Theme 3: Pathogen Detection & Discovery

BRAIN INFECTION AETIOLOGY: SYSTEMATIC REVIEW

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Brain Infections Global

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Background & Rationale

- Lack of large prospective studies of brain infection aetiology in LMICs
- Surveillance data limited by repertoire of available tests
- Makes knowing which pathogens to focus on difficult:
  - Intervention
    - Diagnostics
    - Management algorithms
  - Systematic pathogen detection
  - Gaps for pathogen discovery

Objective

To establish the causes of acute brain infections in Brazil, India and Malawi

Methods: Studies

- Studies reporting aetiology of acute brain infections in Brazil, India and Malawi
  - Any clinical study design considered (preferably observational cross-sectional)
  - As long as denominator is clear (trials/case series possibly problematic)
  - Exclude if only include neonates, or patients with nosocomial or neurosurgical infections
  - >100 participants
- Surveillance data from regional and national laboratory and public health databases within these countries

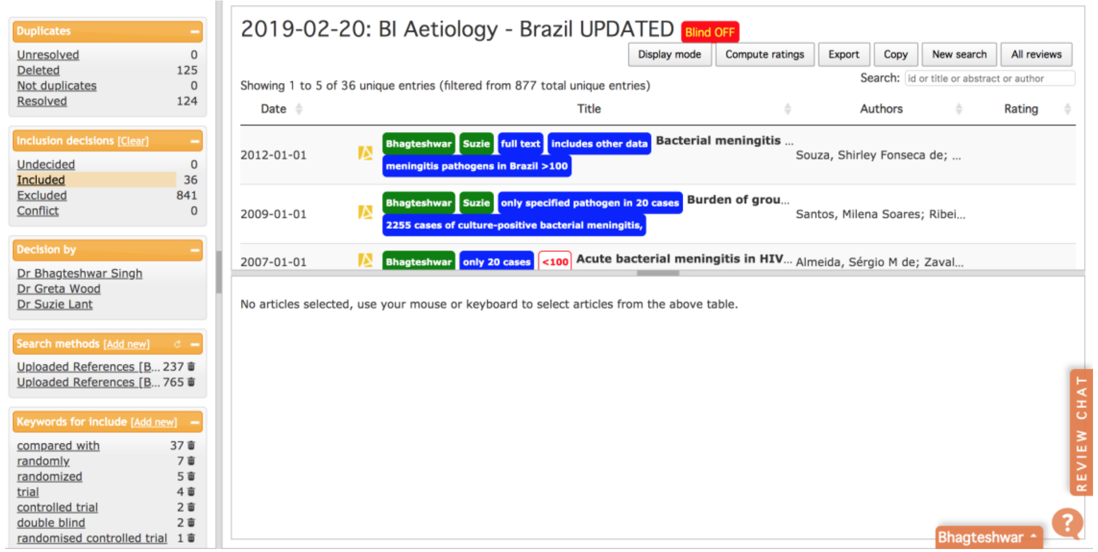
Methods: Search

- Pubmed & Scopus
- Published 1st Jan 1999 to 31st Jan 2019
- Regardless of language or publication status
- Reference lists of included articles

Methods: Data Collection

Study selection:

- Rayyan
- Hierarchical stepwise criteria
- Title -> abstract -> full text



Data extraction:

- Setting, design, years
- Participant recruitment & characteristics
- Diagnostic techniques: approach & tests
- Reported pathogens – proportions of tested and total participants

Risk of bias:

- Bespoke tool modified from existing (JBI)
- 6 questions – score +1 for each “Yes”

All:

- Done by 2 reviewers independently
- 3rd reviewer decides if needed

Methods: Analysis

Meta-analysis:

- R
- Separate for each country
- Pooled proportions of each pathogen
- 95% confidence intervals
- Random-effects DerSimonian and Laird model (anticipated clinical heterogeneity)
- Freeman-Tukey (arcsine square root) transformation

Subgroups:

- Meningitis vs. encephalitis vs. other BI vs. undifferentiated BI vs. suspected BI
- Adults vs. children
- People living with HIV vs. without HIV infection
- Immunosuppressed vs. immunocompetent

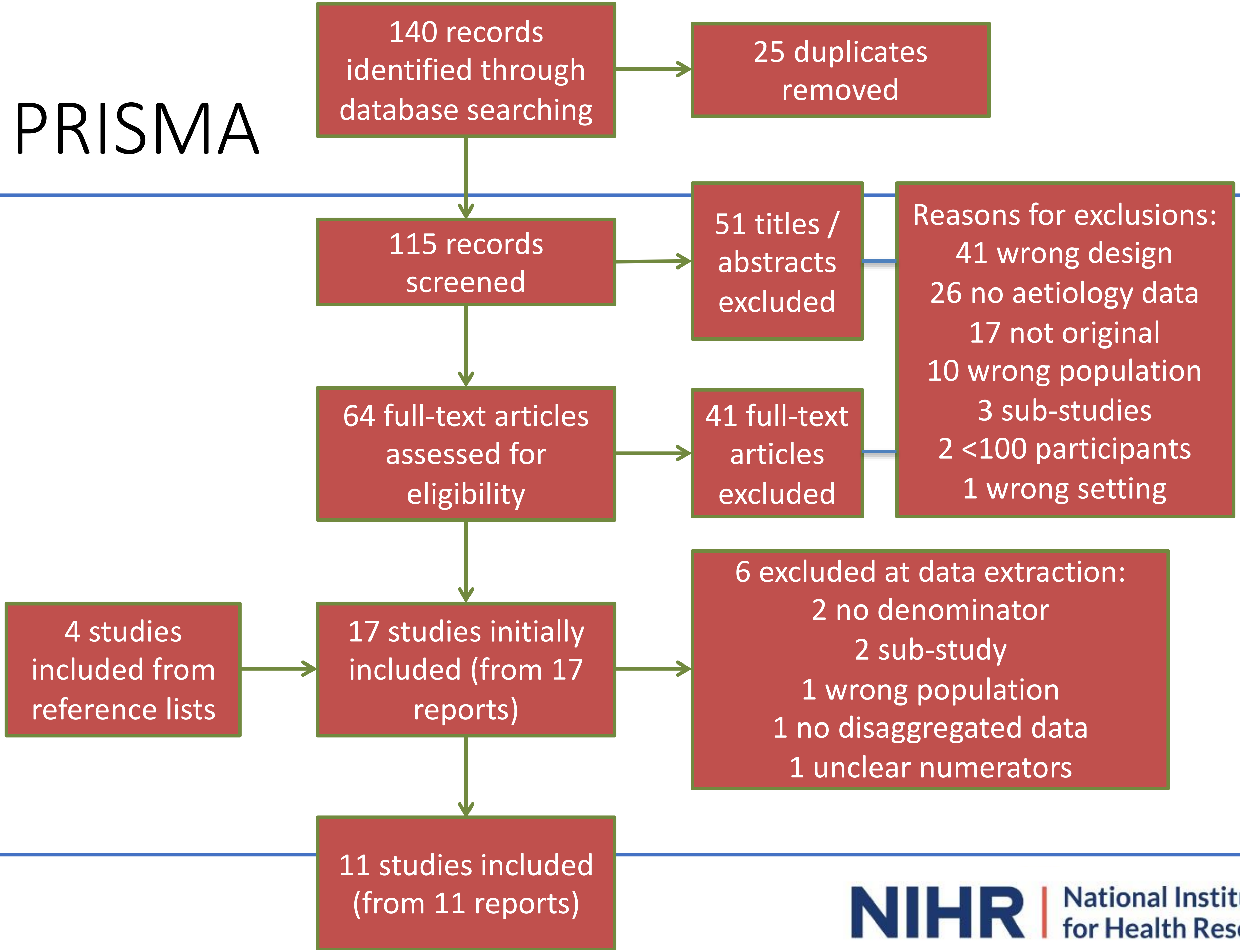
Statistical Heterogeneity:

- Visually inspection of forest plots
- I^2 statistic: >50% = significant heterogeneity

Progress

Country	Search: Records	Screening	Data Extraction	Surveillance Data	Analysis
Brazil	Done: 1002	Done	20/40	Some obtained	
India	Done: 1789	Done	40/100	Pending	
Malawi	Done: 140	Done	Done	Pending	Done (initial)

Malawi: PRISMA

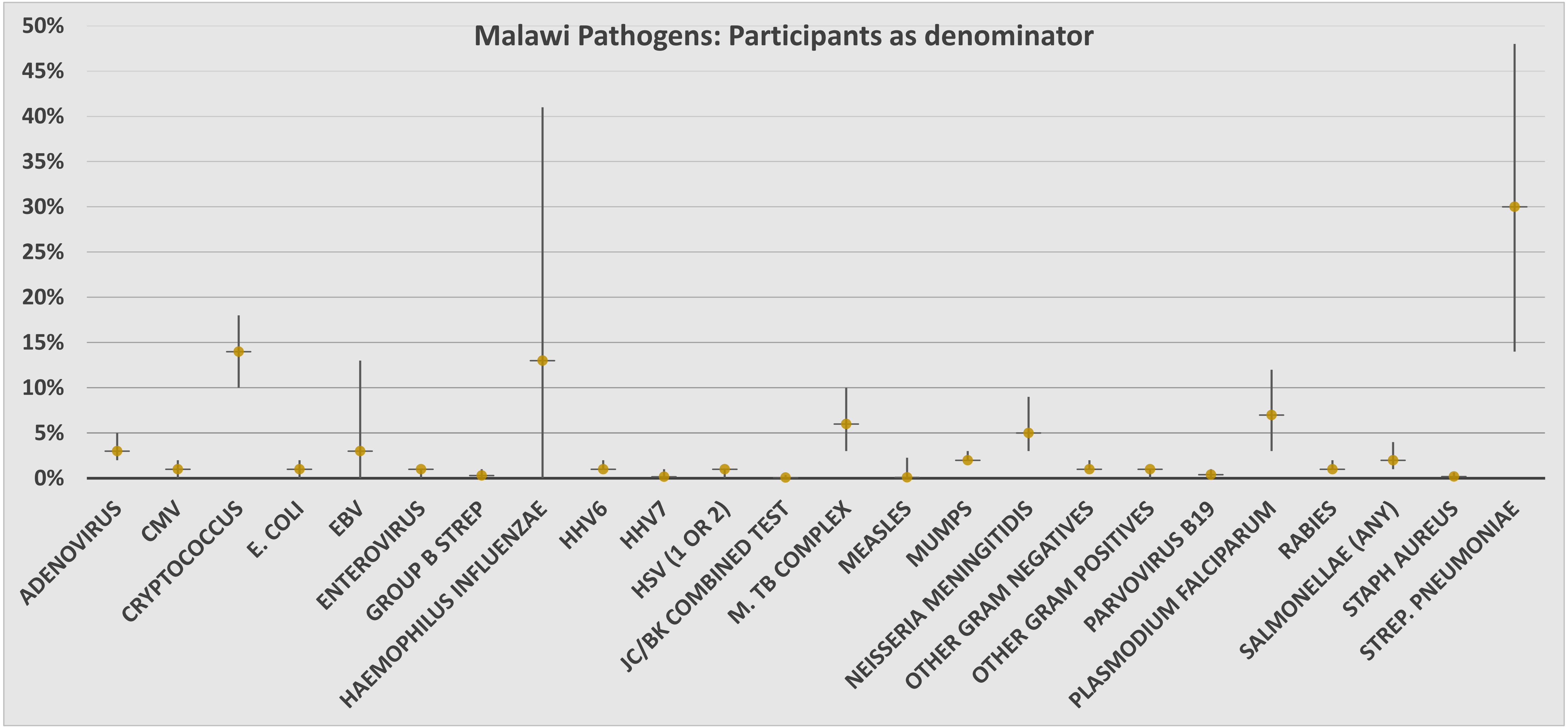


Malawi: Included Studies

- 11 studies
  - 7 prospective observational
  - 4 interventional
- All in single unselected central hospital
  - 1 Lilongwe
  - 10 Blantyre

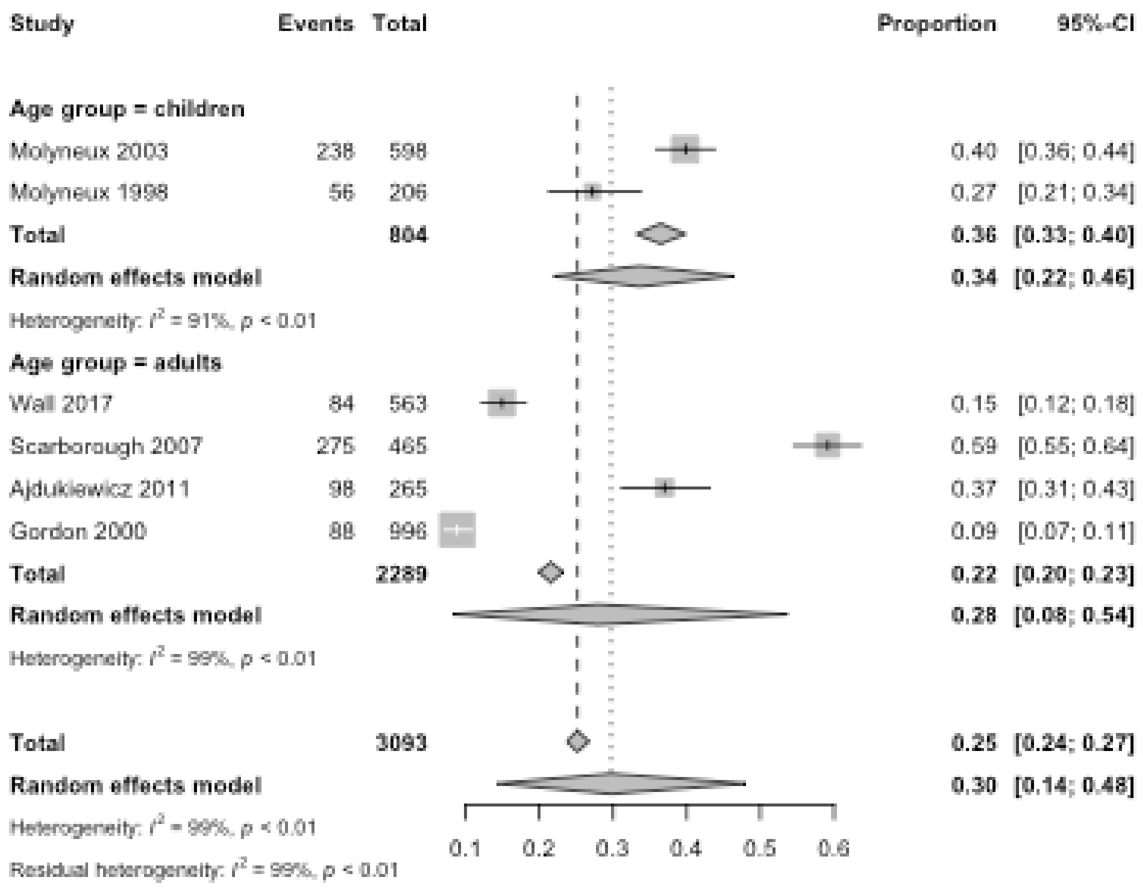
Malawi: Included Studies

Study	Type of Study	Years of Recruitment	Age Groups	Risk of Bias Score	Syndrome	Basis of Inclusion	Testing strategy
Makoka 2012	Prospective	2006 to 2007	All	2	Meningitis	CSF tested	Systematic - single panel
Mallewa 2013	Prospective	2002 to 2004	Children	5	Brain infection	Clinician suspicion	Systematic - staged approach
Molyneux 2003	Interventional	1997 to 2001	Children	6	Meningitis	Confirmed/probable	Systematic - single panel
Maclennan 2017	Prospective	2006	Children	5	Unclear	CSF tested	Systematic - single panel
Wall 2017	Interventional	2012 to 2013	Adults	5	Meningitis	Clinician suspicion	Systematic - single panel
Benjamin 2013	Prospective	2007	Adults	5	Meningitis	Clinician suspicion	Systematic - staged approach
Cohen 2010	Prospective	2007	Adults	5	Brain infection	Clinician suspicion	Systematic - single panel
Scarborough 2007	Interventional	2002 to 2005	Adults	4	Meningitis	Confirmed/probable	Systematic - staged approach
Ajdukiewicz 2011	Interventional	2006 to 2008	Adults	4	Meningitis	Confirmed/probable	Systematic - single panel
Gordon 2000	Prospective	1998 to 1999	Adults	4	Meningitis	Confirmed/probable	Systematic - single panel
Molyneux 1998	Prospective	1996 to 1997	Children	6	Meningitis	Clinician suspicion	Systematic - single panel

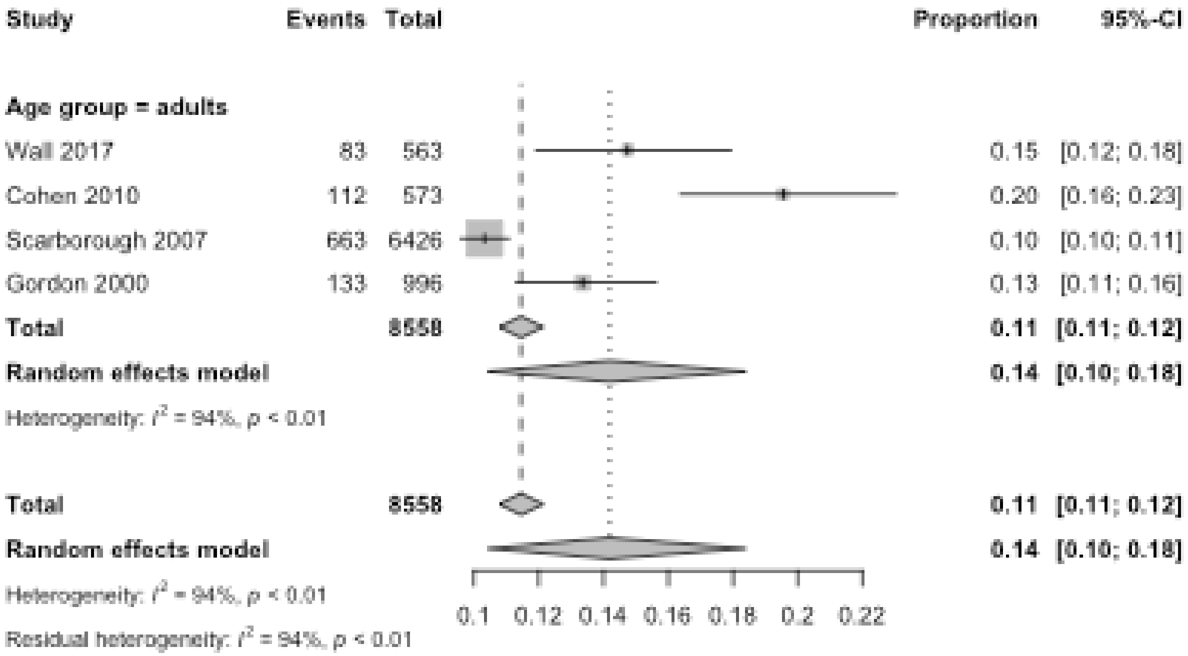

Malawi Pathogens: Participants as denominator
50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
ADENOVIRUS
CMV
CRYPTOCOCCUS
E. COLI
EBV
ENTEROVIRUS
GROUP B STREP
HAEMOPHILUS INFLUENZAE
HHV6
HHV7
HSV (1 OR 2)
JC/BK COMBINED TEST
M. TB COMPLEX
MEASLES
MUMPS
NEISSERIA MENINGITIDIS
OTHER GRAM NEGATIVES
OTHER GRAM POSITIVES
PARVOVIRUS B19
PLASMODIUM FALCIPARUM
RABIES
SALMONELLAE (ANY)
STAPH AUREUS
STREP. PNEUMONIAE

	DENOMINATOR = TOTAL PARTICIPANTS							
PATHOGEN	Participants	Pooled proportion	Lower CI	Upper CI	Adult studies	Child studies	All-age studies	Total studies
Adenovirus	1230	0.03	0.02	0.05	0	1	0	1
CMV	1413	0.01	0	0.02	1	1	0	2
Cryptococcus	8558	0.14	0.1	0.18	4	0	0	4
E. coli	2622	0.01	0	0.02	3	1	0	4
EBV	1413	0.03	0	0.13	1	1	0	2
Enterovirus	1230	0.01	0	0.01	0	1	0	1
Group B strep	598	0.003	0	0.01	0	1	0	1
Haemophilus influenzae	1269	0.13	0	0.41	1	2	0	3
HHV6	1230	0.01	0.01	0.02	0	1	0	1
HHV7	1230	0.0017	0	0.01	0	1	0	1
HSV (1 or 2)	1413	0.01	0	0.01	1	1	0	2
JC/BK combined test	1230	0.0008	0	0.0035	0	1	0	1
M. tb complex	6999	0.06	0.03	0.1	2	0	0	2
Measles	1230	0.001	0	0.0226	0	1	0	1
Mumps	1230	0.02	0.02	0.03	0	1	0	1
Neisseria meningitidis	2828	0.05	0.03	0.09	3	2	0	5
Other Gram negatives	3882	0.01	0.01	0.02	2	2	0	4
Other Gram positives	2059	0.01	0	0.01	2	1	0	3
Parvovirus B19	1230	0.004	0	0.01	0	1	0	1
Plasmodium falciparum	1793	0.07	0.03	0.12	1	1	0	2
Rabies	1230	0.01	0.01	0.02	0	1	0	1
Salmonellae (any)	4088	0.02	0.01	0.04	2	3	0	5
Staph aureus	1461	0.0019	0	0.0064	2	0	0	2
Strep. pneumoniae	3093	0.3	0.14	0.48	4	2	0	6

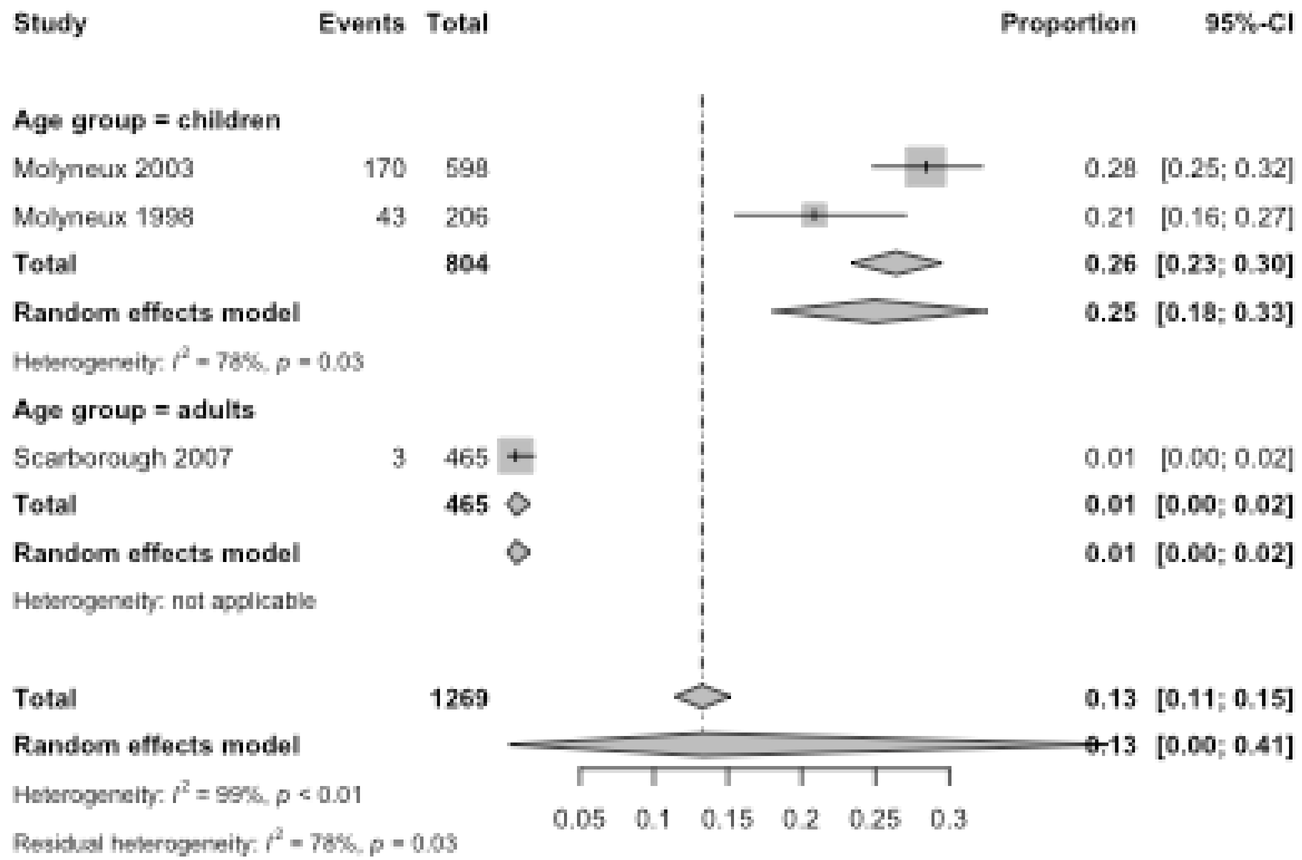
Malawi: *S. pneumoniae*, of total participants



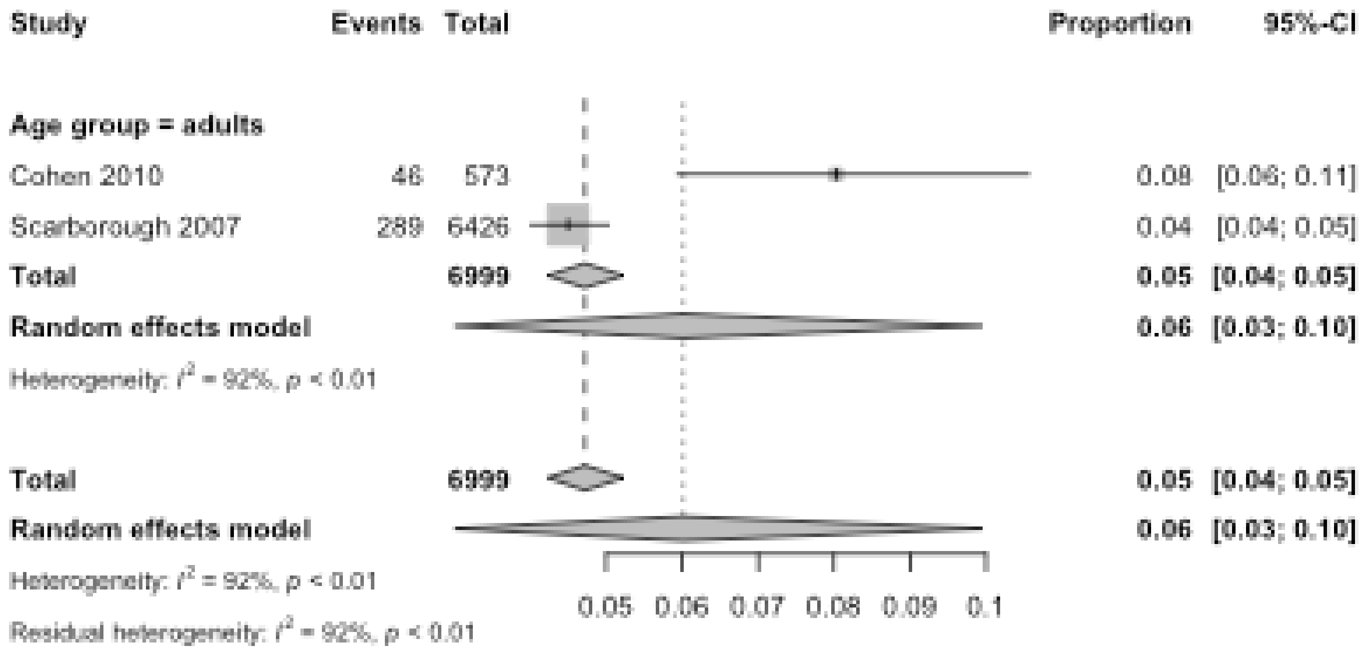
Malawi: *Cryptococcus*, of total participants



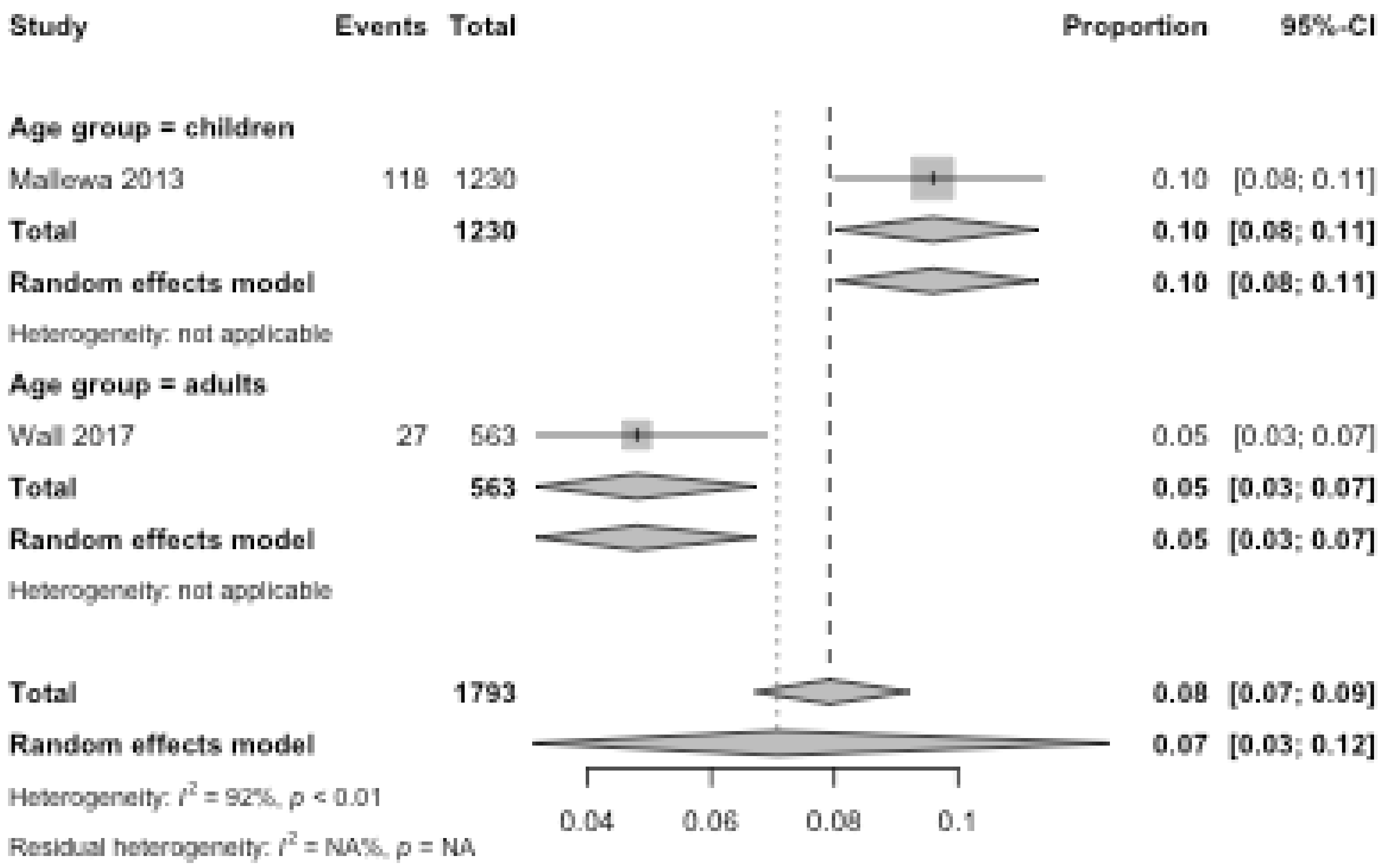
Malawi: *H. influenzae*, of total participants



Malawi: *M. tuberculosis*, of total participants



Malawi: *P. falciparum*, of total participants



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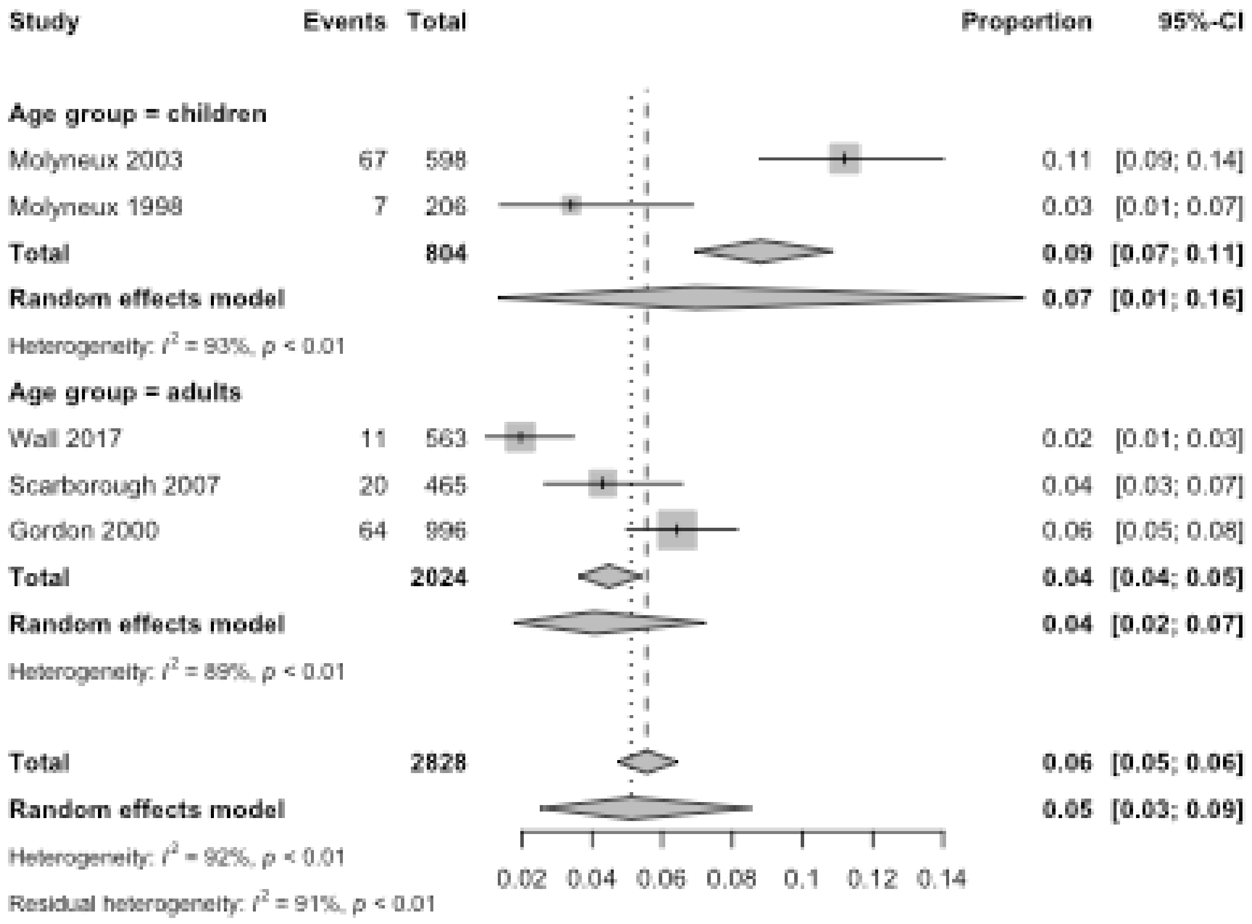
Malawi: *N. meningitidis*, of total participants



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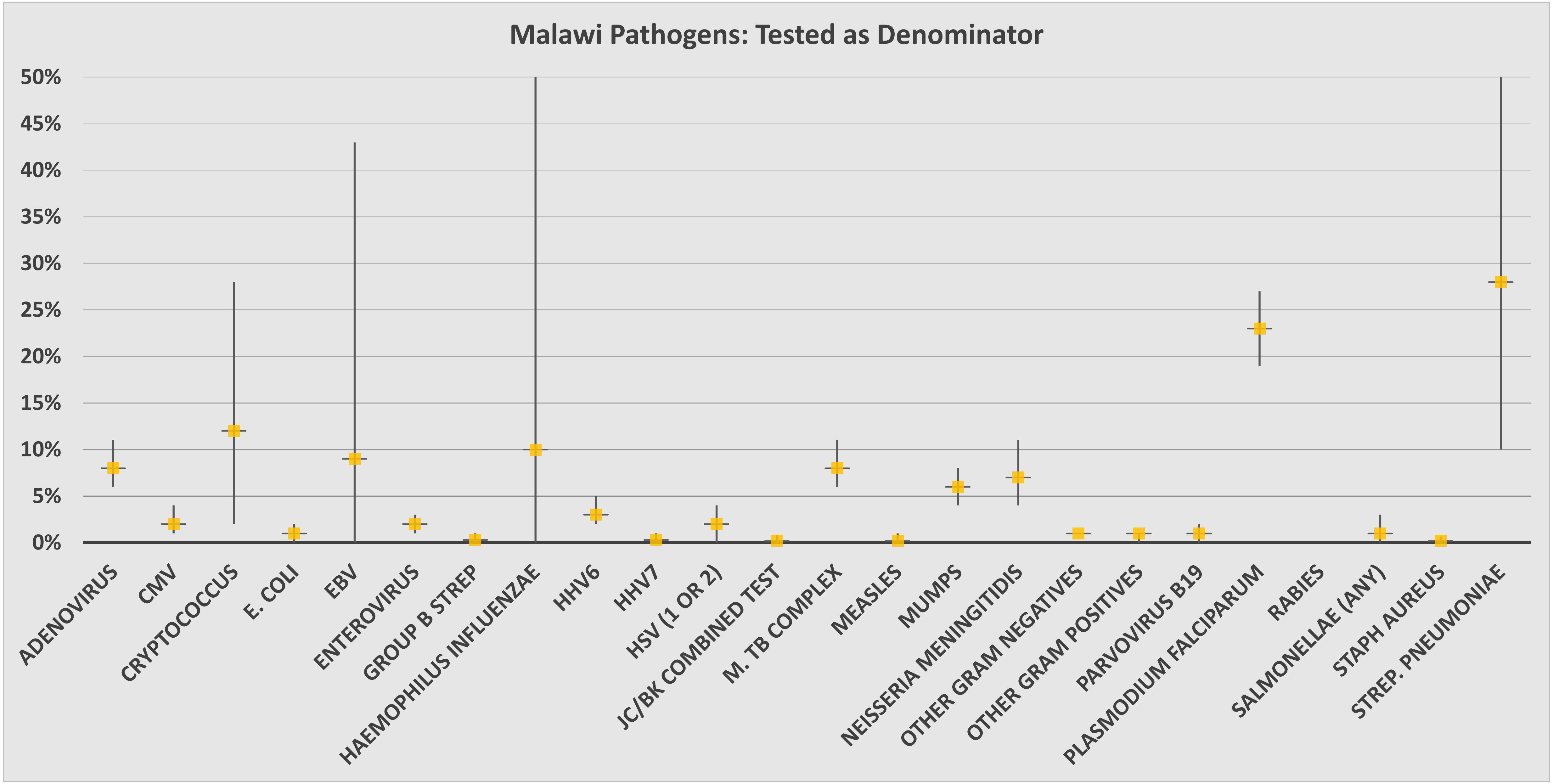

Malawi Pathogens: Tested as Denominator
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PARVOVIRUS B19
PLASMODIUM FALCIPARUM
RABIES
SALMONELLAE (ANY)
STAPH AUREUS
STREP. PNEUMONIAE

	DENOMINATOR = TESTED FOR PATHOGEN							
PATHOGEN	Participants	Pooled proportion	Lower CI	Upper CI	Adult studies	Child studies	All-age studies	Total studies
Adenovirus	513	0.08	0.06	0.11	0	1	0	1
CMV	566	0.02	0.01	0.04	1	1	0	2
Cryptococcus	724	0.12	0.02	0.28	1	0	1	2
E. coli	2059	0.01	0	0.02	2	1	0	3
EBV	566	0.09	0	0.43	1	1	0	2
Enterovirus	513	0.02	0.01	0.03	0	1	0	1
Group B strep	598	0.003	0	0.01	0	1	0	1
Haemophilus influenzae	1063	0.1	0	0.51	1	1	0	2
HHV6	513	0.03	0.02	0.05	0	1	0	1
HHV7	513	0.003	0	0.01	0	1	0	1
HSV (1 or 2)	566	0.02	0	0.04	1	1	0	2
JC/BK combined test	513	0.0019	0	0.0084	0	1	0	1
M. tb complex	573	0.08	0.06	0.11	1	0	0	1
Measles	513	0.002	0	0.01	0	1	0	1
Mumps	513	0.06	0.04	0.08	0	1	0	1
Neisseria meningitidis	2059	0.07	0.04	0.11	2	1	0	3
Other Gram negatives	4033	0.01	0.01	0.01	2	2	1	5
Other Gram positives	2210	0.01	0	0.01	2	1	1	4
Parvovirus B19	513	0.01	0	0.02	0	1	0	1
Plasmodium falciparum	513	0.23	0.19	0.27	0	1	0	1
Rabies								0
Salmonellae (any)	4033	0.01	0	0.03	2	2	1	5
Staph aureus	1461	0.0019	0	0.0064	2	0	0	2
Strep. pneumoniae	2475	0.28	0.1	0.51	3	1	1	5

Comments so far

Heterogeneity:

- Participants
- (India/Brazil) Locations, hospital types
- Statistical

The Team

Malawi:
Sam Moody
Jen Cornick

India:
Chitra Pattabiraman
Divya Mathew
Sharon Jose
Tina Damodar
Priscilla Rupali
V Ravi

Brazil:
Greta Wood
Suzie Lant
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Liverpool:
Fiona McGill
Lance Turtle
Mike Griffiths
Tom Solomon

Pathogen Panels

- Specific to country and adult vs child (and <6mo? – depends on average age of children seen in each centre)
- Aetiology review should help
- Surveillance data may be even more helpful – but struggling to get
- Focus on:
  - Common
  - Treatable
  - Validated test available

Step	Pathogen	Sample	Technique	Common?	Treatable?	Validated test available?
1	Pneumococcus	CSF	PCR	Yes - most common: 30% [14-48] of all participants in meta-analysis (6 studies)	Yes	Yes
	Cryptococcus	CSF/serum	Lateral flow	Yes: 12% [2-28] of those tested for Cryptococcus in meta-analysis (2 studies)	Yes	Yes
	M. tuberculosis	CSF	PCR	Yes: pooled 6% [3-10] of participants in meta-analysis (2 adult studies)	Yes	Yes
	Meningococcus	CSF	PCR	Yes: pooled 5% [4-8] of adults tested for bacteria in meta-analysis (2 studies)	Yes	Yes
	Plasmodium falciparum	Blood/serum ?	PCR/Ag test??	Yes: 7% [3-12] of 1793 participants in 2 studies; but most febrile patients have routine malaria testing	Yes	On stored sample??
2	HSV	CSF	PCR	Probably moderate - 2/53 tested in Benjamin 2013	Yes	Yes
	Enteroviruses	CSF	PCR	Probably - common in HIC; 2% [1-3] in children (Mallewa 2013)	No	Yes
	E. coli	CSF	PCR	Not rare: 1% [0-2] in 2622 participants (4 studies: 3 adult)	Yes	Yes
	Syphilis	CSF/serum	Serology	Uncertain for Malawi, but 1-3% of suspected meningitis in Marks 2017 syst rev	Yes	Yes
	Salmonellae	CSF	PCR	Not rare: 2% [1-4] of 4088 participants (5 studies: 2 adult)	Yes	Yes
3	VZV	CSF	PCR	0/53 PCR-positive in Benjamin 2013, but expected to be prominent with high HIV prevalence	Yes	Yes
	Toxoplasma	CSF/serum	PCR/serolog y	Probably not rare, but not reported in Malawi studies; 25% in HIV-infected adults in Ghana (Opintan 2018)	Yes	Yes but difficult to interpret
	Dengue	CSF/serum	Serology	No Malawi-based studies available, but as seen in India & Brazil, could well be present	No	Yes but difficult to interpret
	Chikungunya	CSF/serum	Serology	No Malawi-based studies available, but as seen in India & Brazil, could well be present	No	Yes but difficult to interpret
	Adenovirus	CSF	PCR	42/513 children tested in Mallewa 2013; adult commonality plausible	No	Yes
	CMV	CSF	PCR	Yes in HIV	Yes	Yes
4 (IF						

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Pathogen Panels

- Specific to country and adult vs child (and <6mo? – depends on average age of children seen in each centre)
- Aetiology review should help
- Surveillance data may be even more helpful – but struggling to get
- Focus on:
  - Common
  - Treatable
  - Validated test available

Outcomes - 3

3.1: Agree on pathogen detection panels

Questions:

1. Do the pathogen panels for each country accurately reflect the pathogens likely to be encountered in each centre?
2. Are the samples and tests correct for each pathogen – in terms of yield, accuracy and practicality?
3. How much CSF and serum will be needed for each test – will 3mL adults/1mL children CSF be enough?
4. We haven't yet planned to take serum (only 2.5mL Paxgene and 2.5mL EDTA blood samples). Should we plan to, and if so, how much?

Questions & Discussion…

Methods: Risk of Bias/Quality – EXTRA SLIDE

- JBI Critical Appraisal Checklist for Studies Reporting Prevalence Data (Munn 2014)
- Developed for population-level cross-sectional studies of prevalence of a specific disease
- Piloted by 3 reviewers
- Decided it (and similar) not applicable to hospital-based patients with a variety of microbiological diagnoses
- Methodological quality – not so helpful in judging confidence in estimates provided in studies
- Risk of bias
- Bespoke – as other studies have done

Questions

1. Which pathogens are responsible for acute brain infections in Brazil, India and Malawi, with particular focus on meningitis, encephalitis and brain abscess?
2. Which tests and testing strategies have been used to determine brain infection aetiologies in Brazil, India and Malawi?