infected is unlikely to have reached levels where susceptible depletion alone would largely be responsible for the decline in R_t .

The spatial distribution of cases also limits the extent to which the decline can reasonably be attributed to infection-derived natural immunity. Transmission was spread across several clusters rather than being concentrated within a cluster, which makes it unlikely that any one pool of susceptible individuals could have been depleted quickly enough to drive a coordinated fall in R_t at the district level. Infection-derived immunity may still have mattered in particular high-risk areas, yet such effects would have been localised and, on their own, are unlikely to account for the sustained decline in R_t observed across Blantyre during the response period. Exploring these local immunity dynamics in more detail will require mechanistic modelling of susceptible depletion which was beyond the scope of this analysis.

The effect of WASH intervention alone on R_t was small with posterior coefficients close to the null. This result should be interpreted with caution as R_t is a distal outcome. The WASH dataset captured only the administrative coverage of CATI activities but did not capture behavioural uptake, sustained use, or changes in environmental contamination at the household level. Furthermore, WASH interventions were delivered intermittently, in a short period and at a stage when transmission was already declining which therefore biases the effect towards the null. Under such conditions, even an intervention with genuine biological and environmental impact may leave only a weak statistical signal when evaluated against R_t at district level.

The effect of WASH in our analysis aligns with the wider literature, which has consistently noted the difficulty of demonstrating population-level effects of WASH interventions on cholera incidence or transmission in emergency settings(313,314). This is particularly evident when evaluations depend on aggregate epidemiological outcomes rather than indicators such as water quality or behavioural adherence. Point-of-use water quality is a direct WASH target that may help to reduce risk of transmission and disease burden during outbreaks, albeit with moderate quality evidence(315).

Our study was not without limitations. Vaccine coverage was measured ecologically at district level using doses delivered, meaning the effect we estimate reflects population-level impact of 1 or more doses rather than individual protection. The difference between the 76% administrative coverage reported by the DHO and the 25% analytical coverage reflects the use of different denominators. The 76% figure was based on doses administered relative to doses supplied to the health facilities, whereas the analytical coverage used the Blantyre population as the denominator. This highlights the importance of clearly documenting doses supplied, doses administered, target populations and denominator definitions during outbreak vaccination campaigns. Routine data can support vaccine impact analyses when these elements are consistently recorded and reported. In addition, WASH data captured operational activity but not adherence or real-world uptake of behaviours promoted during CATI implementation which may underestimate its impact.

Despite these constraints, the study has several strengths. It offers a method that can be deployed in real time using data routinely available to health authorities without the need for recruitment or individual-level linkage. During an outbreak, this approach could support programme monitoring by providing early indications of whether vaccination campaigns and complementary interventions are associated with reductions in transmission. Such information may assist response teams in assessing the timing and intensity of control measures and in guiding decisions on whether additional vaccination rounds or intensified WASH activities are required. The approach incorporates uncertainty rather than masking it and produces interpretable estimates in settings where evidence is urgently needed to guide programme

decisions. The inclusion of WASH activity, even at an ecological level, provides a more realistic estimate of vaccine-attributable impact than models that ignore concurrent interventions.

4.6 Conclusion

This study demonstrates a feasible approach for estimating vaccine-attributable impact whilst accounting for public health and social measures, such as WASH. Using these methods, we show that OCV contributed to a substantial reduction in cholera transmission during the outbreak. More broadly, the work demonstrates that policy makers in LMICs can generate meaningful field VE estimates in resource-constrained settings during outbreaks where traditional observational designs are not feasible and timely evidence is nonetheless required to guide outbreak response.

4.7 Acknowledgements

4.7.1 Funding

This work was supported by the UK National Institute for Health and Care Research (NIHR) Global Health Research Group on Gastrointestinal Infections at the University of Liverpool using UK aid from the UK Government to support global health research (NIHR133066). This work was supported by the Malawi-Liverpool-Wellcome Programme Core Grant from Wellcome (grant number 206545/Z/17/Z). Latif Ndeketa is supported by a studentship from the MRC Discovery Medicine North (DiMeN) Doctoral Training Partnership (MR/W006944/1). Nigel Cunliffe is a NIHR Senior Investigator (NIHR203756) and Khuzwayo Jere is an NIHR Global Health Professor (NIHR306394). Peter Dodd acknowledges research funding through The UK-South East Asia Vaccine Manufacturing Research Hub. This research is funded by the Department of Health and Social Care using UK International Development funding and is managed by the EPSRC. The views expressed in this publication are those of the authors and not necessarily those of the Department of Health and Social Care. Nigel Cunliffe and Daniel Hungerford are also affiliated with the NIHR Health Protection Research Unit in Gastrointestinal Infections at the University of Liverpool, a partnership with the UK Health Security Agency in collaboration with the University of Warwick. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The content is solely the responsibility of the authors

and does not necessarily represent the official views of the National Institutes of Health, the NIHR, the Department of Health and Social Care, the UK government or the UK Health Security Agency.

4.7.2 Conflicts of interest

D.H. and N.F are currently receiving grant support from Seqirus UK Ltd for the evaluation of influenza vaccines and grant support and personal consultancy fees from Merck & Co (Kenilworth, New Jersey, USA) for rotavirus strain surveillance; D.H. received honoraria for a presentation at a Merck Sharp & Dohme (UK) Limited symposium on vaccines; and D.H. has received research grant support from Sanofi Pasteur, and Merck & Co for rotavirus strain surveillance. K.C.J. has received research grant support from GlaxoSmithKline Biologicals for work on rotavirus vaccines. All other authors declare no competing interests.

4.7.3 Contributions

L.N., D.H., N.F, N.A.C and P.D conceived of the study and secured the funding. L.N. performed the analysis, wrote the manuscript. N.F, V.E.P, D.H, P.D assisted with the analysis and interpretation of results and edited the manuscript. K.C.J., M.K., C.B., P.M, U.M, N.K and K.C.J oversaw the data collection, assisted with the interpretation of results and edited the manuscript. All authors assisted with the interpretation of results and edited the manuscript.

5 Estimating RTS,S/AS01 malaria vaccine effectiveness in routine programme settings: comparison of cluster-level and individual-level analyses using the Malawi MVIP endline survey

5.1 Abstract

Background

The RTS,S/AS01 (RTS,S) malaria vaccine was introduced through the World Health Organization-led Malaria Vaccine Implementation Programme (MVIP) in Malawi to generate evidence on safety, feasibility, impact, and effectiveness under routine immunisation conditions. Post-licensure malaria vaccine evaluation is complicated by heterogeneity in transmission, age-dependent malaria risk, incomplete vaccine uptake, and differences between programme-level and individual-level estimates. We compared RTS,S vaccine effectiveness (VE) estimates from cluster-level and individual-level analyses applied to the same Malawi endline household survey dataset.

Methods

We analysed data from 4,868 children aged 5–48 months enrolled in the Malawi MVIP Endline household survey across 46 clusters, with 23 clusters allocated to RTS,S introduction and 23 to control. The endpoint for all analyses was malaria positivity, defined as a positive malaria rapid diagnostic test (mRDT) during the household survey. In the cluster-level analysis, malaria prevalence was compared between intervention and control clusters using weighted regression. Adjusted prevalence ratios were estimated using a two-stage approach that adjusted for age and socioeconomic status (SES) while preserving the cluster-level comparison. We also conducted unmatched and matched case–control analyses using individual vaccination status. Cases were mRDT-positive children and controls were mRDT-negative children. The primary exposure was completion of the three-dose RTS,S primary series. Matching was performed on cluster and age band, with a ±3-month age caliper and Mahalanobis distance based on age and

SES. Conditional logistic regression adjusted for household use of long-lasting insecticidal nets (LLINs).

Results

At the cluster level, malaria prevalence did not differ clearly between intervention and control clusters. The unadjusted risk difference was 0.8 percentage points (95% CI −6.5 to 8.1), and the adjusted prevalence ratio was 0.92 (95% CI 0.55–1.52), corresponding to a VE of 8% (95% CI -52 – 45). In the individual-level analyses, completion of the RTS,S primary series was associated with lower odds of malaria positivity. The adjusted unmatched VE estimate was 41% (OR 0.59, 95% CI 0.46–0.76), while the matched VE estimate was 58.3% (OR 0.42, 95% CI 0.29–0.59). Receipt of any primary dose produced a lower matched VE estimate of 43.8% (OR 0.56, 95% CI 0.41–0.77).

Interpretation

Different analytic approaches applied to the same cross-sectional endpoint data produced different RTS,S VE estimates. The cluster-level endline prevalence comparison had limited precision and did not capture the individual-level protection observed after accounting for age and spatial heterogeneity. These findings suggest that post-licensure malaria vaccine evaluations should prioritise longitudinal and incidence-based clinical endpoints, while interpreting single timepoint mRDT prevalence with caution in high-transmission settings.

Funding

This study was funded by the MRC Discovery Medicine North (DiMeN) Doctoral Training Partnership (UKRI), National Institute for Health and Care Research (NIHR) Global Health Research Group on Gastrointestinal Infections and Wellcome through the core grant to the Malawi-Liverpool-Wellcome Research Programme. The wider MVIP was funded by WHO; Gavi, the Vaccine Alliance; the Global Fund to Fight AIDS, Tuberculosis and Malaria; and Unitaid.

5.2 Introduction

Malaria remains a major cause of morbidity and mortality in children under five years in sub-Saharan Africa (SSA)(1). Malaria is caused by several Plasmodium species, mainly *P. falciparum, P. vivax, P. malariae, P. ovale* and *P. knowlesi*. In 2024, *P. falciparum* accounted for 98% of cases in high-transmission east and southern African countries, including Malawi. The most recent World Health Organization (WHO) estimates show that there were 282 million reported malaria cases globally in 2024, with 94% occurring in SSA(109). This represents an increase of more than 9 million cases from 2023. Of the 610,000 malaria-related deaths reported in the same period, 95% were in SSA and 75% occurred in children under five. The burden of disease therefore remains concentrated in the poorest regions and among the most vulnerable age group.

Global malaria prevention efforts initially led to substantial reductions in malaria incidence, with a 25.5% decline between 2000 and 2015. This progress has slowed, with an 8.5% increase in incidence observed between 2015 and 2024. The recent increase has occurred against a background of persistent transmission, insecticide and antimalarial drug resistance, funding constraints, health-system disruption, population movement and climate-related changes that may affect malaria risk. In this context, the Global Technical Strategy (GTS) for malaria 2016–2030 set targets to reduce malaria incidence and mortality by at least 75% by 2025 and 90% by 2030 relative to 2015 levels(316). Achieving these targets requires strengthening established interventions while introducing additional tools, including malaria vaccines.

Malawi remains a high-transmission setting with malaria endemic across the country and seasonal peaks during the rainy period from November to April(317,318). Over the past two decades, coverage of key malaria control interventions has increased. Insecticide-treated bed net use among children under five rose from 15% in 2004 to 53% in 2024, while use among pregnant women increased from 15% to 46%. Preventive treatment in pregnancy also expanded, with three or more doses of sulfadoxine-pyrimethamine (SP) increasing from 13% in 2012 to 62% in 2024. Case management indicators improved with malaria testing among febrile children increasing from 17% in 2010 to 68% in 2024 and ACT use among treated febrile children rising from 84% to 97%. Malaria incidence declined from 407 per 1,000 population in

2016 to 220 per 1,000 in 2022, approaching but not meeting the national target of 204 per 1,000(318).

Malawi is among five countries that accounted for approximately half of all long-lasting insecticide-treated nets (LLINs) distributed in SSA, receiving an estimated 13 million nets (109). Household ownership of at least one LLIN was 55% in 2021(317). Despite these gains, malaria transmission remains substantial. Current estimates for Malawi indicate a prevalence of 10.5% and approximately 9 malaria-attributable deaths per 100,000 population annually(317). Malaria continues to place a substantial burden on health services in Malawi, with an estimated six million cases annually, accounting for 30% of outpatient visits and 34% of hospitalisations according to the National Malaria Control Programme as reported in the Health Sector Strategic Plan III(319). These patterns suggest that existing control tools, while effective, have not achieved sustained reductions in transmission in high-burden settings.

The fight against malaria is further complicated by emerging resistance to the artemisinin-based combination therapy (ACT) and increasing cost of insecticides that are used for indoor residual spraying (IRS)(320–322). These challenges have limited the long-term effectiveness of existing interventions and have necessitated the development of additional preventative tools such as malaria vaccines. The RTS,S/AS01 (hereafter referred to as RTS,S) was the first WHO-prequalified and recommended malaria vaccine. It targets the circumsporozoite protein of *P. falciparum* and showed moderate protection against clinical malaria in a phase 3 efficacy and safety trial in sub-Saharan Africa. The vaccine schedule includes a primary series of three doses administered between 5, 6 and 7 months of age, followed by a fourth dose given 15–18 months after the third dose. In the phase 3 trial, RTS,S showed moderate protection among children aged 5–17 months at first vaccination, with efficacy estimates of 46% (95% CI: 42–50) against clinical malaria and 38% (95% CI: 18–53) against severe malaria by 18 months after the third dose. Among children in the same age group, a fourth dose administered 18 months after dose three improved longer-term efficacy against clinical malaria from 26% (95% CI: 21–31) to 39% (95% CI: 34–43), and against severe malaria from −2% (95% CI: −31 to 20) to 31.5% (95% CI: 9–48)(122).

These findings led to a positive scientific opinion from the European Medicines Agency (EMA) and a conditional recommendation from the WHO to implement the Malaria Vaccine Implementation Programme (MVIP) in Malawi, Ghana, and Kenya. The MVIP was designed to address three key questions: First, to further evaluate the safety signals observed in the phase 3 trial, including the reported excess of meningitis and cerebral malaria cases and the imbalance in mortality among girls. Second, to assess the vaccine effectiveness (VE) and impact under routine programme conditions. Third, to evaluate the feasibility of delivering RTS,S through existing immunisation systems in moderate to high transmission settings(122,323,324). These objectives were addressed through complementary components within the MVIP. A cluster-based implementation design across 158 clusters in the three countries was used to evaluate programme-level impact, safety, and coverage under routine delivery(324). In parallel, cohort studies (EPI-MAL-003) conducted by the vaccine manufacturer, GSK, in Ghana, Kenya, and Malawi followed children prospectively in vaccine-exposed and unexposed clusters to estimate VE and safety (149).

The MVIP has now produced evidence on RTS,S feasibility, safety, severe malaria impact, and mortality impact. The initial evaluation after two years of implementation found that the first three RTS,S doses could be delivered through national immunisation programmes, with no evidence of the safety signals observed in the phase 3 trial, and a 32% reduction in hospital admission with severe malaria among children eligible to have received three doses compared to those in the vaccine unexposed clusters(324). The same analysis reported a 9% reduction in all-cause mortality excluding injury(324). The final mortality evaluation over 46 months subsequently reported a mortality rate ratio of 0.87 (95% CI 0.77–0.97), indicating a 13% reduction in all-cause mortality excluding injury among children eligible to have received the third RTS,S dose(325). Together, these studies show that RTS,S introduction through routine immunisation had measurable public health impact under programme conditions.

Evaluation of RTS,S in routine immunisation programme settings presents methodological challenges for both effectiveness and impact estimation. Continued evaluation of malaria vaccines will be needed to understand their relative and absolute contribution to malaria control in order to justify continued investment. The contribution of vaccination may increase or

decrease over time depending on changes in transmission intensity, vaccine coverage, LLIN use, insecticide resistance, access to diagnosis and treatment, antimalarial drug resistance, and the delivery of other malaria control measures.

The MVIP therefore provided a rare opportunity to examine these issues. Its phased, cluster-randomised introduction allowed vaccine performance to be evaluated under routine delivery while comparison areas still existed. This opportunity has now largely disappeared, as RTS,S has since been introduced into the former comparison clusters and malaria vaccine implementation has moved into wider roll-out. Future evaluations will therefore have fewer clearly defined unvaccinated comparison populations and will rely more heavily on observational designs, routine surveillance, and repeated programme monitoring.

Cluster-based designs are suited to programme-level questions, including severe malaria and mortality impact, although they can be affected by between-cluster variation in malaria burden, socioeconomic conditions, and vaccine uptake(155,165). Observational approaches such as case–control studies examine the relationship between individual vaccination status and disease, although they remain sensitive to confounding(161). These approaches address related but distinct questions and may produce different estimates, even when applied within the same programme. In this study, we used the Malawi MVIP endline household survey to compare RTS,S effect estimates from cluster-level, unmatched case–control, and matched case–control analyses. We aimed to assess how analytic approach and endpoint definition influence RTS,S estimates and their interpretation in routine programme settings, with a view to informing future evaluation strategies after the MVIP period.

5.3 Methods

5.3.1 Study design and setting

This study used data from the Malawi Endline household survey conducted within the Malawi component of the MVIP. The wider MVIP was a multi-country evaluation implemented in Ghana, Kenya, and Malawi following introduction of RTS,S through routine immunisation services. The programme included cluster-based evaluation of vaccine safety, feasibility, coverage, and programme impact under routine delivery conditions(265,323). In Malawi, the

programme included 11 districts selected based on malaria transmission intensity and disease burden. The districts were divided into 46 clusters with each cluster defined as the catchment area of immunisation clinics and each cluster had a population of at least 100,000 people. Clusters were randomised 1:1 to either introduction of RTS,S through the Expanded Programme on Immunisation (EPI) or continuation of standard EPI services without RTS,S. RTS,S was delivered through a combination of static and outreach clinics within existing EPI delivery structures. Routine malaria control interventions continued to be provided by the Ministry of Health across all clusters, including long-lasting insecticide-treated nets (LLINs), intermittent preventive treatment in pregnancy with sulfadoxine–pyrimethamine (IPTp), and prompt treatment with artemisinin-based combination therapies (ACTs).

5.3.2 Household surveys

The programmatic feasibility component of the MVIP was assessed through three repeated cross-sectional household surveys: baseline, midline, and endline. This analysis used data from the endline survey. The surveys aimed to estimate RTS,S primary series coverage in children aged 12–23 months and fourth-dose coverage in children aged 27–38 months. The endline survey was conducted in nine of the 11 MVIP districts, covering a population of approximately 6.5 million across the 46 clusters. The survey collected demographic, household, and malaria-related variables, including age, malaria test result, vaccination status, LLIN use, fever history, and household assets. Socioeconomic status (SES) was derived from the household wealth index available in the survey dataset. The index incorporated household assets and living conditions, including livestock ownership, transport assets, housing materials, toilet facility, cooking fuel, electricity, and drinking water source. SES was analysed as a categorical variable (low, medium, high) derived from the household wealth index.

5.3.3 Sampling design and participants

The household survey used a two-stage cluster sampling design. The sample size was calculated to estimate vaccine coverage among children aged 27–38 months, assuming an intracluster correlation coefficient (ICC) of ≤0.2, a design effect of 2.1, 90% power, and 5% precision, which gave a minimum required sample of 1,480 children across study arms(326). In the first stage, four enumeration areas (EAs) were selected within each cluster using probability proportional

to population size, giving a total of 184 EAs. Household listing was conducted in all selected EAs to identify households with at least one child aged 5–48 months, which formed the sampling frame for the second stage. In the second stage, 35 households per EA were randomly selected using simple random sampling. Survey teams visited selected households directly and enrolled participants from the first 25 households reached in each EA, with repeat visits where necessary(326). All children aged 5–48 months within selected households were eligible, and caregivers were interviewed following informed consent.

5.3.4 Cluster-randomised analysis

5.3.4.1 Cluster-level analysis

The cluster-randomised analysis was conducted at the cluster level, with each cluster defined as the catchment area of immunisation delivery clinics. A total of 46 clusters were included, with 23 clusters allocated to the malaria vaccine implementation arm and 23 to the control arm. For each cluster, malaria prevalence was calculated as the proportion of children aged 5–48 months in the endline household survey who had a positive mRDT result. Age in months and SES were included as covariates. Age was modelled flexibly using restricted cubic splines to allow for a non-linear association between age and malaria positivity.

5.3.4.2 Endpoint definition

The primary endpoint for both the cluster-level and individual-level analyses was malaria positivity, defined as a positive malaria rapid diagnostic test among children aged 5–48 months enrolled in the endline household survey(326). Malaria testing was conducted in the household by trained nurses using SD Bioline Malaria Ag Pf test kits, in line with Ministry of Health guidelines. Each enrolled child was tested during the survey visit. Children with a positive result received first-line treatment with artemether-lumefantrine, with the first dose administered by the research nurse at the household and the remaining doses given by the caregiver.

5.3.4.3 Intracluster correlation

We assessed clustering of malaria infection using the intracluster correlation coefficient (ICC). A mixed-effects logistic regression model with a random intercept for cluster was fitted, and then

ICC was derived from the cluster-level variance component using the standard logistic distribution variance.

5.3.4.4 Statistical analysis

Unadjusted analysis

Cluster-level malaria prevalence was calculated for each cluster. The difference between trial arms was estimated using weighted linear regression with cluster-level prevalence as the outcome and trial arm as the exposure. Clusters were weighted by cluster size to account for variation in the number of children per cluster. The risk difference and corresponding 95% CIs were obtained from this model.

Adjusted analysis

We used a two-stage approach for the adjusted analyses to preserve the cluster-level comparison while allowing adjustment for individual-level covariates. First, an individual-level logistic regression model was fitted without including the trial arm, modelling malaria positivity as a function of age in months and SES. We obtained predicted probabilities of malaria for each child. These predicted values were then aggregated to the cluster level to obtain expected numbers of cases. Observed and expected counts were compared within each cluster, and the log of the observed-to-expected ratio was modelled using weighted linear regression with trial arm as the exposure. Clusters were weighted by cluster size. Adjusted prevalence ratios (PRs) were obtained by exponentiating the regression coefficients, and VE was calculated as (1 − PR) × 100%.

We also conducted a subgroup analysis restricted to children aged under 12 months to evaluate VE during infancy, the age group targeted for RTS,S primary vaccination. Household LLIN use was not included in the primary adjusted model because it was measured at the endline survey and may reflect post-randomisation behaviour or concurrent malaria control delivery rather than baseline confounding. LLIN use was included in the infant subgroup analysis as an exploratory adjustment to assess whether accounting for household-level malaria prevention altered estimates in the age group targeted for RTS,S primary vaccination.

5.3.5 Matched case–control analysis

5.3.5.1 Study population

The initial dataset had 4,868 children. We restricted the analysis to children with complete data on outcome, matching variables, and covariates, including cluster, age in months, SES, and household LLIN use. Observations with missing data on these variables were excluded from the analytic dataset.

5.3.5.2 Exposure and outcome definition

Cases were defined as children aged 5–48 months who had a positive malaria RDT during the endline household survey, while controls were children in the same survey who had a negative test result. We defined the primary exposure as completion of the RTS,S primary vaccination series (all three primary doses). Our secondary exposure was receipt of at least one primary dose (any primary). The fourth (booster) dose was excluded from the primary analysis due to low uptake.

5.3.5.3 Statistical analysis

We implemented nearest-neighbour matching without replacement using a two-stage process. In the first stage, eligible controls were restricted for each case to children from the same cluster and within ±3 months of the case's age. Within this restricted pool, controls were selected using Mahalanobis distance based on age in months and SES. In the second stage, exact matching on predefined age bands was imposed to further improve age balance. The age bands were chosen to reflect RTS,S eligibility and the strong age dependence of malaria risk in early childhood. We used a 1:2 matching ratio for the primary analysis. Covariate balance after matching was assessed using standardised mean differences (SMDs), with absolute values below 0.1 considered indicative of acceptable balance(327). We estimated matched odds ratios using conditional logistic regression with matched-set strata derived from the matching procedure. Models included completion of the RTS,S primary series (all primary) as the main exposure and adjusted for LLIN use as an additional covariate. VE was calculated as:

$$VE = (1 - OR) \times 100\%$$

where OR is the odds ratio for the vaccination exposure.

5.3.5.4 Sensitivity analyses

We conducted sensitivity analyses to examine the impact of exposure definition and matching specification on the estimated association. First, exposure was redefined as receipt of at least one primary dose (any primary) within the same matched dataset. We then assessed robustness to the matching approach by relaxing exact matching to cluster only with a 1:2 ratio and by increasing the control-to-case ratio to 1:3 under cluster-only matching.

5.3.6 Unmatched case–control analysis

We also performed an unmatched analysis using multivariable logistic regression to estimate odds ratios for malaria positivity associated with vaccination status. Models included completion of the RTS,S primary series (all primary) as the main exposure and adjusted for age in months (continuous), SES and household LLIN use. For exposure-specific analyses, children were restricted to those old enough at the survey to have had the opportunity to receive the relevant RTS,S doses. Receipt of any primary dose was assessed among children aged ≥5 months, while completion of the three-dose primary series was assessed among children aged ≥8 months, corresponding to the age by which dose 3 could have been received under the Malawi EPI.

5.3.7 Ethical considerations and consent

Ethical approval for the Malaria Vaccine Implementation Programme evaluation was obtained through WHO, partner institutional, national, and country-specific review processes. The master and country-specific protocols were reviewed by the European Medicines Agency, WHO, relevant national regulatory authorities, and institutional review boards. National regulatory authorities reviewed RTS,S jointly through the African Vaccine Regulatory Forum, followed by country-level authorisation. In Malawi, the protocols were reviewed by the College of Medicine Research and Ethics Committee, the UNC Chapel Hill Ethics Committee, and the Malawi Pharmacy, Medicines and Poisons Board.

Written informed consent was obtained from a parent or legal guardian for children participating in household surveys. RTS,S vaccination was delivered by the Ministry of Health through routine immunisation services. Consent for vaccination was therefore based on implied

consent, consistent with WHO guidance, with community engagement used to ensure that parents and caregivers had sufficient information to accept or decline RTS,S vaccination for eligible children.

5.4 Results

5.4.1 Cluster-randomised analysis

5.4.1.1 Descriptive statistics

A total of 4,868 children were included across 46 clusters, with 23 clusters allocated to each arm. The median cluster size was 106 children (IQR 103–109; range 99–114). Baseline characteristics were similar between intervention and control clusters, although the age distribution differed modestly between arms (p = 0.008), with a slightly higher proportion of children aged ≥36 months in intervention clusters. Mean age was 24.9 months in control clusters and 25.3 months in intervention clusters (p = 0.33). SES was similar across arms (p = 0.87), and the proportion of children with reported fever was comparable (22.5% vs 21.9%; p = 0.40). Missing data for fever were minimal (<0.5%) and were excluded from regression analyses (Table 5-1).

Table 5-1: Baseline demographic characteristics between vaccinating and non-vaccinating clusters

Characteristic	**Control clusters (n=2430)**	**Vaccinating clusters (n=2438)**	**p-value***
Age (months), mean (SD)	24.94 (11.77)	25.28 (11.89)	0.33
Age category			0.008
0–23 months	1092 (44.9)	1058 (43.4)	
24–35 months	751 (30.9)	714 (29.3)	
36–47 months	520 (21.4)	560 (23.0)	
≥48 months	67 (2.8)	106 (4.3)	
SES			0.87
High	797 (32.8)	785 (32.2)	
Low	905 (37.2)	908 (37.2)	
Medium	728 (30.0)	745 (30.6)	

Fever			0.40
No	1881 (77.4)	1898 (77.9)	
Yes	546 (22.5)	533 (21.9)	
Missing	3 (0.1)	7 (0.3)	

* P-values compare baseline characteristics between vaccinating and non-vaccinating clusters. Continuous variables were compared using t-tests and categorical variables using χ^2 tests, with Fisher's exact tests used where expected cell counts were small. The p-values are descriptive and were used to assess baseline comparability between study arms, rather than to estimate vaccine effect.

5.4.1.2 Intracluster correlation

The intraclass correlation coefficient (ICC) for malaria infection was 0.236, indicating substantial between-cluster variation in malaria risk.

5.4.1.3 Cluster-level vaccine effectiveness

At the cluster level, malaria prevalence was similar in intervention and control clusters. The unadjusted risk difference comparing intervention with control clusters was 0.8 percentage points (95% CI -6.5 – 8.1; p=0.82), with no evidence of a difference between arms. After adjustment for age and SES, the prevalence ratio for intervention compared with control clusters was 0.92 (95% CI 0.55 – 1.52). This corresponded to a VE of 8% (95% CI -52 – 45) against mRDT-confirmed malaria infection. The confidence interval was wide and spanned both meaningful protection and no effect, reflecting the lack of precision in the cluster-level estimates (Table 5-2).

Table 5-2: Cluster-level comparison of malaria prevalence between intervention and control clusters

Measure	**Estimate**	**95% CI**	**p-value**
Risk difference (%)	+0.8	-6.5 to +8.1	0.82
Prevalence ratio	0.92	0.55 – 1.52	–
Vaccine effectiveness (%)	8%	-52 to 45	–
ICC	0.236	–	–

5.4.1.4 Subgroup analysis in children aged under 12 months

A total of 795 children aged under 12 months were included, of whom 61 (7.7%) tested positive for malaria. Malaria prevalence varied across clusters, with an ICC of 0.376, indicating greater between-cluster variation in this age group than in the overall analysis. At the cluster level, there was no evidence of a difference in malaria prevalence between intervention and control clusters. The unadjusted risk difference was 1.2 percentage points (95% CI -5.6–7.9; p=0.73). After adjustment for age, SES, and LLIN use, the prevalence ratio was 1.02 (95% CI 0.59–1.79), corresponding to a VE of -2% (95% CI -79–41).

5.4.2 Matched case–control analysis

5.4.2.1 Descriptive statistics before matching

The analytic dataset comprised 4,700 children, including 562 cases and 4,138 controls. Cases were older than controls, with a mean age of 29.0 months compared with 24.6 months, respectively. SES differed between groups, with a higher proportion of cases in the lowest SES category and a lower proportion in the highest category. These differences in age and SES were addressed in the subsequent matching procedure (Table 5-3).

Table 5-3: Characteristics of the analytic dataset before matching, stratified by case–control status

Characteristic	**Full analytic dataset (n=4700)**	**Cases (n=562)**	**Controls (n=4138)**
Participants	4700 (100%)	562	4138
Age in months, mean (SD)	25.1 (11.8)	29.0 (11.2)	24.6 (11.8)
SES Low, %	36.8	43.4	35.9
SES Medium, %	30.3	30.6	30.3
SES High, %	32.8	26.0	33.8
LLIN use, %	84.4	67.8	86.6
Completed primary series, %	22.0	13.9	23.1
≥1 primary dose, %	28.1	21.2	29.1

Before matching, cases were older than controls and differed modestly in SES. Balance improved after the first-stage match and improved further after the second-stage age-band

restriction. In the final matched dataset, the standardised mean difference for age decreased from 0.39 before matching to 0.03 after matching, while SES differences were reduced to absolute standardised mean differences below 0.1. Balance across clusters and age bands was achieved by design (Table 5-4).

Table 5-4: Covariate balance before and after matching, expressed as standardised mean differences

Variable	**Standardised mean difference (before match)**	**Standardised mean difference (after match)**
Age (months)	0.39	0.03
SES Low	0.15	-0.02
SES Medium	0.01	0.09
SES High	-0.18	-0.07

5.4.2.2 Effectiveness of completing the RTS,S primary series

Completion of the RTS,S primary series was associated with lower odds of malaria positivity. The adjusted odds ratio (aOR) was 0.42 (95% CI 0.29–0.59), corresponding to a VE of 58.3% (95% CI 40.8–70.7). Household LLIN use was also associated with reduced odds of malaria positivity (aOR 0.57, 95% CI 0.44–0.74).

5.4.2.3 Sensitivity analyses

When exposure was defined as receipt of any of the three RTS,S primary doses showed a lower VE of 43.8% (95% CI 23–59). Matching only by cluster with a case to control ratio of 1:2 produced a VE of 59.8% (95% CI 44–71) and the VE increased to 61.2% (95% CI 46–72) when the case to control ratio was increased to 1:3.

Table 5-5: Matched case–control estimates of RTS,S effectiveness under primary and alternative matching specifications

Analysis	**Cases**	**Controls**	**Adjusted OR***	**95% CI**	**VE‡ (%)**	**P-value**
Primary: 1:2 match (cluster + age band), all primary	533	932	0.42	0.29 – 0.59	58.3	<0.001

Sensitivity: same match, any primary	533	932	0.56	0.41 – 0.77	43.8	<0.001
Sensitivity: 1:2 match (cluster only), all primary	560	1022	0.40	0.29 – 0.56	59.8	<0.001
Sensitivity: 1:3 match (cluster only), all primary	560	1373	0.39	0.28 – 0.54	61.2	<0.001

*Matched on cluster and age band using Mahalanobis distance based on age in months and SES. Conditional logistic regression models adjusted for household LLIN use
‡Calculated as (1 – aOR) × 100%

5.4.3 Unmatched case–control analysis

In the unmatched analysis, the number of cases and controls differed between exposure definitions because children were restricted according to their opportunity to receive the relevant RTS,S doses. Among children aged ≥8 months, completion of the RTS,S primary series was associated with lower odds of malaria positivity in crude analysis, corresponding to VE of 49% (OR 0.51, 95% CI 0.39–0.65). After adjustment for age, SES, and household LLIN use, the VE estimate decreased to 41% (OR 0.59, 95% CI 0.46–0.76). A similar pattern was observed when exposure was defined as receipt of any primary dose. Among children aged ≥5 months, receipt of any primary dose was associated with crude VE of 35% (OR 0.66, 95% CI 0.52–0.81) which decreased to 22% after adjustment (OR 0.78, 95% CI 0.62–0.97).

Table 5-6: Unmatched case–control estimates of RTS,S effectiveness

Exposure	**Age-opportunity restriction**	**Cases/controls**	**Crude OR (95% CI)**	**VE (%)**	**Adjusted OR† (95% CI)**	**VE‡ (%)**	**P-value**
All primary	≥8 months	539/3823	0.51 (0.39–0.65)	49	0.59 (0.45–0.76)	41	<0.001
Any primary	≥5 months	559/4132	0.66 (0.52–0.81)	35	0.78 (0.62–0.97)	22	0.029

†Adjusted for age in months, SES and household LLIN use
‡Calculated as (1 – aOR) × 100%

5.5 Discussion

5.5.1 Summary of findings

This analysis compared RTS,S VE estimates from three different approaches applied to the same Malawi MVIP endline survey dataset. At the cluster level, there was little evidence of a difference in malaria prevalence between intervention and control clusters. The unadjusted risk difference was 0.8 percentage points (95% CI −6.5 to 8.1), and the adjusted prevalence ratio was 0.92 (95% CI 0.55–1.52), corresponding to VE of 8% (95% CI −52 to 45). In contrast, individual-level analyses showed moderate protection. Completion of the RTS,S primary series was associated with VE of 41% (OR 0.59, 95% CI 0.46–0.76) in the unmatched analysis and 58% (OR 0.42, 95% CI 0.29–0.59) after matching on cluster and age band. The direction of effect was broadly consistent with efficacy reported in the RTS,S phase 3 trial, although the endpoints and study designs differed(328).

5.5.2 Why the cluster-level and individual-level estimates differed

These approaches used the same cross-sectional endpoint, but they were not comparing the same thing. The cluster-randomised analysis compared malaria prevalence between children living in RTS,S implementation and control clusters at the endline survey. This captures the effect of being in an area where RTS,S was introduced and not necessarily the effect of actually receiving the vaccine. The case–control analysis was different because it compared children according to their own RTS,S vaccination status within the same survey population. This distinction matters because vaccine coverage in the implementation clusters was incomplete. If nearly all eligible children in implementation clusters had received RTS,S, the cluster-level estimate might have been closer to the individual-level case–control estimate, although it would still reflect a programme-level comparison. With incomplete coverage, the cluster-level effect is expected to be diluted because vaccinated and unvaccinated children both contribute to malaria prevalence in the implementation arm. The difference between estimates is therefore not because different outcomes were measured, since both analyses used endline mRDT positivity. It is more likely because the analyses compared different forms of vaccine exposure and handled age, spatial heterogeneity, SES, and individual vaccination status differently. These

differences are consistent with the conceptual framework in Figure 1-7 because the cluster-level and matched case-control analyses estimate vaccine effects at different levels of the vaccine impact pathway.

5.5.3 Interpreting mRDT positivity as an endpoint

A separate issue is that mRDT positivity in the endline survey is not the same as clinical malaria. In the RTS,S phase 3 trial, the primary clinical malaria endpoint required illness with an axillary temperature of at least 37.5°C and *P. falciparum* asexual parasitaemia above 5000 parasites per mm^3(122). In contrast, the endpoint in this analysis was mRDT positivity during a household survey, regardless of whether the child was clinically unwell. In this dataset, malaria prevalence in children aged 5–48 months was 12.0% overall and 48.6% of malaria-positive children were not febrile. This means that nearly half of detected infections were not febrile at the time of the survey. The prevalence endpoint is therefore broader than the clinical malaria endpoints used in the phase 3 trial, and this may have diluted the apparent vaccine effect. A restricted analysis of febrile mRDT-positive children would have been closer to a clinical endpoint, although the number of events is likely to be small and the resulting estimates would probably be imprecise.

5.5.4 Adjustment, matching, and comparability across analyses

A related issue is that the analyses did not adjust for the same covariates in the same way because they were comparing different things. In the cluster-level analysis, the comparison was between children living in RTS,S implementation and control clusters. We therefore kept the analysis at the cluster level and adjusted for age and SES through the two-stage approach before comparing observed and expected malaria cases by cluster. In the case–control analyses, the exposure was individual RTS,S vaccination status, so confounding by factors related to vaccine uptake and malaria risk was more direct. The matched analysis dealt with this by matching children within the same cluster, using age-band matching with a ±3-month age caliper, and including SES in the matching distance. LLIN use was then included in the conditional logistic regression model. The unmatched analysis adjusted for age, SES, and LLIN use using regression alone. The estimates should therefore be interpreted as complementary rather than directly

interchangeable because they differed both in what they compared and in how they accounted for age, SES, spatial heterogeneity, and LLIN use.

5.5.5 Interpretation within the wider MVIP evidence base

The Malawi MVIP endline findings should therefore be interpreted within the wider MVIP evidence base. The pooled MVIP evaluation recruited 652,673 children across 158 clusters in Ghana (66 clusters), Kenya (46 clusters) and Malawi (46 clusters). RTS,S introduction was associated with a 32% (95% CI: 5–51%) reduction in hospitalisation with severe malaria and a 9% (95% CI: 0–18%) reduction in all-cause mortality over two years of follow-up, with no evidence of the safety signals observed in the phase 3 trial(323,324). The cluster-level mRDT prevalence result in our analysis was small and imprecise, but this does not imply absence of public health benefit. The wider MVIP evidence showed reductions in severe malaria admissions and all-cause mortality excluding injury, which are more clinically meaningful endpoints than parasite prevalence measured at a single survey timepoint. These findings suggest that RTS,S can reduce severe outcomes and deaths even where a clear reduction in community mRDT prevalence is not detected in a cross-sectional survey. The case–control analysis adds an individual-level signal in the same direction, since children who had completed the RTS,S primary series had lower odds of mRDT positivity than children who had not completed the series.

5.5.6 Evidence from the EPI-MAL-003 prospective cohort

The EPI-MAL-003 prospective cohort study provides further evidence from the same implementation programme. In that phase 4 study, children in vaccine-exposed and vaccine-unexposed clusters in Ghana, Kenya, and Malawi were followed actively to estimate RTS,S VE against incident malaria outcomes. The study reported a total VE of 30% (95% CI 27–33) against any malaria, 58% (95% CI 40–70) against severe malaria, 36% (95% CI 28–44) against malaria-related hospitalisation, and 21% (95% CI 16–26) against all-cause hospitalisation(123). These findings are not directly comparable with our estimates because the cohort study measured incident outcomes under active follow-up, while our analysis used cross-sectional mRDT positivity at a single survey timepoint. They are nevertheless useful context because they show

that RTS,S effectiveness is more readily demonstrated when outcomes are measured longitudinally and are closer to the clinical endpoints the vaccine is intended to prevent.

5.5.7 Matched and unmatched individual-level estimates

The difference between the unmatched and matched case–control estimates is also informative. The increase in VE from 41% in the unmatched analysis to 58.3% after matching reflects the improved control of age and spatial heterogeneity. Age imbalance was reduced from a standardised mean difference of 0.39 before matching to 0.03 after matching, and balance on SES also improved. These imbalances are likely to have contributed to the difference between estimates. The matched analysis is therefore likely to provide a more internally comparable individual-level estimate in this dataset, particularly given the strong age dependence of malaria risk and the clear variation in transmission across clusters. The lower VE of 43.8% for receipt of any primary dose than for completion of the primary series in the matched case–control shows that partial vaccination was less protective than completion of the three-dose series.

5.5.8 Implications for future evaluation

The main implication of these findings is that the estimated effect of RTS,S depends on the design used, the explanatory variables, and the level at which the comparison is made, even when the same endpoint is used. In this study, all analyses used mRDT positivity as an outcome, but the cluster-level comparison assessed malaria prevalence among children living in RTS,S implementation and control clusters, while the case–control analyses compared children according to their individual vaccination status. This distinction matters because vaccine coverage in the implementation clusters was incomplete. In the cluster-level analysis, vaccinated and unvaccinated children both contributed to malaria prevalence in the implementation cluster, so any vaccine effect would be diluted at the cluster level. If coverage had been close to complete, the cluster-level estimate might have been closer to the individual-level estimate, although it would still have represented programme-level impact rather than individual VE. This should not be taken to mean that cluster-based evaluations are unsuitable for malaria vaccine assessment. The wider MVIP evaluation showed that cluster-based programme evaluation can detect public health impact when outcomes are measured longitudinally and are clinically relevant. The learning point is more specific: a cluster-

randomised approach can measure community-level programme impact but may struggle to separate individual-level vaccine benefit when coverage is moderate and the outcome is a single cross-sectional prevalence measure. Future evaluations should therefore be clear about whether they are estimating programme impact or individual VE, and should prioritise longitudinal follow-up and clinically relevant outcomes where possible.

5.5.9 Strengths and limitations

There are several limitations to our analyses that should be noted. First, the cross-sectional household survey limits causal interpretation because vaccination status and malaria positivity were measured at the same timepoint. Second, the outcome was malaria positivity rather than clinical malaria, which may dilute the apparent vaccine effect in a high-transmission setting where asymptomatic parasitaemia is common. Third, the cluster-level analysis had limited precision. The ICC was 0.236, indicating substantial between-cluster variation in malaria risk. With only 46 clusters, this level of clustering reduces power to detect differences between arms, particularly when the endpoint is cross-sectional prevalence rather than a longitudinal clinical outcome(329,330). Finally, our estimates primarily reflect effectiveness of the primary series because fourth-dose coverage in the survey was 33%. The results therefore provide limited evidence to conclude on protection after the booster dose at either an individual or community level.

Our analysis has several strengths. We applied multiple analytic approaches to the same dataset, allowing direct comparison of how design, exposure definition, and confounding control influence RTS,S estimates. The ability to contrast cluster-level, unmatched, and matched analyses within a single population provides a useful illustration of why estimates may differ even when the same endpoint is used. The matched case–control design provided tighter control of age and spatial heterogeneity than regression adjustment alone. Interpretation was also strengthened by comparison with the wider MVIP evaluation and the EPI-MAL-003 prospective cohort findings, which provide broader context for understanding the Malawi endline survey results.

5.6 Recommendations for vaccine policy and future evaluations

5.6.1 Vaccine policy

- Completion of the three-dose RTS,S primary series was associated with greater protection than partial vaccination, supporting efforts to improve schedule completion in routine programmes.
- Cross-sectional mRDT positivity should be interpreted cautiously as an endpoint for malaria vaccine evaluation in high-transmission settings where asymptomatic parasitaemia is common.
- Estimates based on mRDT prevalence should be interpreted alongside evidence from longitudinal evaluations using clinical and severe malaria endpoints.

5.6.2 Future vaccine evaluations

- Future post-licensure malaria vaccine evaluations should prioritise incidence-based endpoints where feasible, ideally through strengthened routine surveillance and linked programme data rather than expensive stand-alone cohort studies.
- Cluster-based evaluations remain informative for programme assessment when clinically relevant outcomes and sufficient follow-up are incorporated.
- Future evaluations should assess how analytic design, exposure definition, vaccine coverage, and endpoint choice influence effect estimates within the same population.
- Observational analyses should account for spatial heterogeneity and the strong age dependence of malaria risk.

5.7 Conclusion

Different analytic approaches applied to the same dataset produced different RTS,S VE estimates because they captured different aspects of vaccine effect under different assumptions. In individual-level analyses, completion of the RTS,S primary series was associated with moderate protection against mRDT-confirmed malaria infection, with higher estimates after matching on age and cluster. The cluster-randomised endline prevalence comparison was not well suited to detecting individual-level protection in this setting, whereas the matched

case–control analysis and the wider MVIP and prospective cohort evidence provided more consistent evidence of moderate VE. These findings show that evaluation of malaria vaccines in routine settings depends not only on the study design, but also on the exposure being compared, vaccine coverage, and whether the endpoint and follow-up are aligned with the biological and clinical effects the vaccine is expected to produce. The findings also support the role of RTS as an additional malaria control tool, particularly because its greatest public health value is likely to be in reducing severe malaria, hospitalisation and deaths when delivered alongside existing interventions.

5.8 Acknowledgements

5.8.1 Funding

This work was supported by the Malawi-Liverpool-Wellcome Programme Core Grant from Wellcome (grant number 206545/Z/17/Z). Latif Ndeketa is supported by a studentship from the MRC Discovery Medicine North (DiMeN) Doctoral Training Partnership (MR/W006944/1). This work was also supported by the UK National Institute for Health and Care Research (NIHR) Global Health Research Group on Gastrointestinal Infections at the University of Liverpool using UK aid from the UK Government to support global health research (NIHR133066). Peter Dodd acknowledges research funding through The UK-South East Asia Vaccine Manufacturing Research Hub. Daniel Hungerford is affiliated with the NIHR Health Protection Research Unit in Gastrointestinal Infections at the University of Liverpool, a partnership with the UK Health Security Agency in collaboration with the University of Warwick. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health, the NIHR, the Department of Health and Social Care, the UK government or the UK Health Security Agency.

5.8.2 Conflicts of interest

D.H. and N.F are currently receiving grant support from Seqirus UK Ltd for the evaluation of influenza vaccines and grant support and personal consultancy fees from Merck & Co (Kenilworth, New Jersey, USA) for rotavirus strain surveillance; D.H. received honoraria for a presentation at a Merck Sharp & Dohme (UK) Limited symposium on vaccines; and D.H. has received research grant support from Sanofi Pasteur, and Merck & Co for rotavirus strain surveillance. All other authors declare no competing interests.

5.8.3 Contributions

L.N., D.H., N.F, and P.D conceived of the study and secured the funding. L.N. performed the analysis, wrote the manuscript. N.F, D.H, P.D, DM, HM assisted with the analysis and

interpretation of results and edited the manuscript. DM, HM oversaw the data collection, assisted with the interpretation of results and edited the manuscript. All authors assisted with the interpretation of results and edited the manuscript.

6 Discussion

6.1 Reframing vaccine impact in routine settings

Measuring vaccine effectiveness and impact is essential for evaluating new vaccine introductions and monitoring long-term effects. In practice, attributing changes in disease burden to vaccination in settings where multiple public health and social measures (PHSMs) are implemented remains difficult. Most current approaches compare observed post-introduction trends with an expected disease burden in the absence of vaccination(159,160). This comparison depends on whether other determinants of transmission and disease burden remain stable or are adequately captured(159). In this thesis, I set out to examine how vaccine-attributable effects can be more accurately estimated under routine vaccine programme conditions and how these effects can be distinguished from concurrent PHSMs that also influence disease burden. Across the systematic review and empirical chapters, the same issue recurred: the estimated VE changed when design, endpoint or concurrent interventions changed.

In the cholera analysis, inclusion of WASH exposure increased the observed vaccine effect from 53.5% to 62.1%, while increasing the lag period for both vaccine and WASH effects reduced the effect estimate. These shifts occurred without any change in vaccine coverage, biological efficacy, or WASH delivery but they arise from how the model represents the timing and interaction of the interventions. The effect estimate is therefore driven by the structure of the analytical model as much as by the underlying biological effect of the vaccine in the general population.

Pre-licensure clinical trials establish biological efficacy under controlled conditions(79,122,331). Those estimates are often treated as reference points against which post-licensure vaccine performance is judged. The analyses presented in this thesis indicate that this comparison is not straightforward because once vaccines are licensed and introduced in routine immunisation programmes, they work in an environment where disease exposure, susceptibility, and case detection are continuously modified by other interventions and unmeasured stochastic

variation. The observed effect therefore represents a combination of processes rather than a single causal pathway. The central problem is not whether vaccines work, but how much of the observed change in disease burden or transmission can be attributed to vaccination in the presence of other PHSMs.

6.2 Study design as a determinant of observed effect

6.2.1 Divergence across analytical approaches

The main finding for the systematic review was that none of the 64 vaccine post-licensure evaluation studies reported or adjusted for concurrent PHSMs. The systematic review showed that case–control (45.3%) and ecological (37.5%) designs were the most frequently used approaches for post-licensure vaccine evaluation. This distribution is probably driven more by feasibility than by preference for the strongest design(131). That practicality comes at a cost because each design relies on assumptions that are difficult to sustain and are often violated in settings where multiple interventions are implemented concurrently. Case–control studies are sensitive to differences in healthcare-seeking behaviour and incomplete measurement of exposure(332,333). Ecological analyses face a more fundamental constraint. They cannot separate the effect of vaccination from changes in other interventions operating over the same period, making them particularly vulnerable to confounding in outbreak and high-burden settings(334).

The systematic review identified consistent differences in reported VE across study designs. Case–control studies produced higher estimates of direct protection compared to overall impact reported from ecological and time-series analyses. This pattern aligns with the distinction between direct, indirect, and overall effects described by Halloran *et al.*, though the size of the differences suggests that the assumptions built into each design matter more than is usually stated(129,131).

In my rotavirus analyses, the case–control estimate of VE against severe disease was 52% (95% CI: 18–71%). This is broadly consistent with estimates from case–control studies conducted in high-burden, low-income settings identified in the systematic review, which ranged from 52–78% against severe or hospitalised laboratory-confirmed rotavirus(90). In contrast, the

interrupted time-series analysis produced a lower impact estimate of 23%, compared with the 35–81% range observed in the systematic review(90). These estimates are not directly comparable, as they represent different quantities and endpoints. The case–control estimate reflects individual-level protection among those who seek medical care, while the time-series analysis captures population-level changes in incidence that incorporate both direct and indirect effects alongside secular trends. The lower population-level estimate observed here is therefore unlikely to mean there is reduced vaccine performance alone. It is more consistent with concurrent changes in healthcare utilisation, climate, case management, and surveillance sensitivity over time, which influence observed incidence independently of vaccination. For instance, under-five mortality in Malawi declined from 234 deaths per 1,000 live births in 1992 to 48 deaths per 1,000 in 2024, reflecting broad improvements in child survival that extend beyond vaccination alone(335).

6.2.2 What is being estimated

The assumption that vaccine effectiveness or impact is a single quantity is not supported by the analyses in this thesis. Case–control studies estimate direct protection among individuals who interact with the healthcare system. Ecological analyses estimate population-level changes that combine direct and indirect effects with secular trends. Time-series models attempt to reconstruct a counterfactual trajectory, which depends on the stability of pre-intervention patterns and the completeness of the data that is collected in the pre-intervention period. In the presence of concurrent PHSMs, these estimates diverge further.

6.2.2.1 Individual-level estimates and unmeasured exposure differences

A case–control vaccine evaluation that does not account for WASH interventions assumes that WASH is either evenly distributed across the study population or unrelated to vaccination status. In practice, this assumption is unlikely to hold. Systematic reviews in Sub-Saharan Africa (SSA) indicate that higher maternal education (secondary or higher) and higher socioeconomic status (SES) are strongly associated with increased completion of childhood vaccinations. Children of mothers who attained secondary school education or higher are associated with a 2.7 times higher likelihood of completing childhood vaccination compared to having no

education(336). Higher SES status was similarly associated with greater completion of childhood vaccination, with 2.45 times higher odds. These social determinants are linked through several pathways including lower vaccine hesitancy, greater access to information, and better access to health infrastructure(336). A scoping review of WASH practices in Southern Africa identified rurality, low investment in WASH infrastructure, inadequate knowledge of water-borne diseases and weak community engagement as barriers to improved WASH practices. Facilitators to WASH were improved WASH infrastructure, latrine ownership, community engagement and social capital were identified as facilitators(337). WASH interventions are therefore unlikely to be evenly distributed or taken up consistently between vaccinated and unvaccinated children

When WASH and related determinants are not measured, the estimated association between vaccination and disease is driven by both vaccine-induced protection and these underlying differences in exposure risk. This can lead to overestimation of vaccine effectiveness if vaccinated individuals are also less exposed to infection. In other contexts, where vaccination campaigns are targeted towards high-risk populations with poorer WASH conditions, the bias may operate in the opposite direction where the effect estimate is attenuated(26,338). The direction and magnitude of bias therefore depend on how vaccination status co-varies with these often unmeasured factors.

The cholera analysis illustrates this mechanism. Including WASH altered the estimated vaccine effect, showing that WASH could not be treated as independent of vaccination or transmission risk. Although this was modelled directly in the transmission analysis, the same structural problem applies to case–control designs when WASH and related determinants are unmeasured.

6.2.2.2 Population-level estimates and the counterfactual

The same problem appears differently in ecological and ITS analyses, where the challenge is not differences between individuals, but changes within the same population over time. Ecological and ITS designs both estimate change in disease rates within the same population over time. They differ mainly in how often outcomes are measured. Ecological designs in their simplest form compare two aggregate time points, before and after an intervention, whereas interrupted

time-series analyses use repeated observations at regular intervals (usually weekly or monthly) before and after vaccine introduction(158,334). This structure allows ITS models to estimate both level and slope changes while accounting for autocorrelation, seasonality, and underlying secular trends(160).

6.2.2.3 Time-varying confounding in long evaluations

The use of a single population offers an advantage over between-group designs such as case–control studies because comparisons are made within the same population, which reduces selection bias and bias from between-group differences. The pre-intervention period serves as a counterfactual which estimates what would have been observed if the vaccine was not introduced. ITS designs therefore use the same population as its own control.

The limitation is that these models cannot account for time-varying confounders that do not follow the underlying trend(159). Their influence becomes more pronounced when interrupted time-series analyses span long periods, as in the rotavirus vaccine impact in this thesis which was from 1997 to 2019. Across those 22 years, Malawi experienced substantial social and health-system change. These changes are not incidental to vaccine evaluation because they may influence both vaccine uptake and disease transmission(335,339). Maternal education improved markedly, with the proportion of women attaining at least secondary education increasing from 11% in the 1992 Malawi Demographic and Health Survey (DHS) to 29% in the 2024 DHS. Child healthcare utilisation also strengthened. Although the reported two-week prevalence of diarrhoea among children under 5 remained at 22% in both surveys, care-seeking for diarrhoeal illness increased from 45% to 66%, while use of oral rehydration therapy rose from 62% to 78%. WASH indicators show similarly large shifts. The proportion of households relying on unsafe water sources declined from 53% in 1992 to 13% in 2024, and households without hygienic toilet or latrine facilities fell from 27.7% to 5.4%. Therefore, the assumption that these factors remained stable, or were sufficiently captured by the modelled secular trend, is unlikely to hold. Improvements in maternal education, healthcare utilisation and WASH should therefore be treated as part of the interpretive context for long-run vaccine impact estimates, since they may independently reduce enteric disease transmission, alter care-seeking patterns and improve child survival.

6.2.2.4 Controlled ITS and the choice of control

One way to address this limitation is to include a control series within the ITS framework. A controlled interrupted time-series (CITS) extends the basic design by introducing an additional comparison that is exposed to the same underlying temporal processes but not to the intervention or vaccine of interest(159). This allows the counterfactual to be informed not only by the pre-intervention trend but also by contemporaneous changes observed in the control. When a change is observed in the intervention series but not in the control, this strengthens the interpretation that the effect is attributable to the vaccination programme. When similar changes are observed in both series, the more plausible explanation is a co-intervention or external event affecting both outcomes(159). The value of the control series lies in its ability to account for time-varying confounding that cannot be modelled directly. Without this, reductions in disease incidence attributed to vaccination may instead reflect broader system-level changes.

This issue was directly relevant to the rotavirus impact analysis in this thesis. The interrupted time-series model did not rely only on the pre-vaccine trend in rotavirus-positive hospitalisations. Rotavirus test-negative acute gastroenteritis admissions were included as a control series to account for background changes in diarrhoeal admissions that were not expected to be prevented by Rotarix®. This was intended to capture changes in surveillance intensity, healthcare-seeking behaviour, hospital admission patterns, and broader changes in enteric disease risk over time. In this sense, the test-negative series strengthened the counterfactual because it helped distinguish changes specific to rotavirus from changes affecting diarrhoeal admissions more generally.

This control was not perfect. Test-negative diarrhoeal admissions may also be affected by WASH, changes in case management, healthcare access, and diagnostic practice. If those processes changed differently for rotavirus-positive and rotavirus-negative admissions, residual confounding would remain. The use of test-negative AGE therefore improved the ITS analysis by accounting for shared changes in admission and surveillance patterns, though it could not fully separate vaccine impact from all time-varying determinants of diarrhoeal disease.

Choosing a control is rarely straightforward. A valid control must be exposed to the same confounding influences as the intervention series while remaining unaffected by the intervention itself. This requires consideration of the mechanisms through which both the intervention and potential confounders operate. For example, in a rotavirus vaccine impact analysis, one possible control would be hospitalisations due to non-rotavirus diarrhoeal illness such as Shigella. These two conditions are likely to be influenced by the same changes in healthcare access, case management, and WASH interventions that affect rotavirus transmission, while not being directly prevented by the rotavirus vaccine(340,341). In this scenario, Shigella-associated diarrhoeal hospitalisations could serve as a plausible control outcome for broader system-level changes. If rotavirus hospitalisations decline but Shigella-associated diarrhoeal hospitalisations do not, then this would support a vaccine-specific effect. If both decline in parallel, the more plausible explanation is a broader change in the system, such as improved sanitation or healthcare-seeking behaviour.

The difficulty lies in ensuring that the control is truly unaffected by the intervention. In this example, misclassification of diarrhoeal aetiology, changes in diagnostic practices, or indirect effects of vaccination on transmission dynamics could weaken the validity of the control. Similar challenges arise when using alternative outcomes such as geographic controls or untreated populations. Each option introduces its own assumptions. The use of a control series therefore does not remove confounding. It makes the assumptions more explicit and allows them to be examined. The strength of CITS design depends as much on the plausibility of the chosen control as on the statistical model itself. Within this framework, different estimates are expected because each design handles the counterfactual and time-varying processes differently. ITS analyses that do not incorporate an appropriate control remain vulnerable to confounding from concurrent interventions. Controlled designs reduce this risk, though they remain dependent on the validity of the chosen control. The resulting estimates should therefore be interpreted in relation to the assumptions embedded within each design, rather than as directly comparable measures of effect.

6.2.2.5 Surveillance intensity and case ascertainment

A further consideration in interrupted time-series analyses is the comparability of the pre- and post-intervention data. The validity of the counterfactual relies on the assumption that case ascertainment remains consistent over time. In practice, this is often not the case in vaccine evaluations. The introduction of a new vaccine can be accompanied by increased attention to surveillance, including improved diagnostics, more systematic case identification and greater reporting intensity(342). This can produce an apparent increase in the number of detected cases immediately following vaccine introduction, not because true incidence has risen, but because case detection has improved. This pattern was observed in the rotavirus analysis in this thesis, where a surge in reported cases in January 2013 followed vaccine introduction and coincided with enhanced surveillance activity(343).

When pre-intervention data are derived from routine systems and post-intervention data from more intensive surveillance, the resulting time series combines data of differing sensitivity. This introduces bias in the estimation of vaccine impact. Changes in observed incidence reflect both underlying transmission and shifts in detection. The assumption that the pre-intervention trend represents a valid counterfactual is therefore weakened. This issue parallels the requirement in case–control studies that cases and controls arise from the same underlying detection process. Just as differential healthcare-seeking or diagnostic testing can bias case–control estimates, changes in surveillance intensity over time can bias ITS analyses. In both designs, comparability in how outcomes are identified is central to valid inference.

6.3 Analysis approach as a determinant of observed effect

In this section, I will discuss how model specifications, intervention timing and attribution can drive the VE estimate using the cholera and RTS,S analysis as case studies.

6.3.1 Evidence from cholera analyses

The cholera analyses show how easily attribution changes when the model specification changes. The oral Cholera vaccine (OCV) campaign and WASH interventions in Blantyre were implemented around the same month of December 2022. This makes it difficult to separate their individual effects without making assumptions about how they operate. In models that

included vaccination alone, most of the reduction in cholera transmission was attributed to the vaccine, giving a VE of 53.5%. When WASH was added into the model at the same time point, the VE increased to 62.1%. This change does not reflect a difference in how the vaccine induces an immune response, but rather how the model allocates the observed reduction in cases between vaccination and WASH. Treating WASH as having an immediate effect shifts part of that reduction away from WASH and back towards the vaccine, even though this is unlikely to reflect how these interventions act in practice.

When the lag period was increased from 7 to 14 days for both vaccination and WASH, the VE decreased to 35.9%. This specification reallocates early reductions in incidence to delayed WASH effects rather than vaccination. When WASH was modelled as a cumulative rather than short-term exposure, the estimated VE increased to 83%. This shows how the model spreads the effect of WASH over time, rather than attributing it to short-term changes, which in turn shifts more of the observed reduction towards the vaccine. These changes are not driven by differences in the underlying data, but by how the model assigns the observed reduction in cases across the variables included. Each specification makes a different assumption about how the interventions act over time, and there is no direct way to confirm which is most appropriate.

In this context, the choice of specification should be guided by robust evidence and recording of how the interventions are delivered and sustained. WASH is not a single and continuous exposure but a combination of activities which include water buckets for storage, chlorine-based agents for water treatment, health education and measurement of faecal contamination at water sources. Some of these act over short timeframes, while others depend on behaviour change and are difficult to maintain. Adherence is difficult for health authorities to measure because it requires tracking repeated daily behaviours such as handwashing and use of chlorinated water(344). There is, therefore, little evidence to support the assumption that the WASH effects accumulate consistently over time. Modelling WASH as a cumulative exposure is therefore unlikely to reflect how these interventions operate in practice. It risks overstating longer-term effects and shifting too much of the observed reduction away from WASH and towards vaccination. A specification that reflects shorter-term or lagged effects is more

consistent with the way these interventions are delivered and sustained, even if no single approach can fully capture their complexity.

6.3.2 Parallel evidence from RTS,S

The RTS,S/AS01 malaria vaccine analyses make the same point through design rather than exposure specification. Across this chapter, the same underlying population produces markedly different estimates depending on whether the analysis is conducted at the cluster level, through an unmatched case–control design, or after matching on key sources of heterogeneity.

6.3.2.1 Cluster-level comparison and endpoint choice

At the cluster level, there was little evidence of an effect on malaria prevalence. The estimated difference between intervention and control clusters was small, corresponding to a VE of around 8% with very wide confidence intervals (PR 0.92 [95% CI 0.55–1.52]) that included no effect. This could easily be misinterpreted as limited vaccine impact. That interpretation weakens once the endpoint and structure of the analysis are considered. The comparison is based on cross-sectional mRDT positivity at a single timepoint and nearly half (48.6%) of the infections were asymptomatic. The outcome therefore captures infections that are not the primary target of vaccination, which is directed towards clinical and severe disease. At the same time, malaria risk was strongly clustered with an ICC of 0.236. With only 46 clusters available, this level of between-cluster variation reduces precision and makes it difficult to detect differences even where they exist.

6.3.2.2 Matching and individual-level estimates

The individual-level analyses gave a different estimate. In the unmatched case–control analysis, completion of the primary series was associated with a VE of 41%. When the analysis was repeated after matching on cluster and age band and incorporating age and SES into the matching distance, the estimated VE increased to 58%. This shift is not only about improved precision but it also shows that matching changed the comparison being made. Vaccinated and unvaccinated children differed systematically in age and underlying risk before matching. Age imbalance alone was substantial, with a standardised mean difference of 0.39 which was reduced to 0.03 after matching. This imbalance is sufficient to bias the unmatched estimate

given the strong age dependence of malaria risk and the spatial variation in transmission across clusters. Matching does not remove confounding, though it brings the comparison closer to the underlying risk structure of the population. The higher estimate is therefore consistent with improved control of confounding rather than a change in vaccine performance.

6.3.2.3 Placing the estimates within wider MVIP evidence

These patterns are consistent with the wider MVIP evidence. The pooled MVIP evaluation reported a 32% reduction in hospital admission with severe malaria and a 9% reduction in all-cause mortality, while the EPI-MAL-003 cohort reported 58% effectiveness against severe malaria and smaller effects against broader endpoints such as any malaria (30%) and all-cause hospitalisation (21%) in 45,000 children with 5 years follow-up across 12 sites(123). The difference comes down to the endpoint. Longitudinal designs capture incident clinical outcomes over time. Cross-sectional prevalence captures a mixture of symptomatic and asymptomatic infection at a single point, diluting the observed vaccine effect. The estimates derived from post-licensure vaccine evaluations depend on the study design used and the robustness of the endpoint for the vaccine programme aims. Cluster-randomised designs can, in principle, account for spatial heterogeneity and shared exposures by comparing groups rather than individuals. As shown in the RTS,S evaluation chapter, their ability to detect an effect depends on the endpoint used, the number of clusters available, and how variation is distributed across them.

6.3.2.4 What the RTS,S comparison shows

Individual-level approaches estimate protection within the same population, though they rely on comparability between exposed and unexposed groups. Matching and adjustment change that comparison. They do not remove confounding, though they alter how it is expressed in the estimate. By restricting comparisons within matched sets, the control of confounding is no longer achieved at the design stage alone but depends on how those sets are handled analytically. As described by Pearce, matching does not remove confounding but shifts its control to the analysis(327). The resulting estimate is therefore conditional on both the matching variables and the analytical approach used.

In this context, the increase from 41% to 58% is likely due to the matched analysis comparing children who were more similar in age and cluster-level malaria risk. It does not mean the vaccine performed differently, but that the comparison between vaccinated and unvaccinated children was less confounded. The cluster-level estimate of 8%, the unmatched estimate of 41%, and the matched estimate of 58% are not competing answers. They come from the same data but reflect different assumptions about the outcome, the comparison group, and the structure of risk. The variation between them is not inconsistency in vaccine performance. It points to how each design represents the system in which the vaccine is deployed.

6.4 Recommendations from the thesis

The findings of this thesis suggest that vaccine impact estimates should not be interpreted as fixed quantities. For policymakers, this has practical implications for how evidence is used to inform decision making.

6.4.1 Interpret estimates in relation to design and outcome

Estimates derived from different study designs should not be treated as directly comparable. A cluster-level reduction in cross-sectional prevalence, an individual-level odds ratio from a case–control analysis, and an incidence-based estimate from longitudinal follow-up are not measuring the same quantity. Differences between them do not necessarily indicate inconsistency in vaccine performance. The outcome being measured should therefore guide how much weight an estimate carries. For malaria, clinical or severe disease endpoints are more aligned with programme goals than cross-sectional parasite prevalence. For enteric pathogens, hospitalisation or severe disease endpoints are more informative than all-cause diarrhoeal incidence.

6.4.2 Prioritise clinically relevant and incidence-based endpoints

Across the RTS,S analyses, longitudinal follow-up and incident clinical outcomes were more aligned with the expected pattern of vaccine protection than cross-sectional parasite prevalence, which was diluted by asymptomatic infection. For cholera, incidence-based modelling was more useful than relying on crude before-and-after changes because it allowed the timing of OCV and WASH response activities to be linked to changes in transmission over

time. Cross-sectional comparisons, particularly those based on single timepoints are sensitive to underlying heterogeneity and less able to detect meaningful effects. Where feasible, programme evaluations should prioritise designs that capture incident outcomes over time such as cohort designs. These approaches provide a closer representation of how vaccines affect disease burden in real-world settings.

6.4.3 Strengthen routine data quality for attribution

My thesis has shown that vaccine attribution depends not only on the statistical model but also on whether routine programme data capture the right information at the right time. This was clear in my cholera analysis. Incidence-based modelling was only possible because surveillance, vaccination, and WASH activity data were available, though the limitations in these data also show what needs to improve in future outbreaks. Case line lists should be completed from the point at which cases are first detected and should include date of symptom onset, diagnostic status, health-facility catchment, outcome, and vaccination status recorded clearly as vaccinated, unvaccinated, or unknown. In the Blantyre analysis, only 34 reported cases had documented OCV receipt, while vaccination status was missing or unclear for most cases, making it difficult to distinguish true absence of vaccination from absence of documentation.

WASH data also needs to capture the actual interventions that were administered and not only the number of households reached. In the 2022-2023 outbreak, WASH included household and latrine disinfection, safe-water storage buckets, chlorine-based water treatment agents, health education, and water-quality testing, but the dataset did not show whether each household received the full package or only some components. Future response data should therefore preserve time, place, vaccination status, and the specific WASH activities delivered. These data should be checked at cholera admission facilities and district surveillance level before transfer to the national public health institute, because errors introduced at the point of care are difficult to correct later.

6.4.4 Account explicitly for concurrent interventions

In settings where multiple interventions are implemented together, changes in disease burden cannot be attributed to vaccination alone. The cholera analyses in this thesis showed that

including WASH changed the estimated vaccine effect, with different model specifications leading to different conclusions. Concurrent PHSMs such as WASH for diarrhoeal diseases, vector control for malaria, and changes in case management need to be considered alongside vaccination. Where these cannot be measured directly, analyses should examine how different assumptions affect the estimated effect. This is important because estimates may either overestimate or underestimate the attributable effect to vaccination if concurrent PHSMs are not adjusted for.

6.4.5 Use design-based approaches carefully

Approaches such as matching and cluster-based comparisons can improve how comparable groups are, and in doing so reduce some forms of confounding. In the RTS,S analyses, matching on age and cluster changed the estimated effect, bringing vaccinated and unvaccinated children closer in terms of underlying risk. This does not remove confounding. The estimate still depends on which variables are used and how the comparison is constructed. In addition, designs such as cluster-randomised evaluations are not always feasible once a vaccine is widely implemented. Estimates from observational analyses therefore need to be interpreted with these constraints in mind, rather than taken as directly equivalent to trial-based evidence.

6.4.6 Ensure consistency in surveillance before and after introduction

The interpretation of time-series and programme evaluations depends on how comparable the data are over time. Changes in surveillance intensity, diagnostic methods, or reporting can create apparent shifts in incidence that do not reflect underlying transmission. This was evident in the rotavirus analysis, where increased surveillance following vaccine introduction coincided with a rise in detected cases. Maintaining consistent case definitions and surveillance systems before and after introduction is therefore important. Where this is not possible, changes in surveillance need to be accounted for in the analysis, as they can distort estimates of impact and make interpretation more difficult.

6.4.7 Communicate differences in estimates clearly

Differences between vaccine effect estimates need to be communicated clearly to policy makers, ministries of health, programme managers and the general public. A single headline VE

estimate can easily be misread as the effect of the vaccine, when in practice it may represent direct VE, total VE, population impact, or vaccine-attributable effect after adjustment for concurrent interventions. These are not interchangeable estimates. This matters because misinterpretation can affect decision making, particularly for vaccines that provide partial protection. A vaccine such as RTS,S is not expected to replace bed nets, prompt diagnosis and treatment, IPTp, seasonal or perennial chemoprevention, or other malaria control measures. Its value lies in the additional protection it contributes within that package. For these vaccines, the key policy question is not whether the estimate appears high or low in isolation, but how much of the observed reduction in disease can be attributed to vaccination after accounting for the other interventions operating alongside it.

Overstating an estimate may create unrealistic expectations about what vaccination alone can achieve; while understating it may weaken confidence in a vaccine that is providing clinically meaningful protection. Programme evaluation reports should therefore state what each estimate represents, what assumptions it depends on, and how other interventions such as WASH, vector control, treatment access, or surveillance changes may have influenced it.

6.4.8 Avoid reliance on a single estimate

The variation observed across analyses in this thesis, with estimates ranging from minimal effect to substantial protection within the same population, shows the limits of relying on a single measure of vaccine impact. Different designs, endpoints, and data sources answer different questions. Estimates should therefore be interpreted across different approaches rather than in isolation. Confidence in interpretation increases when estimates agree. When they differ, the assumptions built into the analysis should be examined rather than treating one estimate as automatically correct.

6.5 Future directions

I will take this work forward through a one-year Postdoctoral Research Associate Bridging Fellowship that has been awarded to me by the Malawi Liverpool Wellcome Research Programme (MLW) Training and Development Committee. The fellowship builds directly from the analyses in this thesis to develop a framework for interpreting vaccine-attributable impact

under concurrent PHSMs. I will compare analytical approaches on the datasets in my possession and examine how their assumptions, timing structures, and data limitations shape the resulting estimates. The project will deliver a publishable output within 12 months and provide the foundation for my subsequent Wellcome Early-Career Award application. The work will use the three longitudinal datasets that I have developed during this PhD: rotavirus surveillance data from Queen Elizabeth Central Hospital(1997 – 2019), cholera surveillance and oral cholera vaccine coverage data from the 2022–2023 Blantyre outbreak with linked WASH activity, and malaria vaccine implementation data from the MVIP, supplemented by malaria indicator survey data on vector control and malaria prevention practices. These datasets allow the same question to be examined across different pathogens: how much of the observed reduction in disease can be attributed to vaccination when other interventions are operating at the same time.

My main goal in the fellowship will be to adapt the transmission-based approach developed in the cholera analysis to malaria vaccine evaluation. In the cholera chapter, changes in the time-varying reproduction number were modelled in relation to OCV coverage while incorporating WASH intervention activity. The fellowship will apply the same logic to malaria by modelling changes in RDT-confirmed malaria incidence in relation to RTS,S coverage and time-varying malaria control measures. These will include bed-net ownership or use, access to diagnosis through RDT testing, antimalarial treatment, and healthcare-seeking behaviour where these data are available. This is necessary because RDT-confirmed malaria incidence is shaped by transmission, care-seeking, testing access, and diagnostic practice. Treating it as a direct VE measure without accounting for these processes risks attributing changes in malaria burden to vaccination alone.

The cholera analysis is the clearest methodological starting point for this future work because it did something that is rarely possible during outbreaks by estimating vaccine-attributable impact using routine response data while accounting for a concurrent intervention. Its novelty lies in linking changes in the R_t to vaccine coverage and WASH activity, rather than relying on individual vaccination histories that were largely unavailable. This approach estimated a vaccine-associated reduction in transmission of 53.5% before WASH adjustment and 62.1% after

adjustment, despite vaccination status being recorded for fewer than 1% of reported cases. The value of this approach extends beyond cholera but the model cannot simply be transferred without modifying it because different pathogens and interventions act through different pathways. WASH may affect cholera transmission through water safety and household hygiene over short timeframes, while malaria vaccination operates alongside bed nets, RDT-based diagnosis, ACT access, IPTp, and seasonal malaria chemoprevention. The future work will therefore adapt the same principle rather than the same model: using routinely collected intervention and surveillance data to estimate the vaccine-attributable component of observed changes in disease burden.

This extension is motivated by the published interim VE results for the RTS,S from the phase 4 study(123). The study showed 30% VE for any malaria, 58% for severe malaria and 36% for malaria-related hospitalisations among vaccinated children from exposed clusters compared with unvaccinated children from unexposed clusters over one year after the third RTS,S dose. The primary comparison estimated the total VE rather than the vaccine-attributable component alone, because the analysis did not explicitly separate vaccination from differences in malaria transmission intensity, LLIN use, ACT access, or health-seeking behaviour. Establishing the vaccine-attributable effect is very important for this moderately efficacious vaccine. This provides the next methodological step for the fellowship which will be to examine how RTS,S VE estimates change when concurrent malaria interventions are incorporated into the model.

The work will also address a current policy question within the Malawi Immunization Technical Advisory Group (MITAG): whether programme impact would be greater by improving fourth-dose coverage or by introducing a fifth RTS,S dose. These are different questions. Improving fourth-dose coverage, currently at 47%, addresses incomplete delivery of the existing schedule, while introducing a fifth dose addresses waning protection after completion of the current schedule. I will use MVIP data to compare these options through policy simulation, estimating the marginal gains expected under each strategy. This fits within the 12-month fellowship plan, which includes data harmonisation, comparative evaluation of ITS, test-negative and transmission-based approaches. The outputs from this fellowship will include stakeholder consultation with various programmes within the Ministry of Health including the Expanded

Programme on Immunisation and Public Health Institute of Malawi, a peer-reviewed methods manuscript, and a policy technical brief.

The broader purpose of this work is to move beyond pathogen-specific analyses towards a general framework for interpreting vaccine impact in routine settings. By applying related approaches across rotavirus, cholera, and malaria, I will examine where methods converge, where they diverge, and which assumptions drive those differences. This work will extend the analyses in this thesis into a programme of research focused on estimating vaccine-attributable impact in settings where vaccination is delivered alongside other public health interventions.

6.6 Conclusion

This thesis examined how vaccine impact is interpreted in post-licensure settings where multiple interventions operate at the same time. Across the analyses presented here, the differences in estimated effect are driven less by the vaccine itself and more by how the analysis is structured, what outcome is used, and how PHSMs and other interventions are handled. These estimates are not measuring the same thing. Individual-level approaches rely on how comparable exposed and unexposed groups are. Population-level approaches rely on how stable underlying trends are and how well changes over time are captured. When these conditions do not hold, the estimates shift. Vaccine impact cannot therefore be reduced to a single value. What is estimated depends on the design, the endpoint's relation to vaccine programme aim, the quality of data, and the context in which the analysis is conducted. In routine programme settings, where interventions are introduced together and data are incomplete, no single approach captures the full effect. Interpreting these results requires looking across approaches and understanding why they differ. This thesis links those differences to the assumptions built into each design and to the structure of the data available. The aim is not to identify one estimate, but to clarify what each estimate represents and how it should be interpreted in practice. This understanding and evidence is critical for efficient public health decision making under increasingly restricted national and international finances.

References

1. Sharrow D, Hug L, Liu Y, Lindt N, Fell GW, Nie W, et al. Child Mortality Levels and Trends report. 2024. 104 p.

2. Alkema L, Chao F, You D, Pedersen J, Sawyer CC. National, regional, and global sex ratios of infant, child, and under-5 mortality and identification of countries with outlying ratios: A systematic assessment. Lancet Glob Heal [Internet]. 2014 [cited 2026 Feb 16];2(9):e521–30. Available from: https://data.unicef.org/topic/child-survival/under-five-mortality/

3. Liu L, Oza S, Hogan D, Chu Y, Perin J, Zhu J, et al. Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet [Internet]. 2016 Dec 17 [cited 2026 Feb 16];388(10063):3027–35. Available from: https://www.thelancet.com/action/showFullText?pii=S0140673616315938

4. Perin J, Mulick A, Yeung D, Villavicencio F, Lopez G, Strong KL, et al. Global, regional, and national causes of under-5 mortality in 2000–19: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet Child Adolesc Heal [Internet]. 2022 Feb 1 [cited 2026 Feb 16];6(2):106–15. Available from: https://www.thelancet.com/action/showFullText?pii=S2352464221003114

5. Avelino IC, Van-Dúnem J, Varandas L. Under-five mortality and social determinants in africa: a systematic review. Eur J Pediatr [Internet]. 2025 Feb 1 [cited 2026 Feb 16];184(2):150. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11759474/

6. Asare EO, Al-Mamun MA, Armah GE, Lopman BA, Parashar UD, Binka F, et al. Modeling of rotavirus transmission dynamics and impact of vaccination in Ghana. Vaccine. 2020 Jun;38(31):4820–8.

7. Pitzer VE, Ndeketa L, Asare EO, Hungerford D, Lopman BA, Jere KC, et al. Impact of rotavirus vaccination in Malawi from 2012 to 2022 compared to model predictions. npj Vaccines [Internet]. 2024 Dec 1 [cited 2025 Oct 13];9(1):1–8. Available from: https://www.nature.com/articles/s41541-024-01008-6

8. Fung ICH. Cholera transmission dynamic models for public health practitioners. Emerg Themes Epidemiol. 2014;11(1):1–11.

9. Qu Z, Patterson D, Zhao L, Ponce J, Edholm CJ, Prosper Feldman OF, et al. Mathematical modeling of malaria vaccination with seasonality and immune feedback. PLoS Comput Biol [Internet]. 2025;21(5 May):1–32. Available from: https://doi.org/10.1371/journal.pcbi.1012988

10. Afolabi AS, Miswanto M. Modeling the Impacts of Vaccination and other Interventions on Malaria Transmission Dynamics. Stat Optim Inf Comput. 2025;14(6):3667–705.

11. Plotkin SL, Plotkin SA. A short history of vaccination [Internet]. Sixth Edit. Vaccines: Sixth Edition. Elsevier Inc.; 2012. 1–13 p. Available from: http://dx.doi.org/10.1016/B978-1-4557-0090-5.00017-3

12. World Health Organizatio W. Immunization Agenda 2030. 2021;1–24. Available from: https://www.who.int/immunization/ia2030_Draft_One_English.pdf?ua=1

13. Andre FE, Booy R, Bock HL, Clemens J, Datta SK, John TJ, et al. Vaccination greatly reduces disease, disability, death and inequity worldwide. Bull World Health Organ. 2008;86(2):140–6.

14. Fenner F. Global Eradication of Smallpox. Rev Infect Dis [Internet]. 1982 Sep 1 [cited 2026 Mar 22];4(5):916–30. Available from: https://dx.doi.org/10.1093/clinids/4.5.916

15. Breman JG, Arita I. The Confirmation and Maintenance of Smallpox Eradication. N Engl J Med [Internet]. 1980 Nov 27 [cited 2026 Mar 26];303(22):1263–73. Available from: /doi/pdf/10.1056/NEJM198011273032204?download=true

16. Zaffran M, McGovern M, Hossaini R, Martin R, Wenger J. The polio endgame: securing a world free of all polioviruses. Lancet [Internet]. 2018 Jan 6 [cited 2026 Mar 26];391(10115):11–3. Available from: https://www.thelancet.com/action/showFullText?pii=S014067361732442X

17. Antoni S, Ferrari MJ, Nedelec Y, Steulet C, Efe-aluta O, Benassi V, et al. Weekly epidemiological record Relevé épidémiologique hebdomadaire. 2025;(48):591–604.

18. Pollard AJ, Bijker EM. A guide to vaccinology: from basic principles to new developments. Nat Rev Immunol 2020 212 [Internet]. 2020 Dec 22 [cited 2026 Mar 22];21(2):83–100. Available from: https://www.nature.com/articles/s41577-020-00479-7

19. Siegrist CA. Vaccine immunology [Internet]. Sixth Edit. Vaccines: Sixth Edition. Elsevier Inc.; 2012. 14–32 p. Available from: http://dx.doi.org/10.1016/B978-1-4557-0090-5.00004-5

20. Pulendran B, Ahmed R. Immunological mechanisms of vaccination. Nat Immunol 2011 126

[Internet]. 2011 May 18 [cited 2026 Mar 22];12(6):509–17. Available from: https://www.nature.com/articles/ni.2039

21. Sallusto F, Lanzavecchia A, Araki K, Ahmed R. From Vaccines to Memory and Back. Immunity [Internet]. 2010 Oct 29 [cited 2026 Mar 26];33(4):451–63. Available from: https://www.cell.com/action/showFullText?pii=S1074761310003687

22. Djomo PN, Thomas SL, Fine PEM. Correlates of vaccine-induced protection: methods and implications. World Heal Organ [Internet]. 2013;65. Available from: http://apps.who.int/iris/bitstream/10665/84288/1/WHO_IVB_13.01_eng.pdf?ua=1

23. Plotkin SA. Correlates of protection induced by vaccination. Clin Vaccine Immunol [Internet]. 2010 [cited 2026 Mar 27];17(7):1055–65. Available from: /doi/pdf/10.1128/cvi.00131-10?download=true

24. Prunas O, Asare EO, Sajewski E, Li Y, Pithawala Z, Weinberger DM, et al. Global estimates of rotavirus vaccine efficacy and effectiveness: a rapid review and meta-regression analysis. eClinicalMedicine [Internet]. 2025 Mar 1 [cited 2026 Feb 2];81:103122. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11925534/

25. World Health Organizatio W. Measles vaccines: WHO position paper – April 2017. Wkly Epidemiol Rec. 2017;92(17):205–27.

26. World Health Organization. Cholera vaccines: WHO position paper – August 2017. Relev Epidemiol Hebd. 2017;92(34):477–98.

27. Edwards KM, Decker MD. Pertussis vaccines [Internet]. Sixth Edit. Vaccines: Sixth Edition. Elsevier Inc.; 2012. 447–492 p. Available from: http://dx.doi.org/10.1016/B978-1-4557-0090-5.00030-6

28. Chandran A, Watt JP, Santosham M. Haemophilus influenzae vaccines [Internet]. Sixth Edit. Vaccines: Sixth Edition. Elsevier Inc.; 2012. 167–182 p. Available from: http://dx.doi.org/10.1016/B978-1-4557-0090-5.00003-3

29. Jackson LA. Pneumococcal polysaccharide vaccines [Internet]. Sixth Edit. Vaccines: Sixth Edition. Elsevier Inc.; 2012. 542–572 p. Available from: http://dx.doi.org/10.1016/B978-1-4557-0090-5.00033-1

30. Klugman KP, Black S, Dagan R, Malley R, Whitney CG. Pneumococcal conjugate vaccine and

pneumococcal common protein vaccines [Internet]. Sixth Edit. Vaccines: Sixth Edition. Elsevier Inc.; 2012. 504–541 p. Available from: http://dx.doi.org/10.1016/B978-1-4557-0090-5.00032-X

31. Granoff DM, Pelton S, Harrison LH. Meningococcal vaccines. Vaccines Sixth Ed. 2012;388–418.

32. Tiwari TSP, Wharton M. Diphtheria toxoid [Internet]. Sixth Edit. Vaccines: Sixth Edition. Elsevier Inc.; 2012. 153–166 p. Available from: http://dx.doi.org/10.1016/B978-1-4557-0090-5.00024-0

33. Van Damme P, Ward J, Shouval D, Wiersma S, Zanetti A. Hepatitis B vaccines [Internet]. Sixth Edit. Vaccines: Sixth Edition. Elsevier Inc.; 2012. 205–234 p. Available from: http://dx.doi.org/10.1016/B978-1-4557-0090-5.00025-2

34. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N Engl J Med [Internet]. 2020 Dec 31 [cited 2026 Jan 24];383(27):2603–15. Available from: https://www.nejm.org/doi/pdf/10.1056/NEJMoa2034577

35. Bezbaruah R, Chavda VP, Nongrang L, Alom S, Deka K, Kalita T, et al. Nanoparticle-Based Delivery Systems for Vaccines. Vaccines 2022, Vol 10, [Internet]. 2022 Nov 17 [cited 2026 Mar 27];10(11). Available from: https://www.mdpi.com/2076-393X/10/11/1946

36. World Health Organizatio W. WHO issues its first emergency use validation for a COVID-19 vaccine and emphasizes need for equitable global access [Internet]. Who. 2020 [cited 2022 Feb 8]. p. 2020–2. Available from: https://www.who.int/news/item/31-12-2020-who-issues-its-first-emergency-use-validation-for-a-covid-19-vaccine-and-emphasizes-need-for-equitable-global-access

37. Tenforde MW, Self WH, Adams K, Gaglani M, Ginde AA, McNeal T, et al. Association Between mRNA Vaccination and COVID-19 Hospitalization and Disease Severity. JAMA [Internet]. 2021 Nov 23 [cited 2021 Dec 14];326(20):2043–54. Available from: https://jamanetwork.com/journals/jama/fullarticle/2786039

38. Deng S, Liang H, Chen P, Li Y, Li Z, Fan S, et al. Viral Vector Vaccine Development and Application during the COVID-19 Pandemic. Microorg 2022, Vol 10, [Internet]. 2022 Jul 18 [cited 2026 Mar 27];10(7). Available from: https://www.mdpi.com/2076-2607/10/7/1450

39. Ndeketa L, Haine V, Debois M, Asante KP, Agyapong PD, Kaali S, et al. Effectiveness of the RTS,S/AS01E malaria vaccine in a real-world setting over 1 year of follow-up after the three-dose

primary schedule: an interim analysis of a phase 4 study in Ghana, Kenya, and Malawi. Lancet Glob Heal [Internet]. 2026 Jan 1 [cited 2026 Mar 27];14(1):e61–9. Available from: https://www.thelancet.com/action/showFullText?pii=S2214109X25004152

40. Sullivan NJ, Fausther-Bovendo H, Nabel GJ. Ebola vaccine [Internet]. Sixth Edit. Vaccines: Sixth Edition. Elsevier Inc.; 2012. 1060–1067 p. Available from: http://dx.doi.org/10.1016/B978-1-4557-0090-5.00049-5

41. Lahariya C. Vaccine epidemiology: A review. J Fam Med Prim Care [Internet]. 2016 [cited 2026 Mar 26];5(1):7. Available from: https://journals.lww.com/jfmpc/fulltext/2016/05010/vaccine_epidemiology__a_review.3.aspx

42. Vaccine-Specific Recommendations | ACIP Recommendations | CDC [Internet]. [cited 2026 Mar 26]. Available from: https://www.cdc.gov/acip-recs/hcp/vaccine-specific/

43. Anderson RM, May RM. Directly Transmitted Infectious Diseases : Control by Vaccination Author (s): Roy M . Anderson and Robert M . May Published by : American Association for the Advancement of Science Stable URL : http://www.jstor.org/stable/1688362 . Adv Sci. 2011;215(4536):1053–60.

44. Anderson RM, May RM. Vaccination and herd immunity to infectious diseases. Nature [Internet]. 1985 [cited 2026 May 15];318(6044):323–9. Available from: https://www.nature.com/articles/318323a0

45. Anderson RM, May RM. Vaccination against rubella and measles: quantitative investigations of different policies. Epidemiol Infect [Internet]. 1983 [cited 2026 May 15];90(2):259–325. Available from: https://www.cambridge.org/core/journals/epidemiology-and-infection/article/vaccination-against-rubella-and-measles-quantitative-investigations-of-different-policies/4D164C459F5C7775925496F5F6505917

46. Abrignani S, Reali E, Houghton M, De Francesco R. Hepatitis C vaccines. Vaccines Sixth Ed. 2012;1074–84.

47. Gershon AA, Takahashi M, Seward JF. Varicella vaccine [Internet]. Sixth Edit. Vaccines: Sixth Edition. Elsevier Inc.; 2012. 837–869 p. Available from: http://dx.doi.org/10.1016/B978-1-4557-0090-5.00042-2

48. Reef SE, Plotkin SA. Rubella vaccine [Internet]. Sixth Edit. Vaccines: Sixth Edition. Elsevier Inc.; 2012. 688–717 p. Available from: http://dx.doi.org/10.1016/B978-1-4557-0090-5.00038-0

49. Weinberger DM, Malley R, Lipsitch M. Serotype replacement in disease after pneumococcal vaccination. Lancet [Internet]. 2011 Dec 3 [cited 2026 Mar 28];378(9807):1962–73. Available from: https://www.thelancet.com/action/showFullText?pii=S0140673610622258

50. Henderson DA, Klepac P. Lessons from the eradication of smallpox: An interview with D. A. Henderson. Philos Trans R Soc B Biol Sci [Internet]. 2013 Aug 5 [cited 2026 Apr 2];368(1623). Available from: https://dx.doi.org/10.1098/rstb.2013.0113

51. Henderson Ralph H. Expanded programme on immunization of the world health organization: Progress report. Infect Dis Clin Pract [Internet]. 1994;3(6):418–22. Available from: chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://watermark02.silverchair.com/6-Supplement_2-S475.pdf?token=AQECAHi208BE49Ooan9kkhW_Ercy7Dm3ZL_9Cf3qfKAc485ysgAAA5AwggOMBgkqhkiG9w0BBwagggN9MIIDeQIBADCCA3IGCSqGSIb3DQEHATAeBglghkgBZQMEAS4wEQQMUnANF

52. Keja K, Chan C, Hayden G, Henderson RH. Expanded programme on immunization. World Health Stat Q [Internet]. 1988;41(2):59–63. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=med3&NEWS=N&AN=3176515

53. World Health Organizatio W. Hepatitis B vaccines: WHO position paper – July 2017. Relev Epidemiol Hebd. 2017;92(27):369–92.

54. World Health Organizatio W. Haemophilus influenzae type b (Hib) Vaccination Position Paper - July 2013. Wkly Epidemiol Rec. 2013;88(39):413–26.

55. World Health Organizatio W. Rotavirus vaccines: WHO position paper--July 2021. Wkly Epidemiol Rec. 2021;96(28):219–301.

56. Clark A, Mahmud S, Debellut F, Pecenka C, Jit M, Perin J, et al. Estimating the global impact of rotavirus vaccines on child mortality. Int J Infect Dis [Internet]. 2023 Dec 1 [cited 2024 Jul 18];137:90. Available from: /pmc/articles/PMC10689250/

57. Saxenian H, Hecht R, Kaddar M, Schmitt S, Ryckman T, Cornejo S. Overcoming challenges to

sustainable immunization financing: early experiences from GAVI graduating countries. Health Policy Plan [Internet]. 2015 Mar 1 [cited 2026 Apr 8];30(2):197–205. Available from: https://dx.doi.org/10.1093/heapol/czu003

58. GAVI the VA. Gavi Alliance Framework for Gavi Funding to Countries. 2022;1–15.

59. GAVI the VA. Gavi Alliance Co-financing Policy. 2026;(January):1–7.

60. GAVI the VA. Guidelines. 2026.

61. Expanded programme of immunisation M of H, Malawi G of. Malawi National Immunisation Strategy 2023-2030.

62. WHO. Malawi introduces of Pneumococcal Conjugate Vaccine [Internet]. Health (San Francisco). 2011. p. 1–3. Available from: http://www.afro.who.int/en/malawi/press-materials/item/3404-malawi-introduces-pneumococcal-conjugate-vaccine.html

63. Malawi Expanded Programme on Immunisation. District-level vaccine coverage data, Malawi. 2025.

64. Nkambule E, Mbakaya BC. COVID-19 vaccination hesitancy among Malawians: a scoping review. Syst Rev 2024 131 [Internet]. 2024 Feb 28 [cited 2026 Jun 18];13(1):77-. Available from: https://link.springer.com/article/10.1186/s13643-024-02499-z

65. Phiri MM, Osman R, Weldegebriel S, Sabola S, Ongadi B, Beavis C, et al. Exploration of the social determinants of diarrhoea, rotavirus vaccine uptake, and vaccine 'fatigue' in Ethiopia, Kenya, and Malawi. PLoS One [Internet]. 2025 Sep 1 [cited 2026 Jun 22];20(9):e0319691. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0319691

66. Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. Lancet. 2006;367(9524):1747–57.

67. Kyu HH, Vongpradith A, Dominguez RMV, Ma J, Albertson SB, Novotney A, et al. Global, regional, and national age-sex-specific burden of diarrhoeal diseases, their risk factors, and aetiologies, 1990–2021, for 204 countries and territories: a systematic analysis for the Global Burden of Disease Study 2021. Lancet Infect Dis [Internet]. 2025 May 1 [cited 2026 Feb 16];25(5):519–36. Available from: https://www.thelancet.com/action/showFullText?pii=S1473309924006911

68. Troeger C, Khalil IA, Rao PC, Cao S, Blacker BF, Ahmed T, et al. Rotavirus Vaccination and the Global Burden of Rotavirus Diarrhea Among Children Younger Than 5 Years. JAMA Pediatr [Internet]. 2018 Oct 1 [cited 2026 Apr 7];172(10):958–65. Available from: https://jamanetwork.com/journals/jamapediatrics/fullarticle/2696431

69. Greenberg HB, Estes MK. Rotaviruses: From Pathogenesis to Vaccination. Gastroenterology [Internet]. 2009 May 1 [cited 2026 Apr 7];136(6):1939–51. Available from: https://www.gastrojournal.org/action/showFullText?pii=S0016508509003631

70. Clark HF, Offit PA, Parashar UD. Rotavirus vaccines. Vaccines Sixth Ed. 2012;669–87.

71. Hasso-Agopsowicz M, Ladva CN, Lopman B, Sanderson C, Cohen AL, Tate JE, et al. Global Review of the Age Distribution of Rotavirus Disease in Children Aged <5 Years Before the Introduction of Rotavirus Vaccination. Clin Infect Dis [Internet]. 2019 Aug 30 [cited 2026 Apr 27];69(6):1071–8. Available from: https://dx.doi.org/10.1093/cid/ciz060

72. Velázquez FR, Matson DO, Calva JJ, Guerrero ML, Morrow AL, Carter-Campbell S, et al. Rotavirus Infection in Infants as Protection against Subsequent Infections. N Engl J Med [Internet]. 1996 Oct 3 [cited 2026 Apr 27];335(14):1022–8. Available from: https://www.nejm.org/doi/pdf/10.1056/NEJM199610033351404

73. Rennels MB, Ward RL, Mack ME, Zito ET. Concurrent Oral Poliovirus and Rhesus-Human Reassortant Rotavirus Vaccination: Effects on Immune Responses to Both Vaccines and on Efficacy of Rotavirus Vaccines. J Infect Dis [Internet]. 1996 Feb 1 [cited 2023 Aug 22];173(2):306–13. Available from: https://dx.doi.org/10.1093/infdis/173.2.306

74. Murphy T V., Gargiullo PM, Massoudi MS, Nelson DB, Jumaan AO, Okoro CA, et al. Intussusception among Infants Given an Oral Rotavirus Vaccine. N Engl J Med [Internet]. 2001 Feb 22 [cited 2026 May 12];344(8):564–72. Available from: https://www.nejm.org/doi/pdf/10.1056/NEJM200102223440804

75. Burke RM, Tate JE, Kirkwood CD, Steele AD, Parashar UD. Current and new rotavirus vaccines. Curr Opin Infect Dis [Internet]. 2019 Oct 1 [cited 2024 Jul 9];32(5):435. Available from: /pmc/articles/PMC6749957/

76. World Health Organization. Rotavirus [Internet]. [cited 2026 Apr 27]. Available from: https://www.who.int/teams/health-product-policy-and-standards/standards-and-

specifications/norms-and-standards/vaccines-quality/rotavirus

77. Vaselli NM, Iturriza-Gómara[1] M, Hungerford D. Contribution of the EuroRotaNet surveillance network to rotavirus strain surveillance in Europe. Eurosurveillance [Internet]. 2025 Sep 25 [cited 2026 Apr 27];30(38):2400798. Available from: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2025.30.38.2400798

78. Ruiz-Palacios GM, Pérez-Schael I, Velázquez FR, Abate H, Breuer T, Clemens SC, et al. Safety and Efficacy of an Attenuated Vaccine against Severe Rotavirus Gastroenteritis. N Engl J Med [Internet]. 2006 Jan 5 [cited 2026 May 13];354(1):11–22. Available from: https://www.nejm.org/doi/pdf/10.1056/NEJMoa052434

79. Madhi SA, Cunliffe NA, Steele D, Witte D, Kirsten M, Louw C, et al. Effect of human rotavirus vaccine on severe diarrhea in African infants. Malawi Med J [Internet]. 2016 Jan 28 [cited 2026 May 4];28(3):108–14. Available from: https://www.nejm.org/doi/pdf/10.1056/NEJMoa0904797

80. Cunliffe NA, Witte D, Ngwira BM, Todd S, Bostock NJ, Turner AM, et al. Efficacy of human rotavirus vaccine against severe gastroenteritis in Malawian children in the first two years of life: a randomised, double-blind, placebo controlled trial. Vaccine [Internet]. 2012 Apr 27 [cited 2026 Feb 2];30(0 1):A36. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC3982044/

81. Vesikari T, Itzler R, Karvonen A, Korhonen T, Van Damme P, Behre U, et al. RotaTeq®, a pentavalent rotavirus vaccine: Efficacy and safety among infants in Europe. Vaccine [Internet]. 2009 Dec 11 [cited 2026 May 13];28(2):345–51. Available from: https://www.sciencedirect.com/science/article/pii/S0264410X09015710

82. Armah GE, Sow SO, Breiman RF, Dallas MJ, Tapia MD, Feikin DR, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in sub-Saharan Africa: A randomised, double-blind, placebo-controlled trial. Lancet [Internet]. 2010 Aug 21 [cited 2026 May 13];376(9741):606–14. Available from: https://www.thelancet.com/action/showFullText?pii=S0140673610608896

83. Zaman K, Anh DD, Victor JC, Shin S, Yunus M, Dallas MJ, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in Asia: A randomised, double-blind, placebo-controlled trial. Lancet [Internet]. 2010 Aug 21 [cited 2026 May 13];376(9741):615–23. Available from:

https://www.thelancet.com/action/showFullText?pii=S0140673610607556

84. Bhandari N, Rongsen-Chandola T, Bavdekar A, John J, Antony K, Taneja S, et al. Efficacy of a monovalent human-bovine (116E) rotavirus vaccine in Indian infants: A randomised, double-blind, placebo-controlled trial. Lancet [Internet]. 2014 Jun 21 [cited 2026 May 13];383(9935):2136–43. Available from: https://www.thelancet.com/action/showFullText?pii=S0140673613626306

85. Kulkarni PS, Desai S, Tewari T, Kawade A, Goyal N, Garg BS, et al. A randomized Phase III clinical trial to assess the efficacy of a bovine-human reassortant pentavalent rotavirus vaccine in Indian infants. Vaccine [Internet]. 2017 Oct 27 [cited 2026 May 13];35(45):6228–37. Available from: https://www.sciencedirect.com/science/article/pii/S0264410X17312306?via%3Dihub

86. Isanaka S, Langendorf C, McNeal MM, Meyer N, Plikaytis B, Garba S, et al. Rotavirus vaccine efficacy up to 2 years of age and against diverse circulating rotavirus strains in Niger: Extended follow-up of a randomized controlled trial. PLOS Med [Internet]. 2021 Jul 1 [cited 2026 May 13];18(7):e1003655. Available from: https://journals.plos.org/plosmedicine/article?id=10.1371/journal.pmed.1003655

87. Groome MJ, Page N, Cortese MM, Moyes J, Zar HJ, Kapongo CN, et al. Effectiveness of monovalent human rotavirus vaccine against admission to hospital for acute rotavirus diarrhoea in South African children: A case-control study. Lancet Infect Dis [Internet]. 2014 Nov 1 [cited 2026 Mar 8];14(11):1096–104. Available from: https://pubmed.ncbi.nlm.nih.gov/25303843/

88. Duncan Steele A, Groome MJ. Measuring Rotavirus Vaccine Impact in Sub-Saharan Africa. Clin Infect Dis An Off Publ Infect Dis Soc Am [Internet]. 2019 Jun 1 [cited 2025 Nov 26];70(11):2314. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC7245150/

89. Parker EPK, Ramani S, Lopman BA, Church JA, Iturriza-Gómara M, Prendergast AJ, et al. Causes of Impaired Oral Vaccine Efficacy in Developing Countries. Future Microbiol [Internet]. 2018 Jan 1 [cited 2025 Oct 21];13(1):97–118. Available from: https://www.tandfonline.com/doi/pdf/10.2217/fmb-2017-0128

90. Ndeketa L, Vaselli NM, Pitzer VE, Dodd PJ, Hungerford D, French N. Methods of adjustment for non-vaccine interventions in post-licensure vaccine studies in children in sub–Saharan Africa: a systematic review. medRxiv [Internet]. 2026 Apr 2 [cited 2026 May 4];2026.04.01.26349767.

Available from: https://www.medrxiv.org/content/10.64898/2026.04.01.26349767v1

91. Clemens JD, Shin S, Sah BK, Sack DA. Cholera vaccines [Internet]. Sixth Edit. Vaccines: Sixth Edition. Elsevier Inc.; 2012. 141–152 p. Available from: http://dx.doi.org/10.1016/B978-1-4557-0090-5.00023-9

92. Charles RC, Ryan ET. Cholera in the 21st century. Curr Opin Infect Dis [Internet]. 2011 Oct [cited 2026 Apr 7];24(5):472–7. Available from: https://journals.lww.com/co-infectiousdiseases/fulltext/2011/10000/cholera_in_the_21st_century.12.aspx

93. Nelson EJ, Harris JB, Morris JG, Calderwood SB, Camilli A. Cholera transmission: the host, pathogen and bacteriophage dynamic. Nat Rev Microbiol [Internet]. 2009 [cited 2025 Jan 29];7(10):10.1038/nrmicro2204. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC3842031/

94. Chaguza C, Chibwe I, Chaima D, Musicha P, Ndeketa L, Kasambara W, et al. Genomic insights into the 2022–2023Vibrio cholerae outbreak in Malawi. Nat Commun [Internet]. 2024 Dec 1 [cited 2025 Jan 29];15(1):6291. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11282309/

95. Montero DA, Vidal RM, Velasco J, George S, Lucero Y, Gómez LA, et al. Vibrio cholerae, classification, pathogenesis, immune response, and trends in vaccine development. Front Med. 2023 May 5;10:1155751.

96. Gopalakrishnakone P, Balali-Mood M, Llewellyn L, Singh BR. Biological toxins and bioterrorism. Biological Toxins and Bioterrorism. 2015. 1–604 p.

97. Thiagarajah JR, Verkman AS. New drug targets for cholera therapy. Trends Pharmacol Sci [Internet]. 2005 Apr 1 [cited 2026 Apr 8];26(4):172–5. Available from: https://www.cell.com/action/showFullText?pii=S0165614705000489

98. Matanza XM, Clements A. Pathogenicity and virulence of Shigella sonnei: A highly drug-resistant pathogen of increasing prevalence. Virulence [Internet]. 2023 Dec 31 [cited 2026 Apr 8];14(1). Available from: https://www.tandfonline.com/doi/pdf/10.1080/21505594.2023.2280838

99. Thiagarajah JR, Verkman AS. New drug targets for cholera therapy. Trends Pharmacol Sci [Internet]. 2005 Apr 1 [cited 2026 May 13];26(4):172–5. Available from: https://www.cell.com/action/showFullText?pii=S0165614705000489

100. World Health Organization. Cholera in the WHO African Region | WHO | Regional Office for Africa [Internet]. 2024 [cited 2026 Feb 17]. Available from: https://www.afro.who.int/health-topics/disease-outbreaks/cholera-who-african-region?page=0

101. Leung T, Matrajt L. Protection afforded by previous Vibrio cholerae infection against subsequent disease and infection: A review. PLoS Negl Trop Dis [Internet]. 2021 May 1 [cited 2025 Dec 16];15(5):e0009383. Available from: https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0009383

102. World Health Organization. Immunization, Vaccines and Biologicals [Internet]. 2017 [cited 2025 Dec 17]. Available from: https://www.who.int/teams/immunization-vaccines-and-biologicals/diseases/cholera

103. Ryan ET, Leung DT, Jensen O, Weil AA, Bhuiyan TR, Khan AI, et al. Systemic, Mucosal, and Memory Immune Responses following Cholera. Trop Med Infect Dis [Internet]. 2021 Dec 1 [cited 2025 Dec 17];6(4):192. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8628923/

104. Ivers LC, Hilaire IJ, Teng JE, Almazor CP, Jerome JG, Ternier R, et al. Effectiveness of reactive oral cholera vaccination in rural Haiti: a case-control study. Lancet Glob Heal [Internet]. 2015 Mar 1 [cited 2026 Jan 15];3(3):e162. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC4384694/

105. Qadri F, Ali M, Chowdhury F, Khan AI, Saha A, Khan IA, et al. Feasibility and effectiveness of oral cholera vaccine in an urban endemic setting in Bangladesh: A cluster randomised open-label trial. Lancet [Internet]. 2015 Oct 3 [cited 2026 Jan 15];386(10001):1362–71. Available from: https://www.thelancet.com/action/showFullText?pii=S0140673615611400

106. Sialubanje C, Kapina M, Chewe O, Matapo BB, Ngomah AM, Gianetti B, et al. Effectiveness of two doses of Euvichol-plus oral cholera vaccine in response to the 2017/2018 outbreak: a matched case–control study in Lusaka, Zambia. BMJ Open [Internet]. 2022 Nov 11 [cited 2026 Jan 15];12(11):e066945. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC9660660/

107. Wierzba TF, Kar SK, Mogasale V V., Kerketta AS, You YA, Baral P, et al. Effectiveness of an oral cholera vaccine campaign to prevent clinically-significant cholera in Odisha State, India. Vaccine [Internet]. 2015 May 15 [cited 2026 Jan 15];33(21):2463–9. Available from: https://www.sciencedirect.com/science/article/pii/S0264410X15003928?via%3Dihub

108. World Malaria Report 2015. 2015;

109. World Health Organization. World malaria report 2025 [Internet]. [cited 2024 Oct 30]. Available from: https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2023

110. Ojeniyi FD, Ayoola AO, Ibitoye O, Opaleye OO, Olowe OA, Ehigie LO, et al. Performance and challenges of malaria rapid diagnostic tests in endemic regions of Africa. Sci Reports 2025 151 [Internet]. 2025 Dec 11 [cited 2026 Apr 8];15(1):43663-. Available from: https://www.nature.com/articles/s41598-025-97259-x

111. Calderaro A, Piccolo G, Chezzi C. The Laboratory Diagnosis of Malaria: A Focus on the Diagnostic Assays in Non-Endemic Areas. Int J Mol Sci 2024, Vol 25, [Internet]. 2024 Jan 5 [cited 2026 May 13];25(2). Available from: https://www.mdpi.com/1422-0067/25/2/695

112. WHO. Malaria Treatment Guidelines. 2010;9.

113. World Health Organization. World Malaria Report 2019. Geneva. [Internet]. 2019. 1–232 p. Available from: https://www.who.int/publications-detail/world-malaria-report-2019

114. WHO recommends groundbreaking malaria vaccine for children at risk [Internet]. [cited 2026 Apr 8]. Available from: https://www.who.int/news/item/06-10-2021-who-recommends-groundbreaking-malaria-vaccine-for-children-at-risk

115. WHO recommends R21/Matrix-M vaccine for malaria prevention in updated advice on immunization [Internet]. [cited 2026 Apr 8]. Available from: https://www.who.int/news/item/02-10-2023-who-recommends-r21-matrix-m-vaccine-for-malaria-prevention-in-updated-advice-on-immunization

116. World Health Organizatio W. Malaria vaccines (RTS,S and R21) [Internet]. 2024 [cited 2024 Jul 15]. Available from: https://www.who.int/news-room/questions-and-answers/item/q-a-on-rts-s-malaria-vaccine

117. Cohen J, Nussenzweig V, Nussenzweig R, Vekemans J, Leach A. From the circumsporozoite protein to the RTS,S/AS candidate vaccine. Hum Vaccin [Internet]. 2010 [cited 2026 Apr 8];6(1):90–6. Available from: https://www.tandfonline.com/doi/pdf/10.4161/hv.6.1.9677

118. Gordon DM, Mc Govern TW, Krzych U, Cohen JC, Schneider I, La Chance R, et al. Safety,

Immunogenicity, and Efficacy of a Recombinantly Produced Plasmodium falciparum Circumsporozoite Protein-Hepatitis B Surface Antigen Subunit Vaccine. J Infect Dis [Internet]. 1995 Jun 1 [cited 2026 Apr 8];171(6):1576–85. Available from: https://dx.doi.org/10.1093/infdis/171.6.1576

119. Roman F, Burny W, Ceregido MA, Laupèze B, Temmerman ST, Warter L, et al. Adjuvant system AS01: from mode of action to effective vaccines. Expert Rev Vaccines [Internet]. 2024 Dec 31 [cited 2026 Apr 8];23(1):715–29. Available from: https://www.tandfonline.com/doi/pdf/10.1080/14760584.2024.2382725

120. Lell B, Agnandji S, von Glasenapp I, Haertle S, Oyakhiromen S, Issifou S, et al. A Randomized Trial Assessing the Safety and Immunogenicity of AS01 and AS02 Adjuvanted RTS,S Malaria Vaccine Candidates in Children in Gabon. PLoS One [Internet]. 2009 Oct 27 [cited 2026 Apr 8];4(10):e7611. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0007611

121. Kester KE, Cummings JF, Ofori-Anyinam O, Ockenhouse CF, Krzych U, Moris P, et al. Randomized, Double-Blind, Phase 2a Trial of Falciparum Malaria Vaccines RTS,S/AS01B and RTS,S/AS02A in Malaria-Naive Adults: Safety, Efficacy, and Immunologic Associates of Protection. J Infect Dis [Internet]. 2009 Aug 1 [cited 2026 Apr 8];200(3):337–46. Available from: https://dx.doi.org/10.1086/600120

122. RTSS Clinical Trials Partnership. Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: Final results of a phase 3, individually randomised, controlled trial. Lancet [Internet]. 2015 Jul 4 [cited 2026 Apr 8];386(9988):31–45. Available from: https://www.thelancet.com/action/showFullText?pii=S0140673615607218

123. Ndeketa L, Haine V, Debois M, Asante KP, Agyapong PD, Kaali S, et al. Effectiveness of the RTS,S/AS01E malaria vaccine in a real-world setting over 1 year of follow-up after the three-dose primary schedule: an interim analysis of a phase 4 study in Ghana, Kenya, and Malawi. Lancet Glob Heal [Internet]. 2026 Jan 1 [cited 2026 Apr 8];14(1):e61–9. Available from: https://www.thelancet.com/action/showFullText?pii=S2214109X25004152

124. Tsoumani ME, Voyiatzaki C, Efstathiou A. Malaria Vaccines: From the Past towards the mRNA Vaccine Era. Vaccines 2023, Vol 11, [Internet]. 2023 Sep 4 [cited 2026 Apr 8];11(9). Available from: https://www.mdpi.com/2076-393X/11/9/1452

125. Collins KA, Brod F, Snaith R, Ulaszewska M, Longley RJ, Salman AM, et al. Ultra-low dose immunization and multi-component vaccination strategies enhance protection against malaria in mice. Sci Reports 2021 111 [Internet]. 2021 May 24 [cited 2026 Apr 8];11(1):10792-. Available from: https://www.nature.com/articles/s41598-021-90290-8

126. Datoo MS, Dicko A, Tinto H, Ouédraogo JB, Hamaluba M, Olotu A, et al. Safety and efficacy of malaria vaccine candidate R21/Matrix-M in African children: a multicentre, double-blind, randomised, phase 3 trial. Lancet [Internet]. 2024 Feb 10 [cited 2026 Apr 8];403(10426):533–44. Available from: https://www.thelancet.com/action/showFullText?pii=S0140673623025114

127. Tsoumani ME, Voyiatzaki C, Efstathiou A. Malaria Vaccines: From the Past towards the mRNA Vaccine Era. Vaccines 2023, Vol 11, [Internet]. 2023 Sep 4 [cited 2026 May 13];11(9). Available from: https://www.mdpi.com/2076-393X/11/9/1452

128. Verani JR, Baqui AH, Broome C V., Cherian T, Cohen C, Farrar JL, et al. Case-control vaccine effectiveness studies: Data collection, analysis and reporting results. Vaccine [Internet]. 2017;35(25):3303–8. Available from: http://dx.doi.org/10.1016/j.vaccine.2017.04.035

129. Elizabeth Halloran M, Struchiner CJ, Longini IM. Study Designs for Evaluating Different Efficacy and Effectiveness Aspects of Vaccines. Am J Epidemiol [Internet]. 1997 Nov 15 [cited 2026 Feb 27];146(10):789–803. Available from: https://dx.doi.org/10.1093/oxfordjournals.aje.a009196

130. Hungerford D, Vivancos R, Read JM, Bonnett LJ, Bar-Zeev N, Iturriza-Gómara M, et al. Mitigating bias in observational vaccine effectiveness studies using simulated comparator populations: Application to rotavirus vaccination in the UK. Vaccine [Internet]. 2018 Oct 29 [cited 2026 May 12];36(45):6674–82. Available from: https://www.sciencedirect.com/science/article/pii/S0264410X18313203

131. Halloran ME. Designs for Vaccine Studies. Annu Rev Stat Its Appl [Internet]. 2025 Mar 7 [cited 2026 Apr 4];12(1):1–18. Available from: https://www.annualreviews.org/content/journals/10.1146/annurev-statistics-033121-120121

132. Farrington CP. Estimation of vaccine effectiveness using the screening method. Int J Epidemiol. 1993;22(4):742–6.

133. Flannery B, Andrews N, Feikin D, Patel MK. screening method. 2024;52(1):19–21.

134. Orenstein WA, Bernier RH, Dondero TJ, Hinman AR, Marks JS, Bart KJ, et al. Field evaluation of vaccine efficacy. Bull World Health Organ [Internet]. 1985 [cited 2026 Jan 14];63(6):1055. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC2536484/

135. Guris D, Strebel PM, Tachdjian R, Bardenheier B, Wharton M, Hadler SC. Effectiveness of the pertussis vaccination program as determined by use of the screening method: United States, 1992-1994. J Infect Dis. 1997;176(2):456–63.

136. Remschmidt C, Rieck T, Bödeker B, Wichmann O. Application of the screening method to monitor influenza vaccine effectiveness among the elderly in Germany. BMC Infect Dis 2015 151 [Internet]. 2015 Mar 20 [cited 2026 Apr 27];15(1):137-. Available from: https://link.springer.com/article/10.1186/s12879-015-0882-3

137. Araki K, Hara M, Sakanishi Y, Shimanoe C, Nishida Y, Matsuo M, et al. Estimating rotavirus vaccine effectiveness in Japan using a screening method. Hum Vaccin Immunother [Internet]. 2016 May 3 [cited 2026 Apr 27];12(5):1244–9. Available from: https://www.tandfonline.com/doi/pdf/10.1080/21645515.2015.1121337

138. Perumal N, Schönfeld V, Wichmann O. Application of the screening method for estimating COVID-19 vaccine effectiveness using routine surveillance data: Germany's experience during the COVID-19 pandemic, July 2021 to March 2023. Eurosurveillance [Internet]. 2024 Feb 1 [cited 2026 Apr 27];29(8):2300329. Available from: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2023.29.8.2300329

139. Longini IMJ, Halloran ME, Haber M, Chen RT. Measuring vaccine efficacy from epidemics of acute infectious agents. Stat Med. 1993 Feb;12(3–4):249–63.

140. Doll R, Bradfordhill A. The Mortality of Doctors in Relation To Their Smoking Habits A: preliminary report. Br Med J. 1954;1(4877):1451–5.

141. De Serres G, Skowronski DM, Wu XW, Ambrose CS. The test-negative design: Validity, accuracy and precision of vaccine efficacy estimates compared to the gold standard of randomised placebo-controlled clinical trials. Eurosurveillance [Internet]. 2013 Sep 12 [cited 2026 Apr 8];18(37):20585. Available from: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES2013.18.37.20585

142. Doll R, Hill AB. Lung Cancer And Other Causes Of Death In Relation To Smoking A Second Report

On The Mortality Of British Doctors. Br Med J. 1956;2(5001):1071.

143. Euser AM, Zoccali C, Jager KJ, Dekker FW. Cohort Studies: Prospective versus Retrospective. Nephron Clin Pract [Internet]. 2009 Oct 1 [cited 2026 Apr 28];113(3):c214–7. Available from: https://dx.doi.org/10.1159/000235241

144. Pearce N, Checkoway H. Cohort Studies. Wiley StatsRef Stat Ref Online [Internet]. 2014 Sep 29 [cited 2026 Apr 8]; Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/9781118445112.stat03722

145. Paine G, Susser M, Adelstein A. Vital Statistics: A Memorial Volume of Selections from the Reports and Writings of William Farr. J R Stat Soc Ser A. 1977;140(1):109.

146. Lipsitch M, Jha A, Simonsen L. Observational studies and the difficult quest for causality: Lessons from vaccine effectiveness and impact studies. Int J Epidemiol. 2016;45(6):2060–74.

147. Lopez Bernal J, Soumerai S, Gasparrini A. A methodological framework for model selection in interrupted time series studies. J Clin Epidemiol [Internet]. 2018;103:82–91. Available from: https://doi.org/10.1016/j.jclinepi.2018.05.026

148. Praet N, Asante KP, Bozonnat M-C, Akité EJ, Ansah PO, Baril L, et al. Assessing the safety, impact and effectiveness of RTS,S/AS01(E) malaria vaccine following its introduction in three sub-Saharan African countries: methodological approaches and study set-up. Malar J. 2022 Apr;21(1):132.

149. Praet N, Asante KP, Bozonnat M-C, Akité EJ, Ansah PO, Baril L, et al. Assessing the safety, impact and effectiveness of RTS,S/AS01E malaria vaccine following its introduction in three sub-Saharan African countries: methodological approaches and study set-up. Malar J 2022 211 [Internet]. 2022 Apr 25 [cited 2022 May 13];21(1):1–12. Available from: https://malariajournal.biomedcentral.com/articles/10.1186/s12936-022-04144-3

150. Table 2 Summary of WHO Position Papers - Recommended Routine Immunizations for Children [Internet]. [cited 2025 Jul 26]. Available from: https://www.who.int/publications/m/item/table-2-summary-of-who-position-papers-recommended-routine-immunizations-for-children

151. Mwenda JM, Parashar UD, Cohen AL, Tate JE. Impact of rotavirus vaccines in Sub-Saharan African countries. Vaccine [Internet]. 2018;36(47):7119–23. Available from:

https://doi.org/10.1016/j.vaccine.2018.06.026

152. Ngocho JS, Magoma B, Olomi GA, Mahande MJ, Msuya SE, de Jonge MI, et al. Effectiveness of pneumococcal conjugate vaccines against invasive pneumococcal disease among children under five years of age in Africa: A systematic review. PLoS One [Internet]. 2019 Feb 1 [cited 2026 Mar 13];14(2):e0212295. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0212295

153. Higgins JPT, Soares-Weiser K, López-López JA, Kakourou A, Chaplin K, Christensen H, et al. Association of BCG, DTP, and measles containing vaccines with childhood mortality: systematic review. BMJ [Internet]. 2016 Oct 13 [cited 2025 Jul 28];355:5170. Available from: https://www.bmj.com/content/355/bmj.i5170

154. Singh K, Mehta S. The clinical development process for a novel preventive vaccine: An overview. J Postgrad Med [Internet]. 2016 Jan 1 [cited 2022 Nov 22];62(1):4. Available from: /pmc/articles/PMC4944327/

155. Kahn R, Villar SS, Dean NE, Lipsitch M. Vaccine Trial Designs. Princ Pract Emerg Res Response [Internet]. 2024 Aug 31 [cited 2026 Mar 13];585–610. Available from: https://www.ncbi.nlm.nih.gov/books/NBK614024/

156. Hemming K, Taljaard M. Key considerations for designing, conducting and analysing a cluster randomized trial. Int J Epidemiol [Internet]. 2023 Oct 5 [cited 2026 Mar 13];52(5):1648–58. Available from: https://dx.doi.org/10.1093/ije/dyad064

157. Hanquet G, Valenciano M, Simondon F, Moren A. Vaccine effects and impact of vaccination programmes in post-licensure studies. Vaccine [Internet]. 2013 Nov 19 [cited 2026 Mar 13];31(48):5634–42. Available from: https://doi.org/10.1093/oxfordjournals.epirev.a017931

158. Morgenstern H. Ecologic studies in epidemiology: Concepts, principles, and methods. Annu Rev Public Health. 1995;16:61–81.

159. Bernal JL, Cummins S, Gasparrini A. The use of controls in interrupted time series studies of public health interventions. Int J Epidemiol [Internet]. 2018 Dec 1 [cited 2023 Oct 24];47(6):2082–93. Available from: https://dx.doi.org/10.1093/ije/dyy135

160. Bernal JL, Cummins S, Gasparrini A. Interrupted time series regression for the evaluation of public

health interventions: a tutorial. Int J Epidemiol [Internet]. 2017 Feb 1 [cited 2024 Oct 31];46(1):348–55. Available from: https://pubmed.ncbi.nlm.nih.gov/27283160/

161. Pearce N. Analysis of matched case-control studies. BMJ [Internet]. 2016 Feb 25 [cited 2026 Mar 13];352. Available from: https://www.bmj.com/content/352/bmj.i969

162. Pearce N, Checkoway H. Cohort Studies. Encycl Quant Risk Anal Assess Melnick/Risk [Internet]. 2008 Jan 1 [cited 2026 Mar 13];1–12. Available from: /doi/pdf/10.1002/9780470061596.risk0598

163. Wang X, Kattan MW. Cohort Studies: Design, Analysis, and Reporting. Chest [Internet]. 2020 Jul 1 [cited 2026 Mar 13];158(1):S72–8. Available from: https://doi.org/10.1089/jpm.2008.9690

164. Capili B, Anastasi JK. Overview: Cohort Study Designs. Am J Nurs [Internet]. 2021 Dec 1 [cited 2026 Mar 13];121(12):45. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC9536647/

165. Khanam F, Kim DR, Liu X, Voysey M, Pitzer VE, Zaman K, et al. Assessment of vaccine herd protection in a cluster-randomised trial of Vi conjugate vaccine against typhoid fever: results of further analysis. eClinicalMedicine [Internet]. 2023 Apr 1 [cited 2026 Mar 13];58:101925. Available from: https://www.thelancet.com/action/showFullText?pii=S2589537023001025

166. Patel MM, Steele D, Gentsch JR, Wecker J, Glass RI, Parashar UD. Real-world impact of rotavirus vaccination. Pediatr Infect Dis J. 2011;30(SUPPL. 1):1–5.

167. Bar-zeev N, Tate JE, Pecenka C, Chikafa J, Mvula H, Wachepa R, et al. Cost-Effectiveness of Monovalent Rotavirus Vaccination of Infants in Malawi : A Postintroduction Analysis Using Individual Patient – Level Costing Data. 2016;62(Suppl 2).

168. Patriarca PA, Wright PF, John TJ. Factors Affecting the Immunogenicity of Oral Poliovirus Vaccine in Developing Countries: Review. Rev Infect Dis [Internet]. 1991 Sep 1 [cited 2025 Aug 19];13(5):926–39. Available from: https://dx.doi.org/10.1093/clinids/13.5.926

169. Fine PEM. Variation in protection by BCG: implications of and for heterologous immunity. Lancet [Internet]. 1995 Nov 18 [cited 2025 Aug 19];346(8986):1339–45. Available from: https://www.thelancet.com/action/showFullText?pii=S0140673695923489

170. Rayyan: AI-Powered Systematic Review Management Platform [Internet]. [cited 2026 Mar 30]. Available from: https://www.rayyan.ai/

171. Mwenda JM, Tate JE, Parashar UD, Mihigo R, Agócs M, Serhan F, et al. African Rotavirus

Surveillance Network – A Brief Overview. Pediatr Infect Dis J [Internet]. 2014 [cited 2026 Mar 30];33(Suppl 1):S6. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC12046586/

172. Tate JE, Ngabo F, Donnen P, Gatera M, Uwimana J, Rugambwa C, et al. Effectiveness of Pentavalent Rotavirus Vaccine Under Conditions of Routine Use in Rwanda. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2016 May;62 Suppl 2:S208-12.

173. Bonkoungou IJO, Aliabadi N, Leshem E, Kam M, Nezien D, Drabo MK, et al. Impact and effectiveness of pentavalent rotavirus vaccine in children. Vaccine [Internet]. 2018 Nov;36(47):7170–8. Available from: https://pubmed.ncbi.nlm.nih.gov/29290478/

174. Mokomane M, Tate JE, Steenhoff AP, Esona MD, Bowen MD, Lechiile K, et al. Evaluation of the Influence of Gastrointestinal Co-Infections on Rotavirus Vaccine Effectiveness in Botswana. Pediatr Infect Dis J [Internet]. 2018 Mar 1 [cited 2026 Mar 8];37(3):e58. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC5807168/

175. Bar-Zeev N, Jere KC, Bennett A, Pollock L, Tate JE, Nakagomi O, et al. Population Impact and Effectiveness of Monovalent Rotavirus Vaccination in Urban Malawian Children 3 Years after Vaccine Introduction: Ecological and Case-Control Analyses. Clin Infect Dis [Internet]. 2016 May 1 [cited 2021 Jan 6];62(Suppl 2):S213–9. Available from: /pmc/articles/PMC4825885/?report=abstract

176. Beres LK, Tate JE, Njobvu L, Chibwe B, Rudd C, Guffey MB, et al. A Preliminary Assessment of Rotavirus Vaccine Effectiveness in Zambia. Clin Infect Dis [Internet]. 2016 May 1 [cited 2023 Feb 23];62 Suppl 2:S175–82. Available from: https://pubmed.ncbi.nlm.nih.gov/27059353/

177. Jere KC, Bar-Zeev N, Chande A, Bennett A, Pollock L, Sanchez-Lopez PF, et al. Vaccine Effectiveness against DS-1–Like Rotavirus Strains in Infants with Acute Gastroenteritis, Malawi, 2013–2015. Emerg Infect Dis [Internet]. 2019 [cited 2026 Mar 8];25(9):1734. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC6711242/

178. Armah G, Pringle K, Enweronu-Laryea CC, Ansong D, Mwenda JM, Diamenu SK, et al. Impact and Effectiveness of Monovalent Rotavirus Vaccine Against Severe Rotavirus Diarrhea in Ghana. Clin Infect Dis [Internet]. 2016 May 1 [cited 2026 Mar 8];62 Suppl 2(Suppl 2):S200–7. Available from: https://pubmed.ncbi.nlm.nih.gov/27059357/

179. Gastañaduy PA, Steenhoff AP, Mokomane M, Esona MD, Bowen MD, Jibril H, et al. Effectiveness

of Monovalent Rotavirus Vaccine after Programmatic Implementation in Botswana: A Multisite Prospective Case-Control Study. Clin Infect Dis. 2016;62(Suppl 2):S161–7.

180. Platts-Mills JA, Amour C, Gratz J, Nshama R, Walongo T, Mujaga B, et al. Impact of Rotavirus Vaccine Introduction and Postintroduction Etiology of Diarrhea Requiring Hospital Admission in Haydom, Tanzania, a Rural African Setting. Clin Infect Dis [Internet]. 2017 Oct 1 [cited 2026 Mar 8];65(7):1144–51. Available from: https://pubmed.ncbi.nlm.nih.gov/28575304/

181. Groome MJ, Page N, Cortese MM, Moyes J, Zar HJ, Kapongo CN, et al. Effectiveness of monovalent human rotavirus vaccine against admission to hospital for acute rotavirus diarrhoea in South African children: A case-control study. Lancet Infect Dis. 2014;14(11):1096–104.

182. Bar-Zeev N, Kapanda L, Tate JE, Jere KC, Iturriza-Gomara M, Nakagomi O, et al. Effectiveness of a monovalent rotavirus vaccine in infants in Malawi after programmatic roll-out: An observational and case-control study. Lancet Infect Dis [Internet]. 2015 Apr 1 [cited 2021 Jan 6];15(4):422–8. Available from: /pmc/articles/PMC4374102/?report=abstract

183. Abeid KA, Jani B, Cortese MM, Kamugisha C, Mwenda JM, Pandu AS, et al. Monovalent Rotavirus Vaccine Effectiveness and Impact on Rotavirus Hospitalizations in Zanzibar, Tanzania: Data From the First 3 Years After Introduction. J Infect Dis. 2017 Jan;215(2):183–91.

184. Bar-Zeev N, King C, Phiri T, Beard J, Mvula H, Crampin AC, et al. Impact of monovalent rotavirus vaccine on diarrhoea-associated post-neonatal infant mortality in rural communities in Malawi: a population-based birth cohort study. Lancet Glob Heal. 2018 Sep;6(9):e1036–44.

185. Enane LA, Gastañaduy PA, Goldfarb DM, Pernica JM, Mokomane M, Moorad B, et al. Impact of Rotavirus Vaccination on Hospitalizations and Deaths from Childhood Gastroenteritis in Botswana. Clin Infect Dis [Internet]. 2016 May 1 [cited 2021 Jan 6];62(Suppl 2):S168–74. Available from: /pmc/articles/PMC4825886/?report=abstract

186. Tsolenyanu E, Djadou KE, Fiawoo M, Akolly DAE, Mwenda JM, Leshem E, et al. Evidence of the impact of monovalent rotavirus vaccine on childhood acute gastroenteritis hospitalization in Togo. Vaccine [Internet]. 2018;36(47):7185–91. Available from: https://doi.org/10.1016/j.vaccine.2018.01.058

187. de Deus N, Chilaúle JJ, Cassocera M, Bambo M, Langa JS, Sitoe E, et al. Early impact of rotavirus vaccination in children less than five years of age in Mozambique. Vaccine [Internet]. 2018 Nov

12 [cited 2026 Mar 8];36(47):7205–9. Available from: https://pubmed.ncbi.nlm.nih.gov/29128381/

188. Rahajamanana VL, Raboba JL, Rakotozanany A, Razafindraibe NJ, Andriatahirintsoa EJPR, Razafindrakoto AC, et al. Impact of rotavirus vaccine on all-cause diarrhea and rotavirus hospitalizations in Madagascar. Vaccine [Internet]. 2017 Nov 12 [cited 2026 Mar 8];36(47):7198. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC5867203/

189. Tsolenyanu E, Djadou KE, Fiawoo M, Akolly DAE, Mwenda JM, Leshem E, et al. Evidence of the impact of monovalent rotavirus vaccine on childhood acute gastroenteritis hospitalization in Togo. Vaccine [Internet]. 2018 Nov 12 [cited 2026 Mar 8];36(47):7185. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11973829/

190. Jani B, Hokororo A, Mchomvu J, Cortese MM, Kamugisha C, Mujuni D, et al. Detection of rotavirus before and after monovalent rotavirus vaccine introduction and vaccine effectiveness among children in mainland Tanzania. Vaccine [Internet]. 2018 Nov 12 [cited 2026 Mar 8];36(47):7149–56. Available from: https://pubmed.ncbi.nlm.nih.gov/29655631/

191. Mphahlele J, Abebe A, Getahun M, Sl M, Beyene B, Assefa E, et al. Impact of rotavirus vaccine introduction and genotypic. 2018;2011–6.

192. Sibomana H, Rugambwa C, Mwenda JM, Sayinzoga F, Iraguha G, Uwimana J, et al. Impact of routine rotavirus vaccination on all-cause and rotavirus hospitalizations during the first four years following vaccine introduction in Rwanda. Vaccine [Internet]. 2018;36(47):7135–41. Available from: https://doi.org/10.1016/j.vaccine.2018.01.072

193. Mpabalwani EM, Simwaka CJ, Mwenda JM, Mubanga CP, Monze M, Matapo B, et al. Impact of Rotavirus Vaccination on Diarrheal Hospitalizations in Children Aged <5 Years in Lusaka, Zambia. Clin Infect Dis [Internet]. 2016 May 1 [cited 2021 Jan 6];62(suppl 2):S183–7. Available from: https://academic.oup.com/cid/article-lookup/doi/10.1093/cid/civ1027

194. Enweronu-Laryea CC, Armah G, Sagoe KW, Ansong D, Addo-Yobo E, Diamenu SK, et al. Sustained impact of rotavirus vaccine introduction on rotavirus gastroenteritis hospitalizations in children <5 years of age, Ghana, 2009–2016. Vaccine [Internet]. 2018 Nov 12 [cited 2026 Mar 8];36(47):7131. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8848125/

195. Ahmed MLC brahim, Heukelbach J, Weddih A, Filali-Maltouf A, Sidatt M, Makhalla K, et al.

Reduction of hospitalizations with diarrhea among children aged 0–5 years in Nouakchott, Mauritania, following the introduction of rotavirus vaccine. Vaccine [Internet]. 2019 Mar 7 [cited 2026 Mar 8];37(11):1407. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11726317/

196. Diop A, Thiongane A, Mwenda JM, Aliabadi N, Sonko MA, Diallo A, et al. Impact of rotavirus vaccine on acute gastroenteritis in children under 5 years in Senegal: Experience of sentinel site of the Albert Royer Children's Hospital in Dakar. Vaccine [Internet]. 2018 Nov 12 [cited 2026 Mar 8];36(47):7192–7. Available from: https://pubmed.ncbi.nlm.nih.gov/29162319/

197. Mpabalwani EM, Simwaka JC, Mwenda JM, Matapo B, Parashar UD, Tate JE. Sustained impact of rotavirus vaccine on rotavirus hospitalisations in Lusaka, Zambia, 2009–2016. Vaccine [Internet]. 2018 Nov 12 [cited 2026 Mar 8];36(47):7165–9. Available from: https://pubmed.ncbi.nlm.nih.gov/29793891/

198. Maphalala G, Phungwayo N, Masona G, Lukhele N, Tsegaye G, Dube N, et al. Early impact of rotavirus vaccine in under 5 year old children hospitalized due to diarrhea, Swaziland. Vaccine [Internet]. 2018;36(47):7210–4. Available from: http://dx.doi.org/10.1016/j.vaccine.2017.07.072

199. Bennett A, Pollock L, Jere KC, Pitzer VE, Parashar U, Tate JE, et al. Direct and possible indirect effects of vaccination on rotavirus hospitalisations among children in Malawi four years after programmatic introduction. Vaccine [Internet]. 2018 Nov 11 [cited 2024 Jul 9];36(47):7142. Available from: /pmc/articles/PMC6238204/

200. Mujuru HA, Burnett E, Nathoo KJ, Ticklay I, Gonah NA, Mukaratirwa A, et al. Monovalent Rotavirus Vaccine Effectiveness Against Rotavirus Hospitalizations Among Children in Zimbabwe. Clin Infect Dis [Internet]. 2019 Sep 27 [cited 2026 Mar 8];69(8):1339. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8851377/

201. Groome MJ, Zell ER, Solomon F, Nzenze S, Parashar UD, Izu A, et al. Temporal Association of Rotavirus Vaccine Introduction and Reduction in All-Cause Childhood Diarrheal Hospitalizations in South Africa. Clin Infect Dis [Internet]. 2016 May 1 [cited 2026 Mar 8];62(Suppl 2):S188. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11345717/

202. Wandera EA, Mohammad S, Bundi M, Nyangao J, Galata A, Kathiiko C, et al. Impact of rotavirus vaccination on rotavirus hospitalisation rates among a resource-limited rural population in Mbita, Western Kenya. Trop Med Int Health [Internet]. 2018 Apr 1 [cited 2026 Mar 8];23(4):425–32.

Available from: https://pubmed.ncbi.nlm.nih.gov/29432666/

203. Madhi SA, Groome MJ, Zar HJ, Kapongo CN, Mulligan C, Nzenze S, et al. Effectiveness of pneumococcal conjugate vaccine against presumed bacterial pneumonia hospitalisation in HIV-uninfected South African children: a case-control study. Thorax [Internet]. 2015 Dec 1 [cited 2026 Mar 8];70(12):1149–55. Available from: https://pubmed.ncbi.nlm.nih.gov/26092924/

204. Izu A, Solomon F, Nzenze SA, Mudau A, Zell E, O'Brien KL, et al. Pneumococcal conjugate vaccines and hospitalization of children for pneumonia: a time-series analysis, South Africa, 2006–2014. Bull World Health Organ [Internet]. 2017 Sep 1 [cited 2026 Mar 8];95(9):618. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC5578378/

205. Cohen C, Von Mollendorf C, De Gouveia L, Naidoo N, Meiring S, Quan V, et al. Effectiveness of 7-Valent Pneumococcal Conjugate Vaccine Against Invasive Pneumococcal Disease in HIV-Infected and -Uninfected Children in South Africa: A Matched Case-Control Study. Clin Infect Dis [Internet]. 2014 Sep 15 [cited 2026 Mar 8];59(6):808–18. Available from: https://dx.doi.org/10.1093/cid/ciu431

206. Mackenzie GA, Hill PC, Jeffries DJ, Ndiaye M, Sahito SM, Hossain I, et al. Impact of the introduction of pneumococcal conjugate vaccination on invasive pneumococcal disease and pneumonia in The Gambia: 10 years of population-based surveillance. Lancet Infect Dis [Internet]. 2021 Sep 1 [cited 2026 Mar 8];21(9):1293–302. Available from: https://pubmed.ncbi.nlm.nih.gov/34280357/

207. Kambiré D, Soeters HM, Ouédraogo-Traoré R, Medah I, Sangaré L, Yaméogo I, et al. Early impact of 13-valent pneumococcal conjugate vaccine on pneumococcal meningitis-Burkina Faso, 2014-2015. J Infect. 2018 Mar;76(3):270–9.

208. Faye PM, Sonko MA, Diop A, Thiongane A, Ba ID, Spiller M, et al. Impact of 13-Valent Pneumococcal Conjugate Vaccine on Meningitis and Pneumonia Hospitalizations in Children aged <5 Years in Senegal, 2010-2016. Clin Infect Dis [Internet]. 2019 Sep 5 [cited 2026 Mar 8];69(Suppl 2):S66–71. Available from: https://pubmed.ncbi.nlm.nih.gov/31505625/

209. Cohen C, von Mollendorf C, de Gouveia L, Lengana S, Meiring S, Quan V, et al. Effectiveness of the 13-valent pneumococcal conjugate vaccine against invasive pneumococcal disease in South African children: a case-control study. Lancet Glob Heal [Internet]. 2017 Mar 1 [cited 2026 Mar

8];5(3):e359–69. Available from: https://pubmed.ncbi.nlm.nih.gov/28139443/

210. Dondo V, Mujuru H, Nathoo K, Jacha V, Tapfumanei O, Chirisa P, et al. Pneumococcal Conjugate Vaccine Impact on Meningitis and Pneumonia Among Children Aged <5 Years-Zimbabwe, 2010-2016. Clin Infect Dis [Internet]. 2019 Sep 5 [cited 2026 Mar 8];69(Suppl 2):S72–80. Available from: https://pubmed.ncbi.nlm.nih.gov/31505631/

211. Nhantumbo AA, Weldegebriel G, Katsande R, De Gouveia L, Comé CE, Cuco AZ, et al. Surveillance of impact of PCV-10 vaccine on pneumococcal meningitis in Mozambique, 2013 – 2015. PLoS One [Internet]. 2017 Jun 1 [cited 2026 Mar 8];12(6):e0177746. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0177746

212. Jenkins HE, Aylward RB, Gasasira A, Donnelly CA, Abanida EA, Koleosho-Adelekan T, et al. Effectiveness of immunization against paralytic poliomyelitis in Nigeria. N Engl J Med [Internet]. 2008 Oct 16 [cited 2026 Mar 8];359(16):1666–74. Available from: https://pubmed.ncbi.nlm.nih.gov/18923171/

213. Mahamud A, Kamadjeu R, Webeck J, Mbaeyi C, Baranyikwa MT, Birungi J, et al. Effectiveness of oral polio vaccination against paralytic poliomyelitis: a matched case-control study in Somalia. J Infect Dis. 2014 Nov;210 Suppl:S187-93.

214. Chippaux J-P, Diallo A, Marra A, Etard J-F. Impact of previous immunisation on the incidence of meningococcal disease during an outbreak in a Sahelian area of Senegal. Vaccine. 2007 Feb;25(10):1712–8.

215. Kristiansen PA, Diomandé F, Ba AK, Sanou I, Ouédraogo AS, Ouédraogo R, et al. Impact of the serogroup A meningococcal conjugate vaccine, MenAfriVac, on carriage and herd immunity. Clin Infect Dis [Internet]. 2013 Feb [cited 2026 Mar 8];56(3):354–63. Available from: https://pubmed.ncbi.nlm.nih.gov/23087396/

216. Gamougam K, Daugla DM, Toralta J, Ngadoua C, Fermon F, Page A-L, et al. Continuing effectiveness of serogroup A meningococcal conjugate vaccine, Chad, 2013. Emerg Infect Dis. 2015 Jan;21(1):115–8.

217. Sugerman DE, Fall A, Guigui M-T, N'dolie M, Balogun T, Wurie A, et al. Preplanned national measles vaccination campaign at the beginning of a measles outbreak--Sierra Leone, 2009-2010. J Infect Dis. 2011 Jul;204 Suppl:S260-9.

218. Kidd S, Ouedraogo B, Kambire C, Kambou JL, McLean H, Kutty PK, et al. Measles outbreak in Burkina Faso, 2009: a case-control study to determine risk factors and estimate vaccine effectiveness. Vaccine. 2012 Jul;30(33):5000–8.

219. Majwala RK, Nakiire L, Kadobera D, Ario AR, Kusiima J, Atuhairwe JA, et al. Measles outbreak propagated by children congregating at water collection points in Mayuge District, eastern Uganda, July - October, 2016. BMC Infect Dis. 2018 Aug;18(1):412.

220. Mupere E, Karamagi C, Zirembuzi G, Grabowsky M, de Swart RL, Nanyunja M, et al. Measles vaccination effectiveness among children under 5 years of age in Kampala, Uganda. Vaccine. 2006 May;24(19):4111–5.

221. Goodson JL, Wiesen E, Perry RT, Mach O, Kitambi M, Kibona M, et al. Impact of measles outbreak response vaccination campaign in Dar es Salaam, Tanzania. Vaccine. 2009 Sep;27(42):5870–4.

222. Doshi RH, Shidi C, Mulumba A, Eckhoff P, Nguyen C, Hoff NA, et al. The effect of immunization on measles incidence in the Democratic Republic of Congo: Results from a model of surveillance data. Vaccine [Internet]. 2015 Nov 27 [cited 2026 Mar 8];33(48):6786–92. Available from: https://pubmed.ncbi.nlm.nih.gov/26476363/

223. Minetti A, Kagoli M, Katsulukuta A, Huerga H, Featherstone A, Chiotcha H, et al. Lessons and challenges for measles control from unexpected large outbreak, Malawi. Emerg Infect Dis. 2013;19(2):202–9.

224. Doshi RH, Mukadi P, Shidi C, Mulumba A, Hoff NA, Gerber S, et al. Field evaluation of measles vaccine effectiveness among children in the Democratic Republic of Congo. Vaccine [Internet]. 2015 Jun 26 [cited 2026 Mar 8];33(29):3407–14. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0264410X15005435

225. Katz MA, Lebo E, Emukule GO, Otieno N, Caselton DL, Bigogo G, et al. Uptake and Effectiveness of a Trivalent Inactivated Influenza Vaccine in Children in Urban and Rural Kenya, 2010 to 2012. Pediatr Infect Dis J [Internet]. 2016 Mar 1 [cited 2026 Mar 8];35(3):322–9. Available from: https://pubmed.ncbi.nlm.nih.gov/26658627/

226. Ntshoe GM, McAnerney JM, Tempia S, Blumberg L, Moyes J, Buys A, et al. Influenza Epidemiology and Vaccine Effectiveness among Patients with Influenza-Like Illness, Viral Watch Sentinel Sites, South Africa, 2005–2009. PLoS One [Internet]. 2014 Apr 15 [cited 2026 Mar 8];9(4):e94681.

Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0094681

227. Hansen OB, Rodrigues A, Martins C, Rieckmann A, Benn CS, Aaby P, et al. Impact of H1N1 Influenza Vaccination on Child Morbidity in Guinea-Bissau. J Trop Pediatr [Internet]. 2019 Oct 1 [cited 2026 Mar 8];65(5):446–56. Available from: https://dx.doi.org/10.1093/tropej/fmy075

228. McAnerney JM, Walaza S, Tempia S, Blumberg L, Treurnicht FK, Madhi SA, et al. Estimating vaccine effectiveness in preventing laboratory-confirmed influenza in outpatient settings in South Africa, 2015. Influenza Other Respi Viruses [Internet]. 2017 Mar 1 [cited 2026 Mar 8];11(2):177–81. Available from: https://pubmed.ncbi.nlm.nih.gov/27865064/

229. McAnerney JM, Walaza S, Cohen AL, Tempia S, Buys A, Venter M, et al. Effectiveness and knowledge, attitudes and practices of seasonal influenza vaccine in primary healthcare settings in South Africa, 2010-2013. Influenza Other Respi Viruses. 2015 May;9(3):143–50.

230. Lee EHJ, Lewis RF, Makumbi I, Kekitiinwa A, Ediamu TD, Bazibu M, et al. Haemophilus influenzae type b conjugate vaccine is highly effective in the Ugandan routine immunization program: a case-control study. Trop Med Int Health [Internet]. 2008 Apr [cited 2026 Mar 8];13(4):495–502. Available from: https://pubmed.ncbi.nlm.nih.gov/18312475/

231. Madhi SA, Petersen K, Khoosal M, Huebner RE, Mbelle N, Mothupi R, et al. Reduced effectiveness of Haemophilus influenzae type b conjugate vaccine in children with a high prevalence of human immunodeficiency virus type 1 infection. Pediatr Infect Dis J [Internet]. 2002 [cited 2026 Mar 8];21(4):315–21. Available from: https://pubmed.ncbi.nlm.nih.gov/12075763/

232. Fleming JA, Dieye Y, Ba O, Mutombo Wa Mutombo B, Diallo N, Faye PC, et al. Effectiveness of haemophilus influenzae type B conjugate vaccine for prevention of meningitis in Senegal. Pediatr Infect Dis J [Internet]. 2011 [cited 2026 Mar 8];30(5):430–2. Available from: https://pubmed.ncbi.nlm.nih.gov/21099444/

233. Cowgill KD, Ndiritu M, Nyiro J, Slack MPE, Chiphatsi S, Ismail A, et al. Effectiveness of Haemophilus influenzae type b conjugate vaccine introduction into routine childhood immunization in Kenya. JAMA [Internet]. 2006 Aug 9 [cited 2026 Mar 8];296(6):671. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC1592684/

234. Lucas MES, Deen JL, von Seidlein L, Wang X-Y, Ampuero J, Puri M, et al. Effectiveness of mass oral cholera vaccination in Beira, Mozambique. N Engl J Med [Internet]. 2005 Feb 24 [cited 2026 Mar

8];352(8):757–67. Available from: https://pubmed.ncbi.nlm.nih.gov/15728808/

235. Luquero FJ, Grout L, Ciglenecki I, Sakoba K, Traore B, Heile M, et al. Use of Vibrio cholerae vaccine in an outbreak in Guinea. N Engl J Med [Internet]. 2014 May 29 [cited 2026 Mar 8];370(22):2111–20. Available from: https://pubmed.ncbi.nlm.nih.gov/24869721/

236. Tate JE, Ngabo F, Donnen P, Gatera M, Uwimana J, Rugambwa C, et al. Effectiveness of Pentavalent Rotavirus Vaccine Under Conditions of Routine Use in Rwanda. Clin Infect Dis [Internet]. 2016 May 1 [cited 2026 Mar 8];62 Suppl 2(Suppl 2):S208–12. Available from: https://pubmed.ncbi.nlm.nih.gov/27059358/

237. Bar-zeev N, Jere KC, Bennett A, Pollock L, Tate JE, Nakagomi O, et al. Population Impact and Effectiveness of Monovalent Rotavirus Vaccination in Urban Malawian Children 3 Years After Vaccine Introduction : Ecological and Case-Control Analyses. 2016;62(Suppl 2):213–9.

238. Armah G, Pringle K, Enweronu-Laryea CC, Ansong D, Mwenda JM, Diamenu SK, et al. Impact and Effectiveness of Monovalent Rotavirus Vaccine Against Severe Rotavirus Diarrhea in Ghana. Clin Infect Dis [Internet]. 2016 May 1 [cited 2023 Feb 21];62 Suppl 2(Suppl 2):S200–7. Available from: https://pubmed.ncbi.nlm.nih.gov/27059357/

239. Mujuru HA, Burnett E, Nathoo KJ, Ticklay I, Gonah NA, Mukaratirwa A, et al. Monovalent Rotavirus Vaccine Effectiveness Against Rotavirus Hospitalizations Among Children in Zimbabwe. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2019 Sep;69(8):1339–44.

240. Sibomana H, Rugambwa C, Mwenda JM, Sayinzoga F, Iraguha G, Uwimana J, et al. Impact of routine rotavirus vaccination on all-cause and rotavirus hospitalizations during the first four years following vaccine introduction in Rwanda. Vaccine [Internet]. 2018 Nov 12 [cited 2026 Mar 8];36(47):7135. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11973826/

241. Enweronu-Laryea CC, Armah G, Sagoe KW, Ansong D, Addo-Yobo E, Diamenu SK, et al. Sustained impact of rotavirus vaccine introduction on rotavirus gastroenteritis hospitalizations in children <5 years of age, Ghana, 2009–2016. Vaccine [Internet]. 2018 Nov 12 [cited 2021 Jan 6];36(47):7131–4. Available from: https://pubmed.ncbi.nlm.nih.gov/29752020/

242. Ahmed M-LC-B, Heukelbach J, Weddih A, Filali-Maltouf A, Sidatt M, Makhalla K, et al. Reduction of hospitalizations with diarrhea among children aged 0-5 years in Nouakchott, Mauritania, following the introduction of rotavirus vaccine. Vaccine. 2019 Mar;37(11):1407–11.

243. Mpabalwani EM, Simwaka JC, Mwenda JM, Matapo B, Parashar UD, Tate JE. Sustained impact of rotavirus vaccine on rotavirus hospitalisations in Lusaka, Zambia, 2009-2016. Vaccine [Internet]. 2018 Nov 12 [cited 2023 Feb 22];36(47):7165–9. Available from: https://pubmed.ncbi.nlm.nih.gov/29793891/

244. Bennett A, Pollock L, Jere KC, Pitzer VE, Parashar U, Tate JE, et al. Direct and possible indirect effects of vaccination on rotavirus hospitalisations among children in Malawi four years after programmatic introduction. Vaccine. 2018 Nov;36(47):7142–8.

245. Wandera EA, Mohammad S, Bundi M, Nyangao J, Galata A, Kathiiko C, et al. Impact of rotavirus vaccination on rotavirus hospitalisation rates among a resource-limited rural population in Mbita, Western Kenya. Trop Med Int Health [Internet]. 2018 Apr 1 [cited 2023 Feb 22];23(4):425–32. Available from: https://pubmed.ncbi.nlm.nih.gov/29432666/

246. Enane LA, Gastañaduy PA, Goldfarb DM, Pernica JM, Mokomane M, Moorad B, et al. Impact of Rotavirus Vaccination on Hospitalizations and Deaths From Childhood Gastroenteritis in Botswana. Clin Infect Dis [Internet]. 2016 May 1 [cited 2026 Mar 8];62(suppl_2):S168–74. Available from: https://dx.doi.org/10.1093/cid/civ1210

247. Rahajamanana VL, Raboba JL, Rakotozanany A, Razafindraibe NJ, Andriatahirintsoa EJPR, Razafindrakoto AC, et al. Impact of rotavirus vaccine on all-cause diarrhea and rotavirus hospitalizations in Madagascar. Vaccine. 2018 Nov;36(47):7198–204.

248. Tsolenyanu E, Mwenda JM, Dagnra A, Leshem E, Godonou M, Nassoury I, et al. Early Evidence of Impact of Monovalent Rotavirus Vaccine in Togo. Clin Infect Dis [Internet]. 2016 May 1 [cited 2026 Mar 8];62 Suppl 2:S196–9. Available from: https://pubmed.ncbi.nlm.nih.gov/27059356/

249. Bar-Zeev N, Jere KC, Bennett A, Pollock L, Tate JE, Nakagomi O, et al. Population Impact and Effectiveness of Monovalent Rotavirus Vaccination in Urban Malawian Children 3 Years After Vaccine Introduction: Ecological and Case-Control Analyses. Clin Infect Dis [Internet]. 2016 May 1 [cited 2026 Mar 8];62 Suppl 2(Suppl 2):S213–9. Available from: https://pubmed.ncbi.nlm.nih.gov/27059359/

250. Soeters HM, Kambiré D, Sawadogo G, Ouédraogo-Traoré R, Bicaba B, Medah I, et al. Impact of 13-Valent Pneumococcal Conjugate Vaccine on Pneumococcal Meningitis, Burkina Faso, 2016-2017. J Infect Dis [Internet]. 2019 Oct 31 [cited 2026 Mar 8];220(220 Suppl 4):S253–62. Available

from: https://pubmed.ncbi.nlm.nih.gov/31671444/

251. Faye PM, Sonko MA, Diop A, Thiongane A, Ba ID, Spiller M, et al. Impact of 13-Valent Pneumococcal Conjugate Vaccine on Meningitis and Pneumonia Hospitalizations in Children aged <5 Years in Senegal, 2010-2016. Clin Infect Dis [Internet]. 2019 Sep 5 [cited 2020 Dec 18];69(Suppl 2):S66–71. Available from: /pmc/articles/PMC6756160/?report=abstract

252. Dondo V, Mujuru H, Nathoo K, Jacha V, Tapfumanei O, Chirisa P, et al. Pneumococcal Conjugate Vaccine Impact on Meningitis and Pneumonia Among Children Aged <5 Years-Zimbabwe, 2010-2016. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2019 Sep;69(Suppl 2):S72–80.

253. Mupere E, Karamagi C, Zirembuzi G, Grabowsky M, de Swart RL, Nanyunja M, et al. Measles vaccination effectiveness among children under 5 years of age in Kampala, Uganda. Vaccine [Internet]. 2006 May 8 [cited 2026 Mar 8];24(19):4111–5. Available from: https://pubmed.ncbi.nlm.nih.gov/16554111/

254. Sugerman DE, Fall A, Guigui MT, N'dolie M, Balogun T, Wurie A, et al. Preplanned national measles vaccination campaign at the beginning of a measles outbreak--Sierra Leone, 2009-2010. J Infect Dis [Internet]. 2011 Jul 1 [cited 2026 Mar 8];204 Suppl 1(SUPPL. 1). Available from: https://pubmed.ncbi.nlm.nih.gov/21666171/

255. Ntshoe GM, McAnerney JM, Tempia S, Blumberg L, Moyes J, Buys A, et al. Influenza epidemiology and vaccine effectiveness among patients with influenza-like illness, viral watch sentinel sites, South Africa, 2005-2009. PLoS One. 2014;9(4):e94681.

256. McAnerney JM, Walaza S, Cohen AL, Tempia S, Buys A, Venter M, et al. Effectiveness and knowledge, attitudes and practices of seasonal influenza vaccine in primary healthcare settings in South Africa, 2010–2013. Influenza Other Respi Viruses [Internet]. 2015 May 1 [cited 2026 Mar 8];9(3):143. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC4415698/

257. Chippaux JP, Diallo A, Marra A, Étard JF. Impact of previous immunisation on the incidence of meningococcal disease during an outbreak in a Sahelian area of Senegal. Vaccine [Internet]. 2007 Feb 26 [cited 2026 Mar 8];25(10):1712–8. Available from: https://pubmed.ncbi.nlm.nih.gov/17240492/

258. Gamougam K, Daugla DM, Toralta J, Ngadoua C, Fermon F, Page AL, et al. Continuing effectiveness of serogroup A meningococcal conjugate vaccine, Chad, 2013. Emerg Infect Dis

[Internet]. 2015 [cited 2026 Mar 8];21(1):115–8. Available from: https://pubmed.ncbi.nlm.nih.gov/25536336/

259. Mahamud A, Kamadjeu R, Webeck J, Mbaeyi C, Baranyikwa MT, Birungi J, et al. Effectiveness of oral polio vaccination against paralytic poliomyelitis: a matched case-control study in Somalia. J Infect Dis [Internet]. 2014 Nov 1 [cited 2026 Mar 8];210 Suppl 1:S187–93. Available from: https://pubmed.ncbi.nlm.nih.gov/25316835/

260. Ndeketa L, Haine V, Debois M, Asante KP, Agyapong PD, Kaali S, et al. Effectiveness of the RTS,S/AS01E malaria vaccine in a real-world setting over 1 year of follow-up after the three-dose primary schedule: an interim analysis of a phase 4 study in Ghana, Kenya, and Malawi. Lancet Glob Heal [Internet]. 2025 Jan 1 [cited 2026 Jan 7];14(1):e61–9. Available from: https://www.thelancet.com/action/showFullText?pii=S2214109X25004152

261. Bar-Zeev N, Jere KC, Bennett A, Pollock L, Tate JE, Nakagomi O, et al. Population Impact and Effectiveness of Monovalent Rotavirus Vaccination in Urban Malawian Children 3 Years After Vaccine Introduction: Ecological and Case-Control Analyses. Clin Infect Dis [Internet]. 2016 May 1 [cited 2023 Feb 23];62 Suppl 2(Suppl 2):S213–9. Available from: https://pubmed.ncbi.nlm.nih.gov/27059359/

262. Ahmed ST, Haider SS, Hanif S, Anwar HB, Mehjabeen S, Closser S, et al. A scoping review on integrated health campaigns for immunization in low- and middle-income countries. Health Policy Plan [Internet]. 2023 Nov 28 [cited 2026 Jan 14];38(10):1198–224. Available from: https://dx.doi.org/10.1093/heapol/czad082

263. Grimes DA, Schulz KF. Cohort studies: Marching towards outcomes. Lancet [Internet]. 2002 Jan 26 [cited 2026 Jan 14];359(9303):341–5. Available from: https://www.thelancet.com/action/showFullText?pii=S0140673602075001

264. Izu A, Solomon F, Nzenze SA, Mudau A, Zell E, O'Brien KL, et al. Pneumococcal conjugate vaccines and hospitalization of children for pneumonia: a time-series analysis, South Africa, 2006–2014. Bull World Health Organ [Internet]. 2017 Sep 1 [cited 2025 Jul 2];95(9):618. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC5578378/

265. Asante KP, Mathanga DP, Milligan P, Akech S, Oduro A, Mwapasa V, et al. Feasibility, safety, and impact of the RTS,S/AS01E malaria vaccine when implemented through national immunisation

programmes: evaluation of cluster-randomised introduction of the vaccine in Ghana, Kenya, and Malawi. Lancet [Internet]. 2024 Apr 27 [cited 2026 Jan 14];403(10437):1660–70. Available from: https://www.sciencedirect.com/science/article/pii/S0140673624000047

266. Ozawa S, Clark S, Portnoy A, Grewal S, Brenzel L, Walker DG. Return On Investment From Childhood Immunization In Low- And Middle-Income Countries, 2011–20. https://doi.org/101377/hlthaff20151086 [Internet]. 2017 Aug 2 [cited 2026 Jan 15];35(2):199–207. Available from: /doi/pdf/10.1377/hlthaff.2015.1086

267. Sallam M, Sallam M. COVID-19 Vaccine Hesitancy Worldwide: A Concise Systematic Review of Vaccine Acceptance Rates. Vaccines 2021, Vol 9, [Internet]. 2021 Feb 16 [cited 2026 Jan 15];9(2):1–15. Available from: https://www.mdpi.com/2076-393X/9/2/160

268. Madhi SA, Cunliffe NA, Steele D, Witte D, Kirsten M, Louw C, et al. Effect of Human Rotavirus Vaccine on Severe Diarrhea in African Infants. N Engl J Med [Internet]. 2010 Jan 28 [cited 2021 Jan 6];362(4):289–98. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa0904797

269. Hasso-Agopsowicz M, Ladva CN, Lopman B, Sanderson C, Cohen AL, Tate JE, et al. Global Review of the Age Distribution of Rotavirus Disease in Children Aged <5 Years Before the Introduction of Rotavirus Vaccination. Clin Infect Dis [Internet]. 2019 Aug 30 [cited 2025 Oct 21];69(6):1071–8. Available from: https://dx.doi.org/10.1093/cid/ciz060

270. Burnett E, Parashar UD, Tate JE. Rotavirus Infection, Illness, and Vaccine Performance in Malnourished Children: A Review of the Literature. Pediatr Infect Dis J [Internet]. 2021;40(10):930–6. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=med20&NEWS=N&AN=34117200

271. Word Health Organization. Rotavirus: Vaccine Preventable Diseases Surveillance Standards. 2018;1–11. Available from: https://www.who.int/publications/m/item/vaccine-preventable-diseases-surveillance-standards-rotavirus

272. Pitzer VE, Ndeketa L, Asare EO, Hungerford D, Lopman BA, Jere KC, et al. Impact of rotavirus vaccination in Malawi from 2012 to 2022 compared to model predictions. npj Vaccines 2024 91 [Internet]. 2024 Nov 19 [cited 2025 May 18];9(1):1–8. Available from: https://www.nature.com/articles/s41541-024-01008-6

273. Ciarlet M, Sani-Grosso R, Yuan G, Liu GF, Heaton PM, Gottesdiener KM, et al. Concomitant use of

the oral pentavalent human-bovine reassortant rotavirus vaccine and oral poliovirus vaccine. Pediatr Infect Dis J [Internet]. 2008 [cited 2024 Jul 9];27(10):874–80. Available from: https://journals.lww.com/pidj/fulltext/2008/10000/concomitant_use_of_the_oral_pentavalent.4.aspx

274. Patel M, Steele AD, Parashar UD. Influence of oral polio vaccines on performance of the monovalent and pentavalent rotavirus vaccines. Vaccine. 2012 Apr 27;30(SUPPL. 1):A30–5.

275. Emperador DM, Velasquez DE, Estivariz CF, Lopman B, Jiang B, Parashar U, et al. Interference of Monovalent, Bivalent, and Trivalent Oral Poliovirus Vaccines on Monovalent Rotavirus Vaccine Immunogenicity in Rural Bangladesh. Clin Infect Dis [Internet]. 2016 Jan 15 [cited 2024 Oct 14];62(2):150–6. Available from: https://dx.doi.org/10.1093/cid/civ807

276. Okayasu H, Sutter RW, Czerkinsky C, Ogra PL. Mucosal immunity and poliovirus vaccines: impact on wild poliovirus infection and transmission. Vaccine [Internet]. 2011 Oct 26 [cited 2023 Aug 22];29(46):8205–14. Available from: https://pubmed.ncbi.nlm.nih.gov/21896299/

277. Maldonado YA, Peña-Cruz V, De La Sanchez ML, Logan L, Blandón S, Cantwell MF, et al. Host and viral factors affecting the decreased immunogenicity of Sabin type 3 vaccine after administration of trivalent oral polio vaccine to rural Mayan children. J Infect Dis [Internet]. 1997 [cited 2023 Aug 22];175(3):545–53. Available from: https://pubmed.ncbi.nlm.nih.gov/9041324/

278. Ministry of Health. Government of Malawi. EPI Comprehensive Multi-Year Plan Malawi September. 2020;(September 2015):1–147.

279. Turner A, Ngwira B, Witte D, Mwapasa M, Dove W, Cunliffe N. Surveillance of rotavirus gastro-enteritis in children in Blantyre, Malawi. Paediatr Int Child Health [Internet]. 2013 Feb [cited 2026 Jun 20];33(1):42–5. Available from: https://www.tandfonline.com/doi/pdf/10.1179/2046905512Y.0000000015

280. Bar-Zeev N, Jere KC, Bennett A, Pollock L, Tate JE, Nakagomi O, et al. Population Impact and Effectiveness of Monovalent Rotavirus Vaccination in Urban Malawian Children 3 Years After Vaccine Introduction: Ecological and Case-Control Analyses. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2016 May;62 Suppl 2(Suppl 2):S213-9.

281. Cunliffe NA, Ngwira BM, Dove W, Thindwa BDM, Turner AM, Broadhead RL, et al. Epidemiology of Rotavirus Infection in Children in Blantyre, Malawi, 1997–2007. J Infect Dis [Internet]. 2010

Sep 1 [cited 2023 Aug 7];202(Supplement_1):S168–74. Available from: https://dx.doi.org/10.1086/653577

282. Mhango C, Mandolo JJ, Chinyama E, Wachepa R, Kanjerwa O, Malamba-Banda C, et al. Rotavirus Genotypes in Hospitalized Children With Acute Gastroenteritis Before and After Rotavirus Vaccine Introduction in Blantyre, Malawi, 1997–2019. J Infect Dis [Internet]. 2022 Jun 6 [cited 2023 Aug 8];225(12):2127. Available from: /pmc/articles/PMC9200156/

283. Turner A, Ngwira B, Witte D, Mwapasa M, Dove W, Cunliffe N. Surveillance of rotavirus gastro-enteritis in children in Blantyre, Malawi. Paediatr Int Child Health. 2013 Feb;33(1):42–5.

284. Wandera EA, Muriithi B, Kathiiko C, Mutunga F, Wachira M, Mumo M, et al. Impact of integrated water, sanitation, hygiene, health and nutritional interventions on diarrhoea disease epidemiology and microbial quality of water in a resource-constrained setting in Kenya: A controlled intervention study. Trop Med Int Heal [Internet]. 2022 Aug 1 [cited 2024 Jul 9];27(8):669–77. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/tmi.13793

285. Mameli C, Fabiano V, Zuccotti GV. New insights into rotavirus vaccines. Hum Vaccin Immunother [Internet]. 2012 Aug [cited 2025 May 23];8(8):1022. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC3551871/

286. Bar-Zeev N, Kapanda L, Tate JE, Jere KC, Iturriza-Gomara M, Nakagomi O, et al. Effectiveness of a monovalent rotavirus vaccine in infants in Malawi after programmatic roll-out: an observational and case-control study. Lancet Infect Dis. 2015 Apr;15(4):422–8.

287. Mandolo J, Mulira L, Moyo M, Mvula M, Mtonga F, Henrion MYR, et al. Maternal-infant rotavirus-specific antibody kinetics to inform timing of vaccine boosting in Malawi: An observational study. PLOS Med [Internet]. 2025 Sep 1 [cited 2025 Oct 16];22(9):e1004734. Available from: https://journals.plos.org/plosmedicine/article?id=10.1371/journal.pmed.1004734

288. Wenger C, Asare EO, Kwon J, Li X, Mwinjiwa E, Chinkhumba J, et al. Cost-effectiveness analysis of alternative infant and neonatal rotavirus vaccination schedules in Malawi. PLOS Glob Public Heal [Internet]. 2025 Apr 1 [cited 2026 Jan 2];5(4):e0004341. Available from: https://journals.plos.org/globalpublichealth/article?id=10.1371/journal.pgph.0004341

289. World Health Organization. Malawi: WHO and UNICEF estimates of immunization coverage: 2023 revision [Internet]. 2023. Available from: chrome-

extension://efaidnbmnnnibpcajpcglclefindmkaj/https://cdn.who.int/media/docs/default-source/country-profiles/immunization/2024-country-profiles/immunization-2024-mwi.pdf?sfvrsn=b1c8ed49_3&download=true

290. Simbeye AJ, Kumwenda S, Cohee LM, Omondi D, Masibo PK, Wao H, et al. Factors associated with malaria vaccine uptake in Nsanje district, Malawi. Malar J [Internet]. 2024 Dec 1 [cited 2025 Oct 21];23(1):1–10. Available from: https://malariajournal.biomedcentral.com/articles/10.1186/s12936-024-04938-7

291. Greenwood BM, Bradley-Moore AM, Bradley AK, Kirkwood BR, Gilles HM. The immune response to vaccination in undernourished and well-nourished Nigerian children. Ann Trop Med Parasitol [Internet]. 1986 [cited 2025 Oct 21];80(5):537–44. Available from: https://www.tandfonline.com/doi/abs/10.1080/00034983.1986.11812062

292. Chopra K, Kundu S, Chowdhury DS. Antibody Response of Infants in Tropics to Five Doses of Oral Polio Vaccine. J Trop Pediatr [Internet]. 1989 Feb 1 [cited 2025 Oct 21];35(1):19–23. Available from: https://dx.doi.org/10.1093/tropej/35.1.19

293. Saleem AF, Mach O, Quadri F, Khan A, Bhatti Z, Rehman NU, et al. Immunogenicity of poliovirus vaccines in chronically malnourished infants: A randomized controlled trial in Pakistan. Vaccine [Internet]. 2015 Jun 4 [cited 2025 Oct 21];33(24):2757–63. Available from: https://www.sciencedirect.com/science/article/pii/S0264410X15005198?via%3Dihub

294. Morgan B, Lyons EA, Handley A, Bogdanovic-Sakran N, Pavlic D, Witte D, et al. Rotavirus-Specific Maternal Serum Antibodies and Vaccine Responses to RV3-BB Rotavirus Vaccine Administered in a Neonatal or Infant Schedule in Malawi. Viruses [Internet]. 2024 Sep 1 [cited 2025 Oct 21];16(9):1488. Available from: https://www.mdpi.com/1999-4915/16/9/1488/htm

295. At Thobari J, Damayanti W, Haposan JH, Nirwati H, Iskandar K, Samad, et al. Safety and immunogenicity of human neonatal RV3 rotavirus vaccine (Bio Farma) in adults, children, and neonates in Indonesia: Phase I Trial. Vaccine [Internet]. 2021;39(33):4651–8. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=med20&NEWS=N&AN=34244006

296. Witte D, Handley A, Jere KC, Bogandovic-Sakran N, Mpakiza A, Turner A, et al. Neonatal rotavirus vaccine (RV3-BB) immunogenicity and safety in a neonatal and infant administration schedule in Malawi: a randomised, double-blind, four-arm parallel group dose-ranging study. Lancet Infect

Dis [Internet]. 2022 May 1 [cited 2026 Feb 4];22(5):668. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC9021029/

297. WHO, UNICEF. Malawi: WHO and UNICEF estimates of immunization coverage: 2022 revision. 2022;1–23. Available from: https://data.unicef.org/wp-content/uploads/cp/immunisation/mwi.pdf

298. Ganesan D, Gupta S Sen, Legros D. Cholera surveillance and estimation of burden of cholera. Vaccine. 2020 Feb 29;38:A13–7.

299. World Health Organization. Cholera [Internet]. 2024 [cited 2025 Dec 17]. Available from: https://www.who.int/news-room/fact-sheets/detail/cholera

300. Miggo M, Harawa G, Kangwerema A, Knovicks S, Mfune C, Safari J, et al. Fight against cholera outbreak, efforts and challenges in Malawi. Heal Sci Reports [Internet]. 2023 Oct 1 [cited 2025 Jan 29];6(10):e1594. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10551271/

301. UNICEF. Malawi Cholera Response Flash Update - 20 to 26 February 2023 | UNICEF Malawi [Internet]. 2023 [cited 2025 Dec 17]. Available from: https://www.unicef.org/malawi/reports/malawi-cholera-response-flash-update-20-26-february-2023

302. Dunoyer J, Ratnayake R, Moore S, Bulit G, Beaulieu S, Valingot C, et al. Optimizing the implementation of case-area targeted interventions during cholera outbreaks with context-specific delivery mechanisms. PLoS Negl Trop Dis [Internet]. 2025 Sep 1 [cited 2025 Dec 17];19(9):e0013534. Available from: https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0013534

303. Finger F, Bertuzzo E, Luquero FJ, Naibei N, Touré B, Allan M, et al. The potential impact of case-area targeted interventions in response to cholera outbreaks: A modeling study. PLoS Med [Internet]. 2018 Feb 1 [cited 2025 Dec 17];15(2):e1002509. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC5828347/

304. Nash RK, Nouvellet P, Cori A. Real-time estimation of the epidemic reproduction number: Scoping review of the applications and challenges. PLOS Digit Heal [Internet]. 2022 Jun 1 [cited 2025 Dec 17];1(6):e0000052. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC9931334/

305. Malawi National Statistics Office. Population Projections 2018 - 2050 [Internet]. 2018 [cited 2025 Dec 18]. Available from: https://www.nsomalawi.mw/census/2018

306. Severe Malaria Observatory. Malawi health system | smo [Internet]. 2020 [cited 2025 Dec 18]. Available from: https://www.severemalaria.org/countries/malawi/malawi-health-system

307. Cori A, Ferguson NM, Fraser C, Cauchemez S. A New Framework and Software to Estimate Time-Varying Reproduction Numbers During Epidemics. Am J Epidemiol [Internet]. 2013 Nov 1 [cited 2025 Feb 4];178(9):1505–12. Available from: https://dx.doi.org/10.1093/aje/kwt133

308. Chabuka L, Choga WT, Mavian CN, Moir M, Tegally H, Wilkinson E, et al. Genomic epidemiology of the cholera outbreak in Malawi 2022-2023. medRxiv [Internet]. 2023;2(2):2023.08.22.23294324. Available from: https://www.medrxiv.org/content/10.1101/2023.08.22.23294324v1%0Ahttps://www.medrxiv.org/content/10.1101/2023.08.22.23294324v1.abstract

309. Bai J, Perron P. Estimating and Testing Linear Models with Multiple Structural Changes. Econometrica. 1998 Jan;66(1):47.

310. Xu H, Tiffany A, Luquero FJ, Kanungo S, Bwire G, Qadri F, et al. Protection from killed whole-cell cholera vaccines: a systematic review and meta-analysis. Lancet Glob Heal [Internet]. 2025 Jul 1 [cited 2025 Dec 16];13(7):e1203. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC12208782/

311. Malembaka EB, Bugeme PM, Hutchins C, Xu H, Hulse JD, Demby MN, et al. Effectiveness of one dose of killed oral cholera vaccine in an endemic community in the Democratic Republic of the Congo: a matched case-control study. Lancet Infect Dis. 2024;24(5):514–22.

312. Hulland EN, Charpignon M-L, El Hayek GY, Zhao L, Desai AN, Majumder MS. Estimating time-varying cholera transmission and oral cholera vaccine effectiveness in Haiti and Cameroon, 2021-2023. medRxiv [Internet]. 2024 Jun 13 [cited 2025 Dec 16];2024.06.12.24308792. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11343247/

313. Taylor DL, Kahawita TM, Cairncross S, Ensink JHJ. The Impact of Water, Sanitation and Hygiene Interventions to Control Cholera: A Systematic Review. PLoS One [Internet]. 2015 Aug 18 [cited 2025 Dec 17];10(8):e0135676. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC4540465/

314. Brown J, Cavill S, Cumming O, Jeandron A. Water, sanitation, and hygiene in emergencies: summary review and recommendations for further research. Waterlines. 2012 Jan 1;31(1):11–29.

315. Lantagne D, Yates T. Household Water Treatment and Cholera Control. J Infect Dis [Internet]. 2018 Oct 15 [cited 2025 Dec 17];218(Suppl 3):S147. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC6188534/

316. World Health Organization. Global technical strategy for malaria 2016–2030. World Heal Organ [Internet]. 2016;1–35. Available from: https://apps.who.int/iris/bitstream/handle/10665/186671/9789243564999_spa.pdf?sequence=1

317. National Malaria Control Programme (NMCP). Malawi Malaria Indicator Survey 2021. Lilongwe, Malawi; 2021.

318. National Malaria Control Programme (NMCP). NATIONAL MALARIA STRATEGIC PLAN (2023-2030). 2023.

319. Government of Malawi. Health Sector Strategic Plan III 2023 - 2030: Reforming for Universal Health Coverage. GoM [Internet]. 2023;(2023–2030):123. Available from: https://extranet.who.int/countryplanningcycles/sites/default/files/planning_cycle_repository/malawi/health_sector_strategic_plan_ii_030417_smt_dps.pdf

320. Cohee LM, Goupeyou-Youmsi J, Seydel KB, Mangani C, Ntenda P, Sixpence A, et al. Understanding the Intransigence of Malaria in Malawi. Am J Trop Med Hyg. 2022 Oct;107(4_Suppl):40–8.

321. Chipeta M, Terlouw D, Phiri K, Diggle P. Inhibitory geostatistical designs for spatial prediction taking account of uncertain covariance structure. Environmetrics. 2016;

322. Emelda A Okiro, Abdisalan M Noor, Josephine Malinga, Bernard Mitto, Clara W Mundia, Don Mathanga TM& RWS. An epidemiological profile of malaria and its control in Malawi. A report prepared for the Ministry of Health, the Roll Back Malaria Partnership and the Department for International Development, UK. March, 2014. 2014.

323. World Health Organization. Malaria vaccines: WHO position paper. 2024.

324. Asante KP, Mathanga DP, Milligan P, Akech S, Oduro A, Mwapasa V, et al. Feasibility, safety, and

impact of the RTS,S/AS01E malaria vaccine when implemented through national immunisation programmes: evaluation of cluster-randomised introduction of the vaccine in Ghana, Kenya, and Malawi. Lancet [Internet]. 2024 Apr 27 [cited 2026 Apr 22];403(10437):1660–70. Available from: https://www.thelancet.com/action/showFullText?pii=S0140673624000047

325. Mwapasa V, Asante KP, Milligan P, Akech S, Oduro A, Mathanga DP, et al. Impact of introducing RTS , S / AS01 E malaria vaccine on mortality in young children in Ghana , Kenya , and Malawi : an observational evaluation of a cluster-randomised implementation programme. Lancet [Internet]. 2026;407(10541):1796–808. Available from: http://dx.doi.org/10.1016/S0140-6736(26)00248-5

326. Malaria Vaccine Implementation Programme (MVIP). Malawi MVIP Endline Household Survey Report. Vol. 0, MVIP. 2022.

327. Pearce N. Analysis of matched case-control studies. BMJ. 2016;352:1–4.

328. Clinical Trials Partnership S. Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: Final results of a phase 3, individually randomised, controlled trial. Lancet [Internet]. 2015 Jul 4 [cited 2026 Apr 8];386(9988):31–45. Available from: https://www.thelancet.com/action/showFullText?pii=S0140673615607218

329. Richard J. Hayes LHM. Cluster Randomised Trials. Second edi. Vol. 2. 2024. 306–312 p.

330. Hemming K, Taljaard M. Key considerations for designing, conducting and analysing a cluster randomized trial. Int J Epidemiol [Internet]. 2023 Oct 5 [cited 2026 Apr 22];52(5):1648–58. Available from: https://dx.doi.org/10.1093/ije/dyad064

331. Qadri F, Ali M, Lynch J, Chowdhury F, Khan AI, Wierzba TF, et al. Efficacy of a single-dose regimen of inactivated whole-cell oral cholera vaccine: results from 2 years of follow-up of a randomised trial. Lancet Infect Dis [Internet]. 2018 Jun 1 [cited 2026 May 4];18(6):666–74. Available from: https://www.thelancet.com/action/showFullText?pii=S1473309918301087

332. Lewnard JA, Tedijanto C, Cowling BJ, Lipsitch M. Measurement of Vaccine Direct Effects Under the Test-Negative Design. Am J Epidemiol [Internet]. 2018 Dec 1 [cited 2026 May 4];187(12):2686–97. Available from: https://dx.doi.org/10.1093/aje/kwy163

333. Liang Y, Driscoll AJ, Patel PD, Datta S, Voysey M, French N, et al. Typhoid conjugate vaccine effectiveness in Malawi: evaluation of a test-negative design using randomised, controlled clinical

trial data. Lancet Glob Heal [Internet]. 2023 Jan 1 [cited 2026 May 4];11(1):e136–44. Available from: https://www.thelancet.com/action/showFullText?pii=S2214109X22004661

334. Wakefield J. Ecologic studies revisited. Annu Rev Public Health. 2008;29:75–90.

335. National Statistical Office (NSO), Utica International and I. Malawi Demographic and Health Survey 2024: Final Report. 2024.

336. Dzinamarira T, Mano O, Musuka G, Madziva R, Mataruse N, Mbunge E, et al. Prevalence and determinants of full immunization among children under five in sub-Saharan Africa: A systematic review and meta-analysis (2013–2025). Vaccine [Internet]. 2026 Mar 7 [cited 2026 May 4];75:128228. Available from: https://www.sciencedirect.com/science/article/pii/S0264410X26000356?via%3Dihub

337. Tseole NP, Mindu T, Kalinda C, Chimbari MJ. Barriers and facilitators to Water, Sanitation and Hygiene (WaSH) practices in Southern Africa: A scoping review. PLoS One [Internet]. 2022;17(8 August):1–17. Available from: http://dx.doi.org/10.1371/journal.pone.0271726

338. WHO - Global Task Force on Cholera Control. Global Task Force on Cholera Control. Global Road Map To End Cholera by 2030. 2017.

339. National Statistical Office, Macro International Inc. Malawi Demographic and Health Survey 1992. 1994;157.

340. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): A prospective, case-control study. Lancet [Internet]. 2013 Jul 20 [cited 2026 May 4];382(9888):209–22. Available from: https://www.thelancet.com/action/showFullText?pii=S0140673613608442

341. Khanam F, Islam MT, Bhuiyan TR, Hossen MI, Rajib MNH, Haque S, et al. The Enterics for Global Health (EFGH) Shigella Surveillance Study in Bangladesh. Open Forum Infect Dis [Internet]. 2024 Mar 25 [cited 2026 May 4];11(Supplement_1):S76–83. Available from: https://dx.doi.org/10.1093/ofid/ofad653

342. Wang SA, Hyde TB, Mounier-Jack S, Brenzel L, Favin M, Gordon WS, et al. New vaccine introductions: Assessing the impact and the opportunities for immunization and health systems

strengthening. Vaccine [Internet]. 2013 Apr 18 [cited 2026 May 4];31(SUPPL2):B122–8. Available from: https://www.sciencedirect.com/science/article/pii/S0264410X12015927

343. Ndeketa L, Pitzer VE, Jere KC, Bennett A, Cunliffe NA, Dodd PJ, et al. The long-term impact and effectiveness of rotavirus vaccination in Malawi: an interrupted time-series and case-control analysis. medRxiv [Internet]. 2026 Mar 2 [cited 2026 May 4];2026.02.27.26346681. Available from: https://www.medrxiv.org/content/10.64898/2026.02.27.26346681v1

344. Hosking R, O'connor SY, Wangdi K, Kurscheid J, Lal A. Acceptability measures of water, sanitation and hygiene interventions in low- and middle-income countries, a systematic review. PLoS Negl Trop Dis [Internet]. 2022 [cited 2026 May 4];16(9):e0010702. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC9499221/

Supplementary material

Supplementary Table 1: Annual variation in rotavirus vaccine effectiveness (Rotarix®) by age group since introduction showing trends in protection among children under five years of age

Years post vaccine introduction	**<5 IRR (95% CI)**	**<1 IRR (95% CI)**	**≥1 IRR (95% CI)**
1	0.82 (0.58–1.14)	0.59 (0.41 – 0.86)**	1.35 (0.92–1.98)
2	0.69 (0.49–0.96) *	0.60 (0.42 – 0.88)**	0.91 (0.59–1.39)
3	0.77 (0.55–1.08)	0.63 (0.44 – 0.92)*	1.10 (0.73–1.63)
4	0.88 (0.63–1.22)	0.74 (0.51 – 1.09)	0.98 (0.65–1.48)
5	0.72 (0.51–1.02)	0.66 (0.45 – 0.98)*	0.63 (0.39–1.00)
6	0.80 (0.57–1.13)	0.63 (0.42 – 0.93)**	0.93 (0.61–1.40)
7	0.70 (0.48–1.04)	0.56 (0.35 – 0.89)*	0.67 (0.39–1.11)
***<0.05, **<0.01, ***<0.001**			

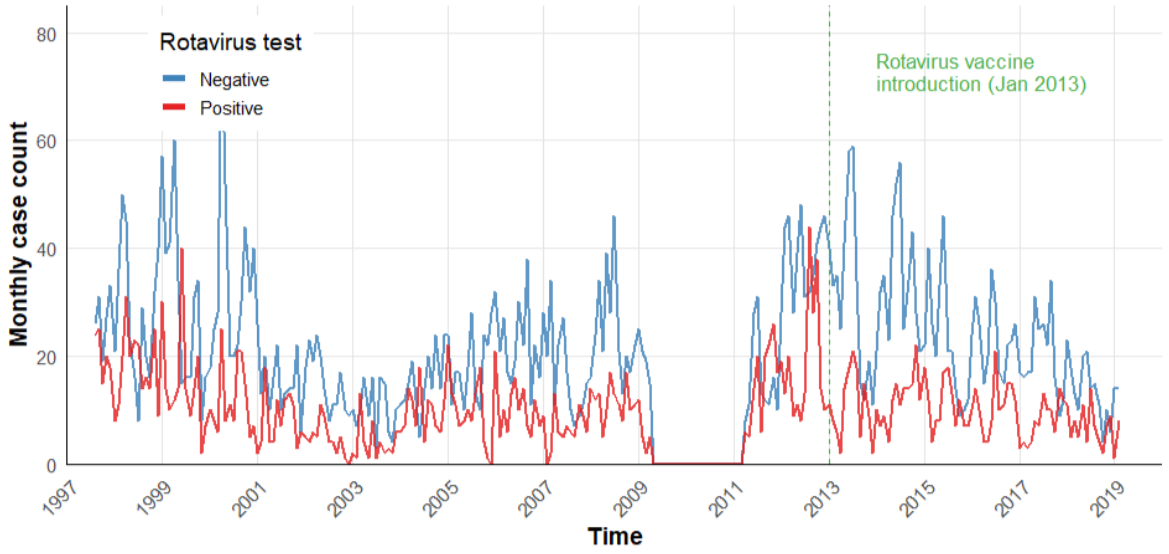
Supplementary Table 2: Characteristics of rotavirus-positive cases and test-negative controls included in the OPV–Rotarix® interaction analysis (children who received both OPV and Rotarix®)

Characteristic	**Controls (N=1,118)**	**Cases (N=504)**
Received bOPV, n (%)	339 (30.3%)	163 (32.3%)
Age category, n (%)		
<1 year	727 (65.0%)	322 (63.9%)
1–<2 years	332 (29.7%)	163 (32.3%)
≥2 years	59 (5.3%)	19 (3.8%)
Male sex, n (%)	693 (62.0%)	310 (61.5%)
Nutritional status, n (%)		
MAM	200 (17.9%)	105 (20.8%)
SAM	151 (13.5%)	43 (8.5%)
Well-nourished	766 (68.6%)	356 (70.6%)

MAM = Moderate acute malnutrition; SAM = Severe acute malnutrition	

Supplementary Table 3: Characteristics of rotavirus-positive cases and rotavirus-negative controls

Characteristic	**Controls (N=1,118)**	**Cases (N=504)**
Received bOPV, n (%)	339 (30.3%)	163 (32.3%)
Age category, n (%)		
<1 year	727 (65.0%)	322 (63.9%)
1–<2 years	332 (29.7%)	163 (32.3%)
≥2 years	59 (5.3%)	19 (3.8%)
Male sex, n (%)	693 (62.0%)	310 (61.5%)
Nutritional status, n (%)		
MAM	200 (17.9%)	105 (20.8%)
SAM	151 (13.5%)	43 (8.5%)
Well-nourished	766 (68.6%)	356 (70.6%)
MAM = Moderate acute malnutrition; SAM = Severe acute malnutrition		



Supplementary Figure 1: Monthly trend of rotavirus positive and negative acute gastroenteritis (AGE) cases before and after Rotarix® introduction among children under five years

Supplementary Table 4: . Definition of variables used in the Rt–vaccine effectiveness analysis

Variable	Description	Source	Role in analysis
weekly_cases	Weekly number of suspected and confirmed cholera cases	Blantyre District Health Office (BDHO) line list	Epidemic description
Median_R (Rt)	Median time-varying reproduction number estimated using EpiEstim	Derived from case time series	Primary outcome
R_lower	Lower bound of the 95% credible interval for Rt	EpiEstim output	Uncertainty quantification
R_upper	Upper bound of the 95% credible interval for Rt	EpiEstim output	Uncertainty quantification
coverage	Cumulative proportion of district population receiving OCV	BDHO vaccination reports	Main exposure. Proportion (0-1)
hh_reached	Number of households reached by WASH teams per day	BDHO WASH activity reports	Raw WASH input
hh_reached_k	Daily households reached scaled per 1,000 households	Derived from WASH data	Standardised WASH metric

wash_roll7_k	Seven-day rolling sum of households reached per 1,000	Derived	Short-term WASH intensity
wash_roll7_k_lag7	Seven-day rolling WASH exposure lagged by seven days	Derived	Lagged WASH exposure
wash_cum_k	Cumulative households reached per 1,000 households	Derived	Total WASH reach
wash_cum_k_lag7	Cumulative WASH exposure lagged by seven days	Derived	Primary WASH covariate in adjusted model
Rt_sample	Simulated Rt draw for each time point	Posterior sampling	Uncertainty propagation

Supplementary Table 4: Classification of reported cholera cases by vaccination status, Blantyre District, 2022–2023

Case classification	Unvaccinated	Vaccinated	Missing vaccination status	Total
Confirmed, RDT-positive	1,071	6	23	1,100
Probable	6,841	28	44	6,913
Total	**7,912**	**34**	**67**	**8,013**

Supplementary Table 5: JBI quality assessment of cohort studies

Study Title	1. Were the two groups similar and recruited from the same population?	2. Were the exposures measured similarly to assign people to both exposed and unexposed groups?	3. Was the exposure measured in a valid and reliable way?	4. Were confounding factors identified?	5. Were strategies to deal with confounding factors stated?	6. Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?	7. Were the outcomes measured in a valid and reliable way?	8. Was the follow up time reported and sufficient to be long enough for outcomes to occur?	9. Was follow-up complete and losses described?	10. Were strategies to address incomplete follow up utilized?	11. Was appropriate statistical analysis used?	C
Impact of H1N1 Influenza Vaccination on Child Morbidity in Guinea-Bissau.	Yes	yes	yes	yes	yes	yes	yes	yes	unclear	unclear	yes	d a c P
Impact of monovalent rotavirus vaccine on diarrhoea-associated post-neonatal infant mortality in rural communities in Malawi: a population-based birth cohort study.	Yes	yes	yes	yes	yes	yes	yes	yes	yes	unclear	yes	d a c P
Measles vaccination effectiveness among children under 5 years of age in Kampala, Uganda.	yes	yes	yes	not reported	not reported	yes	yes	yes	yes	not reported	not reported	y

Reduced effectiveness of Haemophilus influenzae type b conjugate vaccine in children with a high prevalence of human immunodeficiency virus type 1 infection.	yes	yes	yes	not reported	not reported	yes	yes	yes	unclear	not reported	not reported	y th si H in cl in to va ef o
Lessons and challenges for measles control from unexpected large outbreak, Malawi.	Yes	yes	yes	yes	yes	unclear	yes	not applicable - retrospective cohort	not applicable	not applicable	not reported	re co

Supplementary Table 6: JBI quality assessment of ecological studies

Study Title	Was the sample frame appropriate to address the target population?	Were study participants sampled in an appropriate way?	Was the sample size adequate?	Were the study subjects and the setting described in detail?	Was the data analysis conducted with sufficient coverage of the identified sample?	Were valid methods used for the identification of the condition?	Was the condition measured in a standard, reliable way for all participants?	Was there appropriate statistical analysis?	Was the response rate adequate, and if not, was the low response rate managed appropriately?	Comments: Include, exclude % Seek further info
Impact of Rotavirus Vaccination on Hospitalizations and Deaths From Childhood Gastroenteritis in Botswana	Yes	Not applicable	Not clear	yes	yes	yes	yes	yes	Not applicable	Did not adjust for PHSMs
Evidence of the impact of monovalent rotavirus vaccine on childhood acute gastroenteritis hospitalization in Togo.	Yes	Not applicable	Not applicable	yes	yes	yes	yes	yes	yes	Did not adjust for PHSMs
Early impact of rotavirus vaccination in children less than five years of age in Mozambique.	Yes	Not applicable	Not applicable	yes	yes	yes	yes	yes	yes	Did not adjust for PHSMs
Impact of rotavirus vaccine on all-cause diarrhea and rotavirus hospitalizations in Madagascar.	Yes	Not applicable	Not applicable	yes	yes	yes	yes	yes	yes	Did not adjust for PHSMs
Impact and effectiveness of pentavalent	Yes	Not applicable	Not applicable	yes	yes	yes	yes	yes	yes	Did not adjust for PHSMs

rotavirus vaccine in children <5 years of age in Burkina Faso.										
Impact of the serogroup A meningococcal conjugate vaccine, MenAfriVac, on carriage and herd immunity.	Yes	yes	Unclear	yes	yes	yes	yes	yes	yes	Did not adjust for PHSMs
Early Evidence of Impact of Monovalent Rotavirus Vaccine in Togo.	Yes	Not applicable	Not applicable	yes	yes	yes	yes	yes	yes	Did not adjust for PHSMs
Detection of rotavirus before and after monovalent rotavirus vaccine introduction and vaccine effectiveness among children in mainland Tanzania.	Yes	Not applicable	Not applicable	yes	yes	yes	yes	yes	yes	Did not adjust for PHSMs
Impact of rotavirus vaccine introduction and genotypic characteristics of rotavirus strains in children less than 5â€¯years of age with gastroenteritis in Ethiopia: 2011-2016.	Yes	Not applicable	Not applicable	yes	yes	yes	yes	yes	yes	Did not adjust for PHSMs
Impact of routine rotavirus vaccination on all-cause and rotavirus hospitalizations during the first four	Yes	Not applicable	Not applicable	yes	yes	yes	yes	yes	yes	Did not adjust for PHSMs

years following vaccine introduction in Rwanda.										
Impact of Rotavirus Vaccination on Diarrheal Hospitalizations in Children Aged <5 Years in Lusaka, Zambia	Yes	Not applicable	Not applicable	yes	yes	yes	yes	yes	yes	Did not adjust for PHSMs
Impact of 13-Valent Pneumococcal Conjugate Vaccine on Pneumococcal Meningitis, Burkina Faso, 2016-2017.	Yes	Not applicable	Not applicable	yes	yes	yes	yes	yes	yes	Did not adjust for PHSMs
Sustained impact of rotavirus vaccine introduction on rotavirus gastroenteritis hospitalizations in children <5 years of age, Ghana, 2009-2016.	Yes	Not applicable	Not applicable	yes	yes	yes	yes	yes	yes	Did not adjust for PHSMs
Reduction of hospitalizations with diarrhea among children aged 0-5â€¯years in Nouakchott, Mauritania, following the introduction of rotavirus vaccine.	Yes	Not applicable	Not applicable	yes	yes	yes	yes	yes	yes	Did not adjust for PHSMs
Impact of rotavirus vaccine on acute gastroenteritis in children under 5 years in Senegal: Experience of	Yes	Not applicable	Not applicable	yes	yes	yes	yes	yes	yes	Did not adjust for PHSMs

sentinel site of the Albert Royer Children's Hospital in Dakar.										
Impact of measles outbreak response vaccination campaign in Dar es Salaam, Tanzania.	Yes	Not applicable	Not applicable	yes	yes	no	No	yes	Yes	Confirmed measles cases were defined either by a positive result for measles-specific IgM antibodies or by epidemiological linkage
Impact of previous immunisation on the incidence of meningococcal disease during an outbreak in a Sahelian area of Senegal.	yes	Not applicable	Not applicable	yes	yes	No	no	yes	yes	CSF was not taken systematically but only some samples were taken fot serogroup diagnosis. Diagnosis was mainly based on clinical aspects.
Direct and possible indirect effects of vaccination on rotavirus hospitalisations among children in Malawi four years	Yes	Not applicable	Not applicable	yes	yes	yes	yes	yes	yes	Did not adjust for PHSMs

after programmatic introduction										
Impact of 13-Valent Pneumococcal Conjugate Vaccine on Meningitis and Pneumonia Hospitalizations in Children aged <5 Years in Senegal, 2010–2016	Yes	Not applicable	Not applicable	yes	yes	yes	yes	yes	yes	They accounted for seasonality but did not adjust for other PHSMs
Effectiveness of Haemophilus influenzae type b conjugate vaccine introduction into routine childhood immunization in Kenya	yes	not applicable - data is from a disease surveillance programme	not applicable	yes	yes	yes	yes	yes	not applicable	Did not adjust for PHSMs
Pneumococcal conjugate vaccines and hospitalization of children for pneumonia: a time-series analysis, South Africa, 2006-2014.	yes	yes	yes	yes	yes	yes	yes	yes	not reported	Did not adjust for PHSMs
Pneumococcal Conjugate Vaccine Impact on Meningitis and Pneumonia Among Children Aged <5 Years-Zimbabwe, 2010-2016.	yes	not applicable - data is from a disease surveillance programme	not applicable	yes	yes	yes	yes	yes	not applicable	Did not adjust for PHSMs
Impact of rotavirus vaccination on rotavirus	yes	Yes	yes	yes	yes	yes	yes	yes	yes	Did not adjust for PHSMs

hospitalisation rates among a resource-limited rural population in Mbita, Western Kenya.										
Impact of the introduction of pneumococcal conjugate vaccination on pneumonia in The Gambia: population-based surveillance and case-control studies.	yes	not applicable - data is from a disease surveillance programme	not applicable	yes	yes	yes	yes	yes	not applicable	Did not adjust for PHSMs
Pneumococcal Conjugate Vaccine Impact on Meningitis and Pneumonia Among Children Aged <5 Years-Zimbabwe, 2010-2016.										Did not adjust for PHSMs
Pneumococcal Conjugate Vaccine Impact on Meningitis and Pneumonia Among Children Aged <5 Years-Zimbabwe, 2010-2016.	yes	not applicable - data is from a disease surveillance programme	not applicable	yes	yes	yes	yes	yes	not applicable	Did not adjust for PHSMs
Surveillance of impact of PCV-10 vaccine on pneumococcal meningitis in Mozambique, 2013 – 2015	Yes	not applicable - data is from a disease surveillance	not applicable	yes	yes	yes	yes	yes	not applicable	Did not adjust for PHSMs

		programme								
Sustained impact of rotavirus vaccine on rotavirus hospitalisations in Lusaka, Zambia, 2009-2016	Yes	not applicable - data is from a disease surveillance programme	not applicable	yes	yes	yes	yes	yes	not applicable	Did not adjust for PHSMs
Temporal Association of Rotavirus Vaccine Introduction and Reduction in All-Cause Childhood Diarrheal Hospitalizations in South Africa	yes	not applicable - data is from a disease surveillance programme	not applicable	yes	yes	yes	yes	yes	not applicable	Did not adjust for PHSMs
The effect of immunization on measles incidence in the Democratic Republic of Congo: Results from a model of surveillance data	yes	not applicable - data is from a disease surveillance programme	not applicable	yes	yes	yes	yes	yes	not applicable	Did not adjust for PHSMs

Supplementary Table 7: JBI quality assessment of case-control studies

Study Title	**Were the groups comparable other than the presence of disease in cases or the absence of disease in controls?**	**Were cases and controls matched appropriately?**	**Were the same criteria used for identification of cases and controls?**	**Was exposure measured in a standard, valid and reliable way?**	**Was exposure measured in the same way for cases and controls?**	**Were confounding factors identified?**	**Were strategies to deal with confounding factors stated?**	**Were outcomes assessed in a standard, valid and reliable way for cases and controls?**	**Was the exposure period of interest long enough to be meaningful?**	**Was appropriate statistical analysis used?**	**Comments**
Evaluation of the Influence of Gastrointestinal Coinfections on Rotavirus Vaccine Effectiveness in Botswana.	Yes	Not applicable	yes	Yes	Yes	Yes	Yes	Yes	Not applicable	Yes	Did not adjust for concurrent interventions
Field evaluation of measles vaccine effectiveness among children in the Democratic Republic of Congo.	Yes	Not applicable	yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Measles vaccination history was obtained through maternal recall when vaccination cards were unavailable. Did not adjust for concurrent

											interventions
Effectiveness of the 13-valent pneumococcal conjugate vaccine against invasive pneumococcal disease in South African children: a case-control study.	Yes	Yes	yes	yes	yes	yes	yes	yes	yes	yes	They did not clearly define the clinical definition that was similar from cases and controls apart from the strep pneumo positivity. Did not adjust for concurrent interventions
Uptake and Effectiveness of a Trivalent Inactivated Influenza Vaccine in Children in Urban and Rural Kenya, 2010 to 2012.	Yes	Yes	yes	yes	Yes	yes	Yes	yes	yes	Yes	Did not adjust for concurrent interventions
Effectiveness of immunization against paralytic poliomyelitis in Nigeria.	Yes	Yes	yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Did not adjust for concurrent

											interventions
Population Impact and Effectiveness of Monovalent Rotavirus Vaccination in Urban Malawian Children 3 Years After Vaccine Introduction: Ecological and Case-Control Analyses.	yes	Not applicable	yes	yes	yes	yes	Yes	Yes	Yes	Yes	Did not adjust for concurrent interventions
Monovalent Rotavirus Vaccine Effectiveness Against Rotavirus Hospitalizations Among Children in Zimbabwe.	yes	Not applicable	yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Did not adjust for concurrent interventions
A Preliminary Assessment of Rotavirus Vaccine Effectiveness in Zambia.	Yes	Not applicable	yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Did not adjust for concurrent interventions
Effectiveness of pneumococcal conjugate vaccine against presumed bacterial pneumonia hospitalisation in HIV-uninfected South African children: a case-control study.	Yes	Yes	yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	they used both hospital and community controls. Did not adjust for concurrent

											interventions
Influenza epidemiology and vaccine effectiveness among patients with influenza-like illness, viral watch sentinel sites, South Africa, 2005-2009.	Yes	not stated if matched	yes	yes	yes	yes	yes	yes	yes	yes	Did not adjust for concurrent interventions
Effectiveness of haemophilus influenzae type B conjugate vaccine for prevention of meningitis in Senegal.	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	Did not adjust for concurrent interventions
Measles outbreak in Burkina Faso, 2009: a case-control study to determine risk factors and estimate vaccine effectiveness.	yes	yes	yes	yes	yes	no	n/a	yes	yes	yes	Did not adjust for concurrent interventions
Vaccine Effectiveness against DS-1-Like Rotavirus Strains in Infants with Acute Gastroenteritis, Malawi, 2013-2015.	yes	yes	yes	yes	yes	no	n/a	yes	yes	yes	Did not adjust for concurrent interventions
Haemophilus influenzae type b conjugate vaccine is highly effective in the Ugandan routine immunization	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	Did not adjust for concurrent interventions

program: a case-control study.											
Effectiveness of 7-valent pneumococcal conjugate vaccine against invasive pneumococcal disease in HIV-infected and -uninfected children in south africa: a matched case-control study.	yes	yes	yes	yes	yes	yes	yes	yes	yes		Did not adjust for concurrent interventions
Effectiveness of mass oral cholera vaccination in Beira, Mozambique.	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	Did not adjust for concurrent interventions
Estimating vaccine effectiveness in preventing laboratory-confirmed influenza in outpatient settings in South Africa, 2015	YES	not stated if matched	yes	yes	yes	yes	yes	yes	yes	yes	Did not adjust for concurrent interventions
Impact and Effectiveness of Monovalent Rotavirus Vaccine Against Severe Rotavirus Diarrhea in Ghana	yes	not stated if matched	yes	yes	yes	yes	yes	yes	yes	yes	Did not adjust for concurrent interventions
Effectiveness of Pentavalent Rotavirus Vaccine under Conditions of	yes	not stated if matched	yes	yes	yes	yes	yes	yes	yes	yes	Did not adjust for concurre

Routine Use in Rwanda											nt interventions
Effectiveness of Monovalent Rotavirus Vaccine after Programmatic Implementation in Botswana: A Multisite Prospective Case-Control Study	yes	not stated if matched	yes	yes	yes	yes	yes	yes	yes	yes	Did not adjust for concurrent interventions
Effectiveness and knowledge, attitudes and practices of seasonal influenza vaccine in primary healthcare settings in South Africa, 2010-2013	yes	not stated if matched	yes	yes	yes	yes	yes	yes	yes	yes	Did not adjust for concurrent interventions
Use of Vibrio cholerae vaccine in an outbreak in Guinea	yes	not stated if matched	yes	yes	yes	yes	yes	yes	yes	yes	Did not adjust for concurrent interventions
Impact of Rotavirus Vaccine Introduction and Postintroduction Etiology of Diarrhea Requiring Hospital Admission in Haydom, Tanzania, a Rural African Setting.	yes	not stated if matched	yes	yes	yes	yes	yes	yes	yes	yes	Did not adjust for concurrent interventions

Effectiveness of monovalent human rotavirus vaccine against admission to hospital for acute rotavirus diarrhoea in South African children: a case-control study.	yes	not stated if matched	yes	yes	yes	yes	yes	yes	yes	yes	Did not adjust for concurrent interventions
Effectiveness of a monovalent rotavirus vaccine in infants in Malawi after programmatic roll-out: an observational and case-control study.	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	Did not adjust for concurrent interventions
Monovalent Rotavirus Vaccine Effectiveness and Impact on Rotavirus Hospitalizations in Zanzibar, Tanzania: Data From the First 3 Years After Introduction.	yes	not stated if matched	yes	yes	yes	yes	yes	yes	yes	yes	Did not adjust for concurrent interventions
Measles outbreak propagated by children congregating at water collection points in Mayuge District, eastern Uganda, July - October, 2016.	yes	not stated if matched	yes	yes	yes	yes	yes	yes	yes	yes	Did not adjust for concurrent interventions
Effectiveness of oral polio vaccination against paralytic poliomyelitis: a matched case-control study in Somalia.	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	Did not adjust for concurrent interventions

Supplementary Table 8: JBI quality assessment of the screening method study

Study Title	1. Were the criteria for the inclusion in the sample clearly defined?	2. Were the study subjects and the setting described in detail?	3. Was the exposure measured in a valid and reliable way?	4. Were objective, standard criteria used for measurement of the condition?	5. Were confounding factors identified?	6. Were strategies to deal with confounding factors stated?	7. Were the outcomes measured in a valid and reliable way?	8. Was appropriate statistical analysis used?	Comments
Preplanned national measles vaccination campaign at the beginning of a measles outbreak--Sierra Leone, 2009-2010.	Yes	yes	Unclear	yes	yes	Partly/unclear	yes	yes	Restriction by age was used for the VE analysis, but there was no multivariable adjustment. Did not adjust for PHSMs